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NF-κB in solid tumors

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Abbreviations:
c-FLIP, cellular FADD-like interleukin 1beta converting enzyme inhibitory protein
CDK, cyclin dependent kinase
COX, cycloxygenase
CXCR, chemokine receptor
ELAM, endothelial cell leukocyte adhesion molecule
GADD, growth arrest and DNA damage
IAP, inhibitor of apoptosis
ICAM, intercellular adhesion molecule
IKK, IκB kinase
IL, interleukin
IκB, inhibitor of NF-κB
JNK, Jun N-terminal kinase
NEMO, NF-κB essential modulator
NF-κB, nuclear factor-kappa B
PTEN, phosphatase and tensin homologue
ROS, reactive oxygen species

ABSTRACT

Cancer is a multistep process during which cells acquire genetic alterations that drive the progressive transformation of normal cells into highly malignant cells. Self-sufficiency in growth, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis, are signatures of transformed cells. NF-κB is a key actor in tumorigenesis given its ability to control the expression and the function of a number of genes involved in these processes. Indeed, constitutive activation of NF-κB is a common feature of many human tumors, while its sustained activation during inflammation predisposes normal cells to neoplastic transformation. Since suppression of NF-κB has been shown to inhibit oncogenic potential of transformed cells, targeting it should be effective in the prevention and treatment of cancer.

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TNF, tumor necrosis factor
TRAIL, TNF-related apoptosis-inducing ligand
VCAM, vascular cell adhesion molecule

1. Introduction

Several lines of evidence show that NF-κB plays a role in cancer. Its activity has been found constitutively elevated in many types of human tumors from either haematological or solid origin, such as melanomas [1,2], breast [3,4], prostate [5,6], ovarian [7,8], pancreatic [9,10], colon [11,12] and thyroid carcinomas [13,14] (Table 1). The role of NF-κB in solid tumors has been well documented in several studies performed on primary tumors and neoplastic cell lines derived from different human tissues. These studies show that the inhibition of constitutive NF-κB activity blocks the oncogenic potential of neoplastic cells by different ways: by sensitizing tumor cells to chemotherapeutic drug-induced apoptosis, by decreasing the highly proliferative rate which characterizes transformed cells, by inhibiting tissue invasiveness and metastatic potential of highly malignant cells [15]. In addition, since it is now generally accepted that chronic inflammation contributes to the genesis of many solid tumors, such as gastric, colon or hepatic carcinomas, it has been recently shown that activation of NF-κB by the classical IKKβ-dependent pathway, is a crucial mediator of inflammation-induced tumor growth and progression in animal models of inflammation-associated cancer [16,17]. Therefore, NF-κB is able to regulate the expression and the function of a wide spectrum of genes involved in the control of cell cycle, apoptosis, cell growth, tissue invasiveness and inflammation. It is just this ability that makes NF-κB the crucial point of convergence of a number of stimuli that can influence different aspects of cellular homeostasis and, therefore, can lead to the onset of cancer. This chapter will focus on different aspects of NF-κB-mediated tumorigenesis.

2. Evidence for a role for NF-κB in tumorigenesis

The earliest evidence for a role for NF-κB in cell transformation comes from the finding that v-Rel was identified as the transforming gene of an avian retroviruses which is highly oncogenic and causes aggressive tumors in chickens [18]. Other viral proteins that mediate tumorigenesis also signal through NF-κB activation. For example, the Tax oncoprotein from the human T-cell leukemia virus (HTLV-I), induces NF-κB activity, and NF-κB activation is required for transformation of rat fibroblast by the HTLV-I Tax protein. Also, the latent membrane protein 1 (LMP1) of Epstein-Barr virus (EBV), an essential component of the viral machinery that orchestrates cellular transformation and oncogenesis, functions mimicking of activated cell surface receptors of the tumor necrosis factor (TNF) superfamily, resulting in the activation of NF-κB.

3. Genetic associations of NF-κB and human cancer

The cellular homolog of v-Rel, c-Rel, transforms cells in vitro and is frequently amplified in different lymphomas [19]. The transforming activity of c-Rel is likely to reside in its DNA-binding domain given that the sequences in the Rel homology

Table 1 – Constitutive NF-κB activity in human cancer cells

Type of cancer	Proposed mechanism
Hodgkin's disease	Constitutive IKK activity; *iκba*, *iκbe* mutations
Acute lymphoblastic leukemia	Constitutive IKK activity; *nfκb1* chromosomal rearrangement
T-cell leukemia	Increased IκBα degradation
Diffuse large cell lymphoma	*c-rel* amplification/rearrangement/overexpression
Follicular lymphoma	*c-rel* rearrangement/overexpression
B-cell non-Hodgkin's lymphoma	*relA*, *nfκb2*, *bcl3* chromosomal rearrangements
Multiple myeloma	*relA* amino acid substitution
Head and neck squamous cell carcinoma	Defective IκBα activity
Breast	Defective IκBα activity
Colon	Defective IκBα activity
Ovary	Defective IκBα activity
Pancreas	Defective IκBα activity
Thyroid	Defective IκBα activity
Kidney	Defective IκBα activity
Bladder carcinoma	Defective IκBα activity
Liver	Defective IκBα activity
Prostate	Defective IκBα activity
Astrocytoma/glioblastoma	Defective IκBα activity
Melanoma	Defective IκBα activity

domain (RHD) are highly similar between chicken, mouse and human c-Rel, while there is much less similarities in the transactivation domain. Because RelA and RelB, similarly to c-Rel, are potent transcription activators, one might expect to find RelA and RelB amplified in human cancer. However, there is no convincing evidence for amplification of RelA and RelB in human malignancies and, in addition, neither RelA nor RelB transform cells ''in vitro''. It is then possible that c-Rel overexpression (mediated by either ectopic expression ''in vitro'' or amplification ''in vivo'') drives transcription of specific genes which are not targeted by RelA and RelB.

Other members of the NF-κB family have been reported to be involved in rearrangement and amplification. The t(10:14) chromosomal translocation breakpoint associated with *nfκb2* originally found in a case of B cell non-Hodgking's lymphoma, is found in numerous lymphoid neoplasms. The truncated p100 proteins generated by this translocation may undergo unregulated processing, resulting in the altered production of p52. Consistent with unchecked production of p52 contributing to oncogenesis, mice with homozygous deletion of the C-terminal ankirin repeats of the p100 precursor exhibited increased number of T lymphocytes as well as enlarged lymph nodes and gastric hyperplasia [20]. However, the lymphoma-derived p100 proteins have not been demonstrated to be directly oncogenic, so far.

Similarly, the *bcl-3* gene was originally identified in a t(14:19) chromosomal translocation in a subset of chronic lymphocytic leukemias [21]. Bcl-3 protein is a member of the IκB family of NF-κB inhibitors with the unique feature that is largely a nuclear protein and that can enhance transcription of NF-κB target genes through formation of heterocomplexes with p50 or p52 homodimers. Bcl-3 can directly transform cell ''in vitro'' and, accordingly, transgenic mice overexpressing Bcl-3 in B cells develop splenomegaly and show excess of mature B cells in lymph nodes and bone marrow [22].

In addition to chromosomal translocation and gene amplification, also mutations of the *iκba* gene have been described. The Reed–Sternberg cells of the Hodgkin's lymphoma have constitutive active NF-κB and blockage of NF-κB activity by using a super-repressor form of IκBα can induce apoptosis [23]. Constitutive NF-κB activity due to defective IκB activity has been demonstrated in Hodgkin's lymphoma, where loss of function mutations are sometimes found.

4. What is the role of NF-κB in oncogenesis?

According to Hanahan and Weinberg [24], six essential alterations in cell physiology characterize a tumor cell: self-sufficiency in growth, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Many of the genes able to mediate such effects are under transcriptional control of NF-κB [25] (Fig. 1). In fact, the activity and the expression of Cyclin D1 [26], CDK2 kinase [27], c-myc [28], which are involved in the control of cell cycle and are altered in several types of cancer, such as breast, prostate and ovarian tumors, are regulated by NF-κB. The expression and the function of numerous cytokines, that are growth factors for tumor cells, are NF-κB-dependent. Among them are: IL-1β, a growth factor for acute myeloid leukemia (AML), TNF, a growth factor for Hodgkin's lymphoma, cutaneous T cell lymphoma and gliomas, interleukin (IL)-6, a growth factor for multiple myeloma [29]. Some growth factors, such as epidermal growth factor (EGF), or receptors for growth factors, such as HER2, able to promote growth of solid tumors, activate NF-κB [30]. Tissue invasion and metastasis, two crucial events of tumor progression, are regulated by NF-κB-dependent genes, including matrix metalloproteinases (MMPs) [31], urokinase type of plasminogen activator (uPA) [32], IL-8 [33], the adhesion molecules VCAM-1, ICAM-1 and ELAM-1 [34] and chemokine receptors such as CXCR4 [35]. NF-κB activity is also involved in the regulation of angiogenesis, the process by which tumor cells promote neo-vascularization, an essential step for their growth and invasiveness. Vascular endothelial growth factor (VEGF), which is the main member of angiogenic factors family, is under transcriptional control of NF-κB [36]. Finally, altered expression of genes involved in regulation of apoptosis, which is a feature of neoplastic cells, is often due to deregulated NF-κB activity. TNF receptor associated factor (TRAF)1/2 [37], IAP proteins (XIAP, cIAP-1 and -2) [37,38], members of Bcl-2 family [39], c-FLIP [40], GADD45β [41], Ferritin Heavy Chain [42] are all anti-apoptotic genes. By promoting cell survival, these genes lead to the maintaining of cell-transformed state and are responsible for the resistance to chemotherapeutic drugs. Therefore, it is not surprising that altered NF-κB promotes neoplastic transformation by controlling expression of these genes. In fact, inactivation of NF-κB in different cell lines derived from tumors displaying high constitutive NF-κB activity, leads to the loss of their tumorigenic potential due to either an increased susceptibility to apoptosis or a decrease of their uncontrolled proliferative rate. Moreover, in some cases, blocking NF-κB activity inhibits the metastatic potential of many cancer cell lines or reduces the tumor size.

What is the cause of NF-κB constitutive activation in tumors? The mechanisms determining persistent and deregulated NF-κB activity in cancer cells are not well understood. Since NF-κB signaling pathway is tightly controlled by several regulatory proteins, the constitutive activation of NF-κB in solid tumors has been mainly attributed to disruption of this process (i.e., defective IκBα activity, constitutive IKK activity, enhanced proteasomal activity), consistent with scenario observed in NF-κB-associated haematological malignancies. Another possibility is that constitutive NF-κB activation could be determined by prolonged and persistent stimulation of cancer cells by factors promoting induction of NF-κB signaling by autocrine or paracrine fashion. Thus, NF-κB activity is held at high levels until this autocrine or paracrine loop is not interrupted.

5. How does NF-κB suppress apoptosis?

Apoptosis is a genetically encoded, highly regulated process enabling cells to undergo death following certain stimuli. It is characterized by a number of unique features, including cytoplasmic fragmentation, intranucleosomal DNA fragmentation, phosphatydilserine exposure and, finally, fragmentation

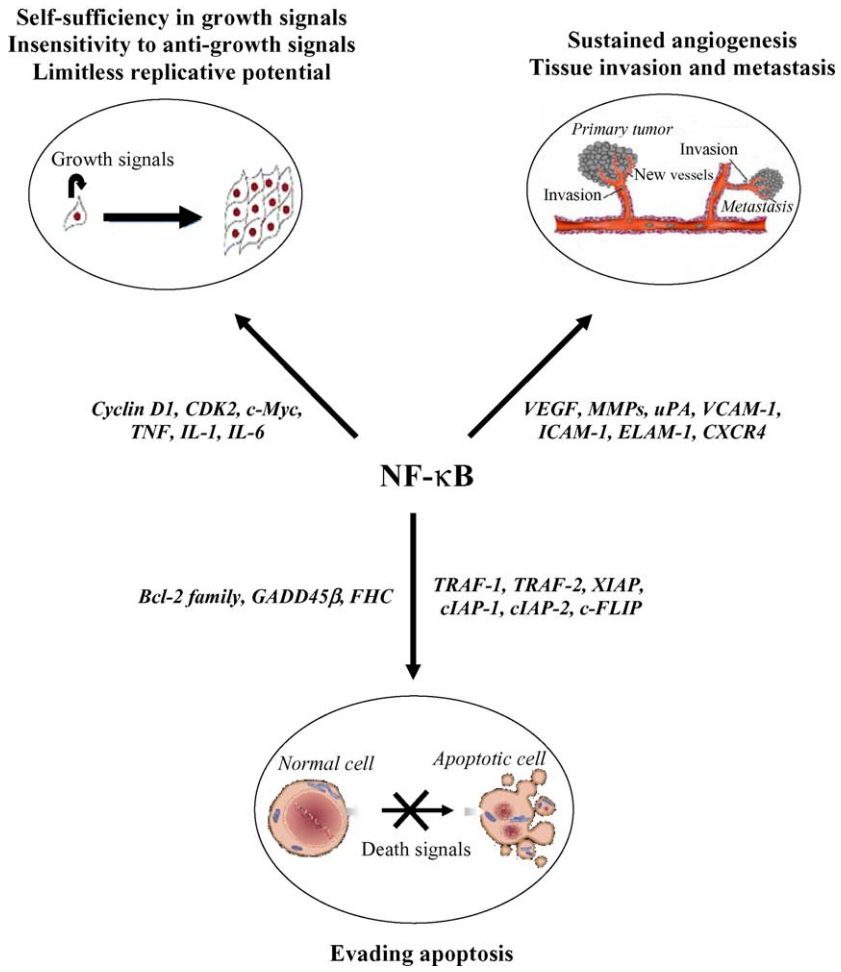

Fig. 1 – NF-κB contributes to cancer development through regulation of different genes involved in oncogenesis. According to Hanahan and Weinberg, six essential alterations in cell physiology characterize a tumor cell: self-sufficiency in growth, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Many of the genes able to mediate such effects are under transcriptional control of NF-κB.

into membrane-enclosed apoptotic bodies sequestered by macrophages and other phagocytic cells. Apoptosis is critical for maintenance of normal homeostasis together with other important processes such as proliferation and differentiation. Defects in apoptosis contribute to tumor pathogenesis and progression in several ways. For example, allowing neoplastic cells to survive beyond their normal lifespan, providing protection against hypoxia as tumor mass expands, promoting angiogenesis and invasiveness during tumor progression, allowing tumor cells to become resistant to radio- and chemotherapy. They also promote resistance to immune system given that as many of the weapons cytolytic T-cells and NK cells use for killing tumor cells depends on the integrity of the apoptotic machinery. Thus, de-regulated apoptosis is a fundamental aspect of the biology of cancer. The first indication that suppression of apoptosis is an important NF-κB function came from the evidence that RelA$^{-/-}$ mice died during embryonic development as a result of massive liver apoptosis [43]. The massive liver apoptosis, which is also displayed by mice that lack IKKβ or NEMO/IKKγ, is mediated by TNF and is completely suppressed in absence of TNF or TNFR1. Further experiments showed that NF-κB is a critical regulator of apoptotic response in different physiological and pathological contexts. The survival of peripheral B cells in response to antigen depends on B-cell receptor (BCR)-mediated activation of NF-κB and the induction of anti-apoptotic target genes [44]. The full activation of naïve T cells via T-cell receptor (TCR) leads to NF-κB activation and consequent cell survival via induction of anti-apoptotic genes [45]. A link between NF-κB and apoptosis has also been demonstrated in the regulation of innate immune response, which plays a fundamental role in the detection and elimination of pathogens [46]. The inhibition of NF-κB activity in mouse xenograft models of chemo resistant tumors provokes tumor regression by sensitizing them to chemotherapeutic drug treatments [47]. Consistent with this work, NF-κB inhibition in many human tumor-derived cell lines, including malignant Reed–Sternberg (H-RS) cells of Hodgkin's disease, colon carcinoma cells, lung adenocarcinoma cells, Ewing sarcoma cells, prostate carcinoma cells, thyroid carcinoma cells and many others, induces spontaneous apoptosis or/and sensitizes them to killing by TNFα, TRAIL or anti-cancer drugs.

Mechanisms for NF-κB -mediated protection from apoptosis are essentially based on its ability to activate the transcription of a number of genes capable of suppressing cell death [48]. Among them are: (1) pro-survival members of the mammalian Bcl-2 gene family, Bcl-xL and Bfl-1/A1, which suppress the release of pro-apoptotic cytochrome c and Smac/Diablo from mitochondria, thereby blocking programmed cell death in response to TNFα and chemotherapeutic drugs [39,49]; (2) the cellular inhibitors of apoptosis c-IAP1, c-IAP2, TRAF1 and TRAF2, the zinc-finger protein A20, c-FLIP, all of them able to suppress cell death induced by TNFα, death receptors or anti-cancer drugs [37,38,40]; (3) the anti-apoptotic genes XIAP, that inhibits the processing of procaspase-9 and the activities of caspase-7 and -3, and GADD45β, that belongs to the GADD45 family of factors involved in cell cycle control and DNA repair, both of them implicated in NF-κB-mediated suppression of pro-apoptotic JNK signaling in response to TNFα [41,50]. A similar mechanism of action is also displayed by Ferritin Heavy Chain (FHC) that controls ROS production responsible, in turn, to mediated sustained activation of the pro-apoptotic JNK pathway [41]. All these anti-apoptotic proteins may work in a coordinated manner to interfere with apoptosis at multiple steps along the apoptotic signalling cascade (Fig. 2).

6. NF-κB has pro-apoptotic functions

Under certain circumstances NF-κB can promote or amplify cell death. Most of the effects are dependent on the induction of genes that code for the death receptors DR4, DR5 and Fas [51,52]. Induction of death receptors, however, is not the only way NF-κB induces cell death. Different groups reported that treatment of different cancer cell lines with Daunorubicin and Doxorubicin causes IκB degradation and NF-κB nuclear translocation, but repression of antiapoptotic genes. This repressive effect seems to be mediated by the lack of RelA phosphorylation and acetylation [53,54,55]. These studies suggest that activation of NF-κB by several distinct stimuli can produce repressive form of NF-κB. However, it is presently unclear which signaling events ultimately determine whether NF-κB activation will lead to pro- or anti-apoptotic response.

7. NF-κB links inflammation and cancer

For many years, a link between inflammation and cancer has been strongly suspected. The Greek doctor Galen two millenniums ago, noticed a link between inflammation and cancer. In the 19th century Wirkow suggested that tumors may arise in area of chronic inflammation. More recently, population based studies show that susceptibility to cancer increases when tissues are chronically inflamed, and long-term use of non-steroidal anti-inflammatory drugs reduces the risk of several cancer.

Inflammation is central to control our fight against pathogens, and to regulate wound healing. These are very intricate biological responses that involved complex interactions between different cell types that regulate the expression of biological mediators which promote cell chemotaxis, cell migration and cell proliferation. If inflammation is not ordered and timely, the resulting chronic inflammation can contribute to a variety of diseases including cancer. Cells of the innate immune system neighbouring the tumor (inflamed area) secrete pro-inflammatory cytokines, such as TNFα, IL-1, IL-6 and IL-8, as well as extracellular matrix-degrading enzymes, growth factors and ROS. This microenvironment enhances cell proliferation, cell survival, cell migration and angiogenesis, thereby promoting tumor development. Chronic inflammation due to viruses or bacteria infections can directly induce mutations in tumor suppressors or oncogenes of epithelial cells, but can also stimulate the formation of carcinomas through an indirect mechanism involving activation of surrounding inflammatory cells. In addition, activated innate immune cells can interfere with T cell response to cancer.

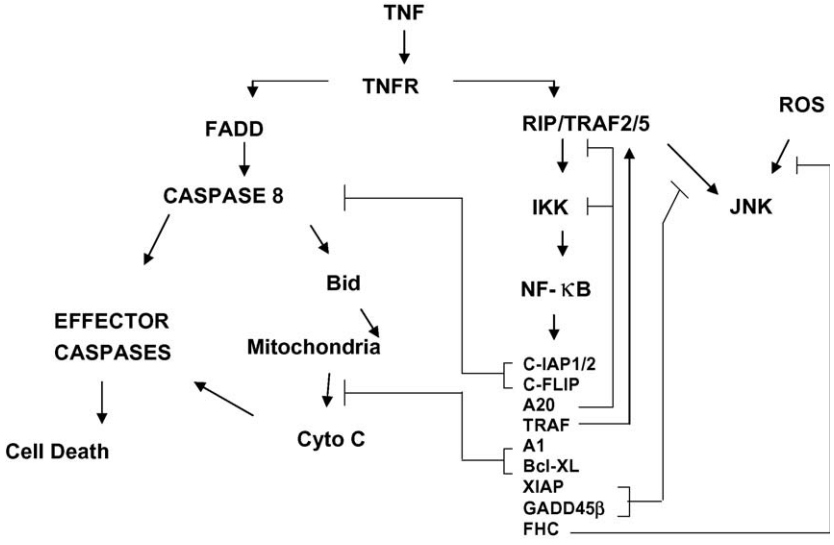

Fig. 2 – NF-κB controls transcription of different genes interfering with different steps in the apoptotic process. NF-κB drives transcription of genes controlling caspase activation (c-IAP, c-FLIP), release of cytocrome C from the mitochondria (A1, Bcl-xL), NF-κB activation (TRAFs, A20), JNK activation XIAP, GADD45β and Ferritin Heavy Chain.

Macrophages can inhibit antigen presentation by dendritic cells, directly suppress T cell response, and indirectly down regulate immunity by inducing regulatory T cell through affects of IL-10 and TGFβ. Thus, the ongoing inflammatory response has the capacity to profoundly alter the ability of the host to mount adaptive immune response within the inflammatory stroma of tumors. The interplay between epithelial and inflammatory cells is thought to be crucial for the genesis and the establishment of carcinomas. One of the main actors in inflammatory process is NF-κB which, by regulating the expression and the function of different cytokines and chemokines in inflammatory cells, stimulates the growth and its own activity in epithelial cells. Thus, NF-κB establishes a network that, after prolonged time, can lead epithelial cells to undergo malignant transformation. For example, one of the main risk factors linked to gastric cancer is *Helicobacter pylori* (*H. pylori*) infection. *H. pylori* is a potent NF-κB activator [56]: once NF-κB is activated in gastric cells, it induces transcription of IL-1, IL-6, IL-8, TNFα and other growth factors that can promote uncontrolled proliferation either by autocrine stimulation or by paracrine stimulation of surrounding inflammatory cells. On the other hand, *H. pylori* induces NF-κB-dependent production of COX2, ROS, iNOS that determine DNA damage, thereby increasing the susceptibility to malignant transformation of gastric cells. Another example for the role played by NF-κB in controlling inflammation and cancer is represented by colitis-associated cancer. Ulcerative colitis is a chronic inflammatory bowel disease that, together to Crohn's disease, shows persistent NF-κB activation in tissue macrophages and epithelial cells of the colonic mucosa. By a mechanism similar to that of gastric cells, NF-κB is able to promote colorectal cancer. These two examples demonstrate that NF-κB activates a network between epithelial cells and inflammatory cells that not only leads to neoplastic transformation, but also sustains the malignant phenotype of epithelial cells. To analyze in more detail the molecular mechanisms regulating these phenomena, Karin [16] and Ben-Neriah [17] have independently developed two animal models of inflammation-associated cancer, a colitis-associated cancer (CAC) model and a genetic model (MDR2$^{-/-}$ mice) of cholangitis (bile-duct inflammation), which leads to hepatocarcinoma.

Karin and colleagues showed that the administration of dextran sulfate to mice caused chronic colitis which greatly enhanced the incidence of colon carcinomas following administration of the pro-carcinogen azoxymethane. Chronic colitis was determined by disruption of intestinal barrier and exposure of macrophages in the lamina propria to enteric bacteria. The exposure of macrophages to bacteria induced the activation of NF-κB in these cells, leading to the production and secretion of pro-inflammatory cytokines that activated NF-κB in intestinal epithelial cells. It is also possible that necrotic tumor cells may activate adjacent macrophages and/or other myeloid cells which than secrete growth factors and mediators of inflammation. The sustained activation of NF-κB in enterocytes increased the tumor susceptibility following azoxymethane stimulation. If IKKβ was deleted in enterocytes, tumor incidence but not tumor size strongly decreased, indicating that the IKKβ-dependent NF-κB-activation pathway operated during early phases of tumorigenesis. However, deleting IKKβ in myeloid cells, which are important for the development of CAC, decreased not only tumor incidence but also tumor size, as a consequence of diminished proliferation of transformed enterocytes which requires growth factors produced by myeloid cells [16]. Thus, in this model, NF-κB promotes survival of malignant cells either in early or late phases of tumorigenesis, depending on cell types (epithelial or inflammatory cells) in which it has been activated.

Similarly, Ben-Neriah and Co. demonstrated that the appearance of hepatocarcinoma in MDR2$^{-/-}$ mice, a genetic model of cholangitis, was due to prolonged TNF-dependent NF-κB activation of hepatocyte by inflammatory cells surrounding them in the liver. This caused increased survival of dysplastic hepatocyte by up-regulation of anti-apoptotic genes such as GADD45β and BFL1. The inactivation of NF-κB by hepatocyte-specific expression of IκBα super-repressor, or by suppressing TNF activity blocked tumor development. This effect was seen only when NF-κB activity was inhibited between 7 and 14 months after mice birth, during late phases of tumor promotion, while its inactivation during the first 7 months caused no beneficial effects [17]. Therefore, in this mouse model, the role of NF-κB was prominent in maintaining rather than in promoting malignant transformation of hepatocyte.

The data obtained in these two cancer models, suggest that the NF-κB pathway does not affect initiation but has multiple actions in tumor promotion, by preventing apoptosis of cells with malignant potential, by stimulating the production of pro-inflammatory cytokines in the inflammatory cells infiltrating the tumor mass (Fig. 3). This model is consistent with the observed correlation between the number of inflammatory cells, level of cytokines and tumor aggressiveness and prognosis in both human and mouse. However, the transformed cells themselves can contribute to the overall level of secreted pro-inflammatory cytokines, and to maintain the NF-κB pathway activated. In fact, it is possible to keep malignant cells in culture without the support of the innate immunity and still detect constitutive activation of NF-κB. In addition, at least for some cell types, such as undifferentiated thyroid carcinomas, it is possible to detect an NF-κB modulating activity secreted by the transformed cells.

Based on these observations, NF-κB inhibition is considered a very promising weapon to fight cancer. Recently, however several studies of mouse models of cancer suggest that, at least in some cases, NF-κB inhibition may induce tumorigenesis. For example, in the chemical hepatocarcinogenesis model, inhibiting NF-κB in the liver accelerate tumorigenesis induced by diethylnitrosamine (DEN). DEN is metabolized into an alkylating agent that induces DNA damage and mutations, as well as hepatocytes death. According to its antiapoptotic activity, loss of IKKβ in hepatocytes augments DEN-induced death, but the increased cell death potentiates carcinogenesis by inducing production of growth factors by Kupfer cells, that stimulate proliferation of hepatocytes harbouring DEN-induced mutation [57]. This result is in apparent contrast with the above mentioned studies of Pikarsky and colleagues and Greten and colleagues, where the inhibition of NF-κB blocked tumor development. A possible explanation for this seemingly in contrast result may be the different experimental model used. In the chemical liver carcinogenesis model, NF-κB activation is limited to the acute response following administration of the carcinogen, and results in increased cell death

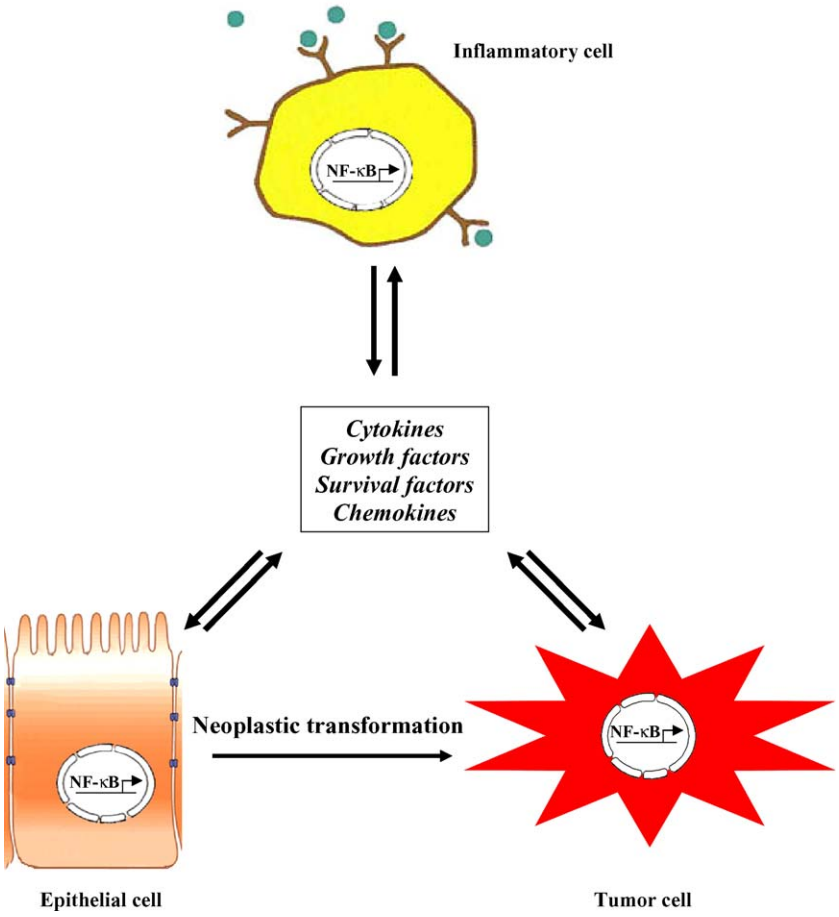

Fig. 3 – Mechanisms by which NF-κB contributes to cancer development during inflammation. The interplay between epithelial and inflammatory cells is thought to be crucial for the genesis and the establishment of carcinomas. One of the main actors in inflammatory process is NF-κB which, by regulating the expression and the function of different cytokines and chemokines in inflammatory cells, stimulates the growth and its own activity in epithelial cells. Thus, NF-κB establishes a network that can sustain cell proliferation and resistance to apoptosis.

and compensatory hyperproliferation of DEN-mutated hepatocytes. In chronic hepatitis model, such as the model used by Pikarsky and colleagues, NF-κB is activated over a prolonged period of time.

8. NF-κB and skin carcinogenesis

The most convincing evidence that inhibition of NF-κB may induce tumorigenesis, came from studies on the role of NF-κB in skin, where interfering with NF-κB activation induces squamous cell carcinomas (SCC) [58,59]. The constitutive expression of a superepressor form of IκB in the epidermidis, drives the development of a chronic inflammatory skin disease with massive dermal infiltration by neutrophilis, epidermal hyperplasia and eventually SCC. At least part of these effects seems to be due to the increased production of TNF in the skin. Crossing the mice expressing IκB superepressor with a TNFRI KO strain, results in blockade of both inflammation and tumor formation [60]. Similar data were reported by Pasparakis et al. [61], who generated mice with an epidermidis-specific deletion of IKKβ. These mice develop a severe skin inflammation mediated by TNF shortly after birth, followed by hypeproliferation of keratinocytes. Importantly, purified keratinocytes proliferate normally indicating that the observed high proliferative rate in absence of IKKβ is not a cell autonomous defect. The role of TNF is further supported by the mouse model described by Kavary and colleagues [62]. They transplanted a foetal skin from a $p65^{-/-}$ mouse to adult mice. The p65 graft develops keratinocyte hyperplasia and SSC. Skin graft from p65/TNFR1 double KO mice did not develop tumors, further suggesting that TNF signalling is responsible for SSC formation. In a different set of experiments, Balkwill's group has convincingly shown that TNF is a key mediator of epidermal carcinogenesis [63].

Therefore, the scenario that arises from these studies is that inhibition of NF-κB in the epidermidis, results in increased production of TNF, in turn responsible for the inflammatory response, the keratinocyte hyperproliferation and the development of SSC. TNF action seems to be mediated by JNK which concomitantly with the inflammatory process, leads to development of cancer [64,65]. The pro-tumorigenic effect of NF-κB inhibition seems to be due to an indirect effect, i.e., up-regulation of TNF, rather than to a direct antineoplastic activity of NF-κB itself.

Such results suggest that the role played by NF-κB on cancer development may be cell type or model specific. It is likely that if inhibition is inducing inflammation, mediated for example by TNF, as in skin, or cell death as in the chemical liver carcinogenesis model, it would facilitate tumorigenesis. If NF-κB inhibition affects survival of transformed cells, it will block tumorigenesis.

9. Interactions of the NF-κB pathway with some tumor suppressors

While it is well established the role of NF-κB in tumor progression, it is still debated if NF-κB can fuction as a tumor suppressor. Recent evidences have shown that NF-κB can inhibit tumor growth by repressing, rather than activating, the expression of tumor-promoting genes. In addition, it has been reported that tumor suppressors, such as p53 and the ADP-ribosylation factor (ARF), can modulate NF-κB activity [66,67]. In fact, p53 and RelA could mutually inhibit the transcriptional activity of each other, establishing a functional relationship between these pathways [66]. A consequence of this relationship is the ability of RelA to facilitate p53-induced cell death, probably because p53 converts NF-κB to a repressor of anti-apoptotic gene expression, thus facilitating the p53-mediated apoptosis [68]. Moreover, it has been found that p53 could also regulate the activity of the p52 subunit of NF-κB, inducing a switch from p52-Bcl3 activator complexes to p52-histone deacetylase (HDAC) repressor complexes, resulting in the inhibition of the cyclin D1 promoter [69]. Accordingly, the tumor suppressor ARF, which represents the first line of defence against oncogene transformation, could induce the association of RelA with histone deacetylase 1 (HDAC1) thereby turning it into a repressor of gene expression without inducing, inhibiting or affecting NF-κB DNA-binding activity [67]. By this way, the transcription of the Bcr-Abl oncogene is repressed [69]. Similarly, the expression of Bcl-X_L, an anti-apoptotic gene, is inhibited so that cancer cells acquire an increased susceptibility to drug-induced apoptosis [69].

Other tumor suppressors, such as $p16^{INK4a}$ and PTEN, are able to inhibit the transcriptional activity of NF-κB [70,71], but it is unknown if these proteins can also induce association of NF-κB subunits with co-repressor complexes, a common mechanism through which many tumor suppressor proteins work. The general idea is that in normal cells tumor suppressor genes are activated in response to an oncogenic signal and can modulate NF-κB complexes, whose activity is preexisting or induced by cellular neoplastic transformation. Consequently, the oncogenic potential of NF-κB is neutralized and its activity is switched on a tumor suppressor program. However, as neoplastic cells accumulate additional mutations, the tumor suppressor activity of NF-κB is lost and its oncogenic functions can contribute to the growth and the development of cancer cells.

Even so several reports suggest that NF-κB could function as a physiological suppressor of tumors (for example, *rela*$^{-/-}$ mouse fibroblasts form colonies on soft agar and tumors in nude mice [72], the inhibition of NF-κB in skin cells stimulates the development of squamous cell carcinomas [59], the over-expression of p65 reduces the tumorigenicity of MCF7 cells in nude mice [73]), no clear evidence for active NF-κB-dependent tumor suppression currently exists on the base of knockout mouse studies or human tumors. At this time, it seems improbable that NF-κB is a sufficiently potent tumor suppressor, whereby its inhibition would result in the formation of cancer cells in humans.

10. NF-κB as therapeutic target in cancer

The finding that NF-κB is a key player in cancer has prompted researchers to focus their efforts in looking for drugs able to suppress NF-κB activity in malignant cells. NF-κB activation could be blocked at different levels targeting the various components of its signaling cascade, such as the IKK complex, the IκBα inhibitory protein, the p65 subunit of the transcriptionally active heterodimer, the proteasome. Therefore, an increasing number of compounds able to block NF-κB by inhibiting one or more of the molecules involved in the pathway activating it, have been tested and have shown to suppress the growth of those cancer cells whose tumorigenicity depends on NF-κB activity [15]. Many of these drugs have given promising results in preclinical models for NF-κB-dependent solid tumors (breast, lung, colon, bladder, ovary, pancreas and prostate cancers), but their clinical efficacy have shown to be poorly appreciable. Actually, the only pharmacological inhibitors of NF-κB activation approved for clinical use are represented by proteasome inhibitors for treatment of some haematological malignancies, such as multiple myeloma, or adult T-cell leukemia, for whose pathogenesis it has been clearly demonstrated the key role of NF-κB [74,75].

One of the most problematic aspects of a cancer therapy based on inhibition of NF-κB activity is represented by the difficulty to find compounds which block the oncogenic activity of NF-κB without interfering with its physiological roles in immunity, inflammation and cellular homeostasis. Unfortunately, most of the drugs analyzed so far also affect other cellular signaling pathways involved in the regulation of apoptosis and proliferation other than of inflammatory and immunological response, thereby determining a number of highly toxic side effects. To this end, all the efforts are now concentrated on the ability to identify novel NF-κB targets specifically activated in tumors, but not in normal cells. Thus, the inhibition of these targets should block the oncogenic potential of NF-κB in cancer cells without affect its role in normal tissues.

11. Conclusions

In the last few years, an enormous amount of basic and clinical observations strongly implicate NF-κB in a variety of human cancer. However, recently this proposal has been challenged by the evidence that, at least for skin cancer, NF-κB activation has been postulated as a safeguard against cancer. One of the major challenges is then to figure out the molecular mechanisms that dictate the pro-oncogenic or anti-oncogenic activity of NF-κB. A second challenge is to find compounds which block the oncogenic activity of NF-κB without interfering with its physiological roles in immunity, inflammation and cellular homeostasis.

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REFERENCES

[1] Shattuck-Brandt RL, Richmond A. Enhanced degradation of I-kappaB alpha contributes to endogenous activation of NF-κB in Hs294T melanoma cells. Cancer Res 1997;57:3032–9.
[2] Amiri KI, Richmond A. Role of nuclear factor-kappa B in melanoma. Cancer Metastasis Rev 2005;24:301–13.
[3] Sovak MA, Bellas RE, Kim DW, Zanieski GJ, Rogers AE, Traish AM, Sonenshein GE. Aberrant nuclear factor-kappaB/Rel expression and the pathogenesis of breast cancer. J Clin Invest 1997;100:2952–60.
[4] Kalaitzidis D, Gilmore TD. Transcription factor cross-talk: the estrogen receptor and NF-kappaB. Trends Endocrinol Metab 2005;16:46–52.
[5] Huang S, Pettaway CA, Uehara H, Bucana CD, Fidler IJ. Blockade of NF-kappaB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene 2001;20:4188–97.
[6] Sweeney C, Li L, Shanmugam R, Bhat-Nakshatri P, Jayaprakasan V, Baldridge LA, et al. Nuclear factor-kappaB is constitutively activated in prostate cancer in vitro and is overexpressed in prostatic intraepithelial neoplasia and adenocarcinoma of the prostate. Clin Cancer Res 2004;10:5501–7.
[7] Huang S, Robinson JB, Deguzman A, Bucana CD, Fidler IJ. Blockade of nuclear factor-kappaB signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. Cancer Res 2000;60:5334–9.
[8] Mabuchi S, Ohmichi M, Nishio Y, Hayasaka T, Kimura A, Ohta T, et al. Inhibition of NF-kappaB increases the efficacy of cisplatin in in vitro and in vivo ovarian cancer models. J Biol Chem 2004;279:23477–85.
[9] Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ. The nuclear factor-kappa B RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. Clin Cancer Res 1999;5:119–27.
[10] Sclabas GM, Uwagawa T, Schmidt C, Hess KR, Evans DB, Abbruzzese JL, et al. Nuclear factor kappa B activation is a potential target for preventing pancreatic carcinoma by aspirin. Cancer 2005;103:2485–90.
[11] Kojima M, Morisaki T, Sasaki N, Nakano K, Mibu R, Tanaka M, et al. Increased nuclear factor-κB activation in human colorectal carcinoma and its correlation with tumor progression. Anticancer Res 2004;24:675–81.
[12] Dejardin E, Deregowski V, Chapelier M, Jacobs N, Gielen J, Merville MP, Bours V. Regulation of NF-kappaB activity by I kappaB-related proteins in adenocarcinoma cells. Oncogene 1999;18:2567–77.
[13] Visconti R, Cerutti J, Battista S, Fedele M, Trapasso F, Zeki K, et al. Expression of the neoplastic phenotype by human thyroid carcinoma cell lines requires NFkappaB p65 protein expression. Oncogene 1997;15:1987–94.
[14] Pacifico F, Mauro C, Barone C, Crescenzi E, Mellone S, Monaco M, et al. Oncogenic and anti-apoptotic activity of NF-kappa B in human thyroid carcinomas. J Biol Chem 2004;279:54610–9.
[15] Karin M, Cao Y, Greten FR, Li ZW. NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2002;2:301–10.
[16] Greten FR, Eckmann L, Greten FT, Park JM, Li ZW, Egan LJ, et al. IKKβ links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 2004;118:285–96.
[17] Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, et al. NF-κB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–6.
[18] Gilmore TD. Multiple mutations contribute to the oncogenicity of the retroviral oncoprotein v-Rel. Oncogene 1999;18:6925–37.
[19] Rayet B, Gelinas C. Aberrant *rel/nfκb* genes and activity in human cancer. Oncogene 1999;18:6938–47.
[20] Ishikawa H, Carrasco D, Claudio E, Ryseck RP, Bravo R. Gastric hyperplasia and increased proliferative responses of lymphocytes in mice lacking the COOH-terminal ankyrin domain of NF-kappaB2. J Exp Med 1997;186:999–1014.
[21] Ohno H, Takimoto G, McKeithan TW. The candidate proto-oncogene bcl-3 is related to genes implicated in cell lineage determination and cell cycle control. Cell 1990;60:991–7.
[22] Ong ST, Hackbarth ML, Degenstein LC, Baunoch DA, Anastasi J, McKeithan TW. Lymphadenopathy, splenomegaly, and altered immunoglobulin production in BCL3 transgenic mice. Oncogene 1998;16:2333–43.
[23] Bargou RC, Emmerich F, Krappmann D, Bommert K, Mapara MY, Arnold W, et al. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's disease tumor cells. J Clin Invest 1997;100:2961–9.
[24] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
[25] Aggarwal BB. Nuclear factor-κB: the enemy within. Cancer Cell 2004;6:203–8.
[26] Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin Jr AS. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. Mol Cell Biol 1999;19:5785–99.
[27] Perkins ND, Felzien LK, Betts JC, Leung K, Beach DH, Nabel GJ. Regulation of NF-kappaB by cyclin-dependent kinases associated with the p300 coactivator. Science 1997;275:523–7.
[28] Kim DW, Gazourian L, Quadri SA, Romieu-Mourez R, Sherr DH, Sonenshein GE. The RelA NF-kappaB subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. Oncogene 2000;19:5498–506.
[29] Pahl HL. Activators and target genes of Rel/NF-κB transcription factors. Oncogene 1999;18:6853–66.
[30] Biswas DK, Cruz AP, Gansberger E, Pardee AB. Epidermal growth factor-induced nuclear factor kappa B activation: a major pathway of cell-cycle progression in estrogen-receptor negative breast cancer cells. Proc Natl Acad Sci USA 2000;97:8542–7.
[31] Farina AR, Tacconelli A, Vacca A, Maroder M, Gulino A, Mackay AR. Transcriptional up-regulation of matrix metalloproteinase-9 expression during spontaneous epithelial to neuroblast phenotype conversion by SK-N-SH neuroblastoma cells, involved in enhanced invasivity, depends upon GT-box and nuclear factor kappaB elements. Cell Growth Differ 1999;10:353–67.
[32] Novak U, Cocks BG, Hamilton JA. A labile repressor acts through the NF-κB-like binding sites of the human urokinase gene. Nucleic Acids Res 1991;19:3389–93.
[33] Kunsch C, Rosen CA, NF-kappa. B subunit-specific regulation of the interleukin-8 promoter. Mol Cell Biol 1993;13:6137–46.
[34] van de Stolpe A, Caldenhoven E, Stade BG, Koenderman L, Raaijmakers JA, Johnson JP, et al. 12-O-tetradecanoylphorbol-13-acetate- and tumor necrosis factor alpha-mediated induction of intercellular adhesion molecule-1 is inhibited by dexamethasone. Functional

analysis of the human intercellular adhesion molecular-1 promoter. J Biol Chem 1994;269:6185–92.
[35] Helbig G, Christopherson 2nd KW, Bhat-Nakshatri P, Kumar S, Kishimoto H, Miller KD, et al. NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem 2003;278:21631–8.
[36] Kiriakidis S, Andreakos E, Monaco C, Foxwell B, Feldmann M, Paleolog E. VEGF expression in human macrophages is NF-kappaB-dependent: studies using adenoviruses expressing the endogenous NF-kappaB inhibitor IkappaBalpha and a kinase-defective form of the IkappaB kinase 2. J Cell Sci 2003;116:665–74.
[37] Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin Jr AS. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 1998;281:1680–3.
[38] Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. Eur Mol Biol Org J 1998;17:2215–23.
[39] Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993;74:597–608.
[40] Yeh WC, Itie A, Elia AJ, Ng M, Shu HB, Wakeham A, et al. Requirement for Casper (c-FLIP) in regulation of death receptor-induced apoptosis and embryonic development. Immunity 2000;12:633–42.
[41] De Smaele E, Zazzeroni F, Papa S, Nguyen DU, Jin R, Jones J, et al. Induction of gadd45beta by NF-kappaB downregulates pro-apoptotic JNK signalling. Nature 2001;414:308–13.
[42] Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, et al. Ferritin heavy chain upregulation by NF-kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. Cell 2004;119:529–42.
[43] Li Q, Verma IM. NF-kappaB regulation in the immune system. Nat Rev Immunol 2002;2:725–34.
[44] Bendall HH, Sikes ML, Ballard DW, Oltz EM. An intact NF-kappa B signaling pathway is required for maintenance of mature B cell subsets. Mol Immunol 1999;36:187–95.
[45] Jamieson C, McCaffrey PG, Rao A, Sen R. Physiologic activation of T cells via the T cell receptor induces NF-kappa B. J Immunol 1991;147:416–20.
[46] Naumann M. Nuclear factor-kappa B activation and innate immune response in microbial pathogen infection. Biochem Pharmacol 2000;60:1109–14.
[47] Greten FR, Karin M. The IKK/NF-kappaB activation pathway-a target for prevention and treatment of cancer. Cancer Lett 2004;206:193–9.
[48] Kucharczak J, Simmons MJ, Fan Y, Gelinas C. To be or not to be: NF-κB is the answer – role of Rel/NF-κB in the regulation of apoptosis. Oncogene 2003;22:8961–82.
[49] Wang CY, Guttridge DC, Mayo MW, Baldwin Jr AS. NF-kappaB induces expression of the Bcl-2 homologue A1/Bfl-1 to preferentially suppress chemotherapy-induced apoptosis. Mol Cell Biol 1999;19:5923–9.
[50] Tang G, Minemoto Y, Dibling B, Purcell NH, Li Z, Karin M, et al. Inhibition of JNK activation through NF-kappaB target genes. Nature 2001;414:313–7.
[51] Farhana L, Dawson M, Fontana JA. Apoptosis induction by a novel retinoid-related molecule require NF-κB activation. Cancer Res 2005;65:4909–17.
[52] Jin F, Liu X, Zhou Z, Yue P, Lotan R, Khuri F, et al. Activation of NF-κB contributes to the induction of death receptors and apoptosis by the synthetic retinoid CD437 in DU145 human prostate cancer cells. Cancer Res 2005;65:6354–63.
[53] Ho WC, Dickson KM, Barker P. NF-κB induced by Doxorubicin is deficient in phosphorylation and acetylation and represses NF-κB-dependent transcription in cancer cells. Cancer Res 2005;65:4273–81.
[54] Campbell KJ, Rocha S, Perkins N. Active repression of antiapoptotic gene expression by RelA/p65 NF-κB. Mol Cell 2004;13:853–65.
[55] Campbell KJ, Witty JM, Rocha S, Perkins N. Cisplatin mimics ARF tumor suppressor regulation of RelA/p65 NF-κB transactivation. Cancer Res 2006;66:929–35.
[56] Keates S, Hitti YS, Upton M, Kelly CP. Helicobacter pylori infection activates NF-kappa B in gastric epithelial cells. Gastroenterology 1997;113:1099–109.
[57] Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKβ couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–90.
[58] Dajee M, Lazarov M, Zhang JY, Cai T, Green CL, Russell AJ, et al. NF-kappaB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. Nature 2003;421:639–43.
[59] van Hogerlinden M, Rozell BL, Ahrlund-Richter L, Toftgard R. Squamous cell carcinomas and increased apoptosis in skin with inhibited Rel/nuclear factor-kappaB signaling. Cancer Res 1999;59:3299–303.
[60] Lind MH, Rozell B, Wallin RP, van Hogerlinden M, Ljunggren HG, Toftgard R, et al. Tumor necrosis factor receptor 1-mediated signaling is required for skin cancer development induced by NF-kappaB inhibition. Proc Natl Acad Sci USA 2004;10:4972–7.
[61] Pasparakis M, Courtois G, Hafner M, Schmidt-Supprian M, Nenci A, Toksoy A, et al. TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. Nature 2002;417:861–6.
[62] Zhang JY, Green CL, Tao S, Khavari PA. NF-kappaB RelA opposes epidermal proliferation driven by TNFR1 and JNK. Genes Dev 2004;18:17–22.
[63] Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, et al. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med 1999;5:828–31.
[64] Arnott CH, Scott KA, Moore RJ, Hewer A, Phillips DH, Parker P, et al. Tumour necrosis factor-alpha mediates tumour promotion via a PKC alpha- and AP-1-dependent pathway. Oncogene 2002;21:4728–38.
[65] Zhang JY, Tao S, Kimmel R, Khavari PA. CDK4 regulation by TNFR1 and JNK is required for NF-kappaB-mediated epidermal growth control. J Cell Biol 2005;168:561–6.
[66] Webster GA, Perkins ND. Transcriptional cross talk between NF-κB and p53. Mol Cell Biol 1999;19:3485–95.
[67] Rocha S, Campbell KJ, Perkins ND. p53- and Mdm2-independent repression of NF-kappa B transactivation by the ARF tumor suppressor. Mol Cell 2003;12:15–25.
[68] Ryan KM, Ernst MK, Rice NR, Vousden KH. Role of NF-kappaB in p53-mediated programmed cell death. Nature 2000;404:892–7.
[69] Rocha S, Martin AM, Meek DW, Perkins ND. p53 represses cyclin D1 transcription through down regulation of Bcl-3 and inducing increased association of the p52 NF-kappaB subunit with histone deacetylase 1. Mol Cell Biol 2003;23:4713–27.
[70] Wolff B, Naumann M. INK4 cell cycle inhibitors direct transcriptional inactivation of NF-kappaB. Oncogene 1999;18:2663–6.
[71] Mayo MW, Madrid LV, Westerheide SD, Jones DR, Yuan XJ, Baldwin Jr AS, et al. PTEN blocks tumor necrosis factor-induced NF-kappa B-dependent transcription by inhibiting the transactivation potential of the p65 subunit. J Biol Chem 2002;277:11116–25.

[72] Gapuzan ME, Yufit PV, Gilmore TD. Immortalized embryonic mouse fibroblasts lacking the RelA subunit of transcription factor NF-kappaB have a malignantly transformed phenotype. Oncogene 2002;21:2484–92.
[73] Ricca A, Biroccio A, Trisciuoglio D, Cippitelli M, Zupi G, Del Bufalo D. relA over-expression reduces tumorigenicity and activates apoptosis in human cancer cells. Br J Cancer 2001;85:1914–21.
[74] Richardson PG, Hideshima T, Mitsiades C, Anderson K. Proteasome inhibition in hematologic malignancies. Ann Med 2004;36:304–14.
[75] Nasr R, El-Sabban ME, Karam JA, Dbaibo G, Kfoury Y, Arnulf B, et al. Efficacy and mechanism of action of the proteasome inhibitor PS-341 in T-cell lymphomas and HTLV-I associated adult T-cell leukemia/lymphoma. Oncogene 2005;24:419–30.