TO NFκB OR NOT TO NFκB:
THE DILEMMA ON HOW TO INHIBIT A CANCER CELL FATE REGULATOR

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Abstract- Nuclear factor κB (NFκB) is a transcription factor that plays an important role in carcinogenesis as well as in the regulation of inflammatory response. NFκB is constitutively expressed in tumours where it induces the expression of genes which promote cell proliferation, apoptotic events, angiogenesis, invasion and metastasis. Furthermore, many cancer cells show aberrant or constitutive NFκB activation that mediates resistance to chemo- and radio-therapy. Therefore, the inhibition of NFκB activity appears a potential therapeutic strategy for cancer treatment. In this review, we focus on the role of NFκB in carcinogenesis and summarize actual inhibitors of NFκB that could be potential therapeutic target in cancer therapy.

Keywords- transcription factors; IκB, GRK5, cancer

I. INTRODUCTION

Human cancer is a complex disease based on multiple etiologies, multiple cell targets, and distinct developmental stages. Cancer cells share common features that regulate cell proliferation and homeostasis [1] including resistance to growth inhibitory signals, self-sufficiency in growth, resistance to apoptosis, extended replication potential, enhanced angiogenic potential, and the ability to invade local tissue and to metastasize to distant sites [1]. Autonomous cell growth characterizes cancer cells and depends on impaired expression of growth factors or growth factor receptors, leading to uncontrolled cell proliferation. Thus, a fairly common mechanism in cancer is the up-regulation of expression of members of the epidermal growth factor receptor family such as EGF receptor or Her2/ErbB2. Furthermore, certain cancer cells produce growth factors such as PDGF and TGF-α, which can promote cell proliferation in an autocrine manner [1,2]. Mutations in proteins that regulate cell proliferation are also relatively common in cancer. For example, resistance to growth inhibitory signals are due to mutations in tumour suppressor genes such as p53, Rb, Arf, and APC, or in receptors such as those for TGFβ. Additionally, up-regulation of expression of cyclin D1 or c-myc, or activating mutations in transcription factors can promote cell proliferation or cell growth [1,2]. A key process in the ability of tumour cells to spread is the suppression of apoptotic potential. Resistance to apoptosis can involve the activation of expression of anti-apoptotic factors, such as Bcl-2 or Bcl-xL, or the loss of expression or mutation of pro-apoptotic factors, such as p53 [2]. Additionally, mutation in tumour suppressors such as PTEN leads to the activation of intracellular signalling pathways (in this case, the PI3 kinase/Akt pathway) that suppress apoptosis [3]. An additional mechanism of suppression of cancer cell apoptosis can be derived from release of cytokines from the tumour stroma [2]. The ability of cancer cells to metastasize depends on angiogenesis which in turn is mediated through a complex interplay of regulatory factors, including vascular endothelial growth factor (VEGF). In fact, many tumors exhibit up-regulation of VEGF [1]. Local invasion is mediated by changes in expression of cell adhesion molecules and integrins, and in changes in expression of extracellular proteases such as MMP-2 and MMP-9. In some situations, the matrix-degrading proteases are produced by the tumour-associated stromal and inflammatory cells [2].

II. TRANSCRIPTION FACTORS AND NFκB

Transcription factors are gene regulatory proteins that bind to the promoter or enhancer regions of target genes and induce either transcriptional repression or activation [4]. The basic structure of a transcription factor mainly contains a DNA-binding domain and an activator domain. DNA-binding motifs include zinc-finger, helix-loop-helix, helix-turn-helix, leucine zipper and high-mobility groups, based on which transcription factors are classified [4,5]. The activator domain of these transcription factors interacts with components of transcription machinery such as RNA polymerases and associated transcription regulators. Transcription factors regulate gene expression in different ways: they stabilize or block the binding of RNA polymerase to DNA; catalyse the acetylation or
NFκB belongs to the Rel family, comprising the following proteins: RelA (p65), c-Rel, RelB, NFκB 1 (p50/p105) and NFκB 2 (p52/100) [10,11]. While RelA, c-Rel and RelB are synthesized as final proteins, p50 and p52 derive from large precursors p105 and p100, respectively, after processing by the proteasome. The nuclear activity of NFκB is controlled by shuttling from the cytoplasm to the nucleus in response to cell stimulation. It has been demonstrated that NFκB activation depends on two different signalling pathways, which can be referred to as canonical and non-canonical pathway [12]. In the canonical pathway, NFκB dimers containing RelA or c-Rel are retained in the cytoplasm through interaction with the inhibitors of NFκB (IκBαs). In response to a variety of stimuli, IκBαs are phosphorylated (Ser32 and Ser36 for IκBα and Ser19 and Ser21 for IκBβ) by the activated IκB kinase (IKK) complex, followed by rapid ubiquitin-dependent degradation by the proteasome [12,13]. This allows NFκB dimers to translocate to the nucleus, where they stimulate the expression of target genes. IKK is composed of two catalytic subunits, IKKα and IKKβ (also known as IKK1 and IKK2), and an essential regulatory subunit, IKKγ (also known as NEMO) [12]. While IKKβ is mostly required for the canonical NFκB pathway that depends on IκB degradation [14-16], IKKα is involved in a non-canonical NFκB pathway that regulates, at least, the RelB/p52 dimer [17]. In resting cells, RelB is associated with p100 in the cytoplasm. Upon cell stimulation, the IκB-like C terminus of p100 is degraded, and the resulting RelB-p52 dimers translocate to the nucleus [18] (fig 1).

IV. NFκB ACTIVATION IN CANCER

Strong evidences suggest a key role of NFκB in cancer. According to Hanahan and Weinberg, the tumour genesis requires six essential alterations to normal cell physiology: self-sufficiency in growth signals; insensitivity to growth inhibition; evasion of apoptosis; immortalization; sustained angiogenesis; and tissue invasion and metastasis [1]. NFκB is able to regulate several of these cellular alterations (fig 2), and has been shown to be constitutively activated in some types of cancer cell [2,19,20].
This phenomenon seems to be dependent on several mechanisms in different cancers: aberrant IKK activity, a shorter half-life of IκB in B-cell lymphoma, mutated IκB in Hodgkin’s lymphoma, IL-1β production in acute myelogenous leukaemia, TNF production in cutaneous T-cell lymphoma and Burkitt’s lymphoma [21,22]. It has been shown that the avian REV-T oncovirus produces the constitutively active v-REL oncoprotein, which causes rapidly progressing lymphomas and leukemias [19,23]. The TAX oncoprotein of human T-cell leukaemia virus (HTLV)-1 has been shown to directly interact with the IKK complex, inducing its constitutively activating which results in the activation of both NFκB signalling pathways [19,24]. Other viral oncoproteins have also been shown to activate NFκB by means of different mechanisms [25]. Moreover, chromosomal amplification, rearrangement and other genetic aberrations of genes coding for NFκB family members are present in many solid tumours and cause NFκB activation [20]. Indeed, cancer-associated genetic modifications of genes encoding for NFκB and IκB proteins induce uncoupling of NFκB factors from their regulators, causing constitutive NFκB activation [19]. Finally, autocrine and paracrine production of pro-inflammatory cytokines, oncogenic activation of upstream signalling molecules and chronic infections have been shown to persistently stimulate IKK activity, which leads to constitutive NFκB activation [19].

V. EFFECTS OF NFκB ACTIVATION ON TUMOUR GROWTH

NFκB activation regulates tumour genesis by inducing the expression of target genes which promote cell proliferation, inhibition of apoptosis, angiogenesis, invasion and metastasis, resistance to chemo- and radiotherapy (fig 2).

A. NFκB and apoptosis

It has been demonstrated that NFκB exerts a dual function on apoptosis, either as an inhibitor or an activator, depending on stimuli, cell type and subunit involved [26-29].

For instance, it is generally accepted that NFκB activation is responsible of induction of apoptosis in cardiac cells [28] and apoptosis resistance in cancer cells [27]. This latter event occurs by inducing the expression of multiple anti-apoptotic proteins and interfering with the expression or activity of pro-apoptotic proteins. Indeed, NFκB may activate the transcription of several genes involved in the suppression of cell death by both mitochondrial (intrinsic) and death receptor (extrinsic) pathways [30]. The release of cytochrome c from mitochondria directly triggers caspase-3 activation through formation of the cytochrome c/Apaf-1/caspase-9-containing apoptosisome complex [31]. It has been demonstrated that NFκB activation suppresses mitochondrial release of cytochrome c through the activation of the Bcl-2 family member A1/Bfl-1 [32]. NFκB may up-regulate the expression of proteins that interfere with the death receptor apoptotic pathway such as the FLICE-like inhibitory protein (FLIP) [33,34]. FLIP competes with caspase-8 for the binding to the Death-Inducing Signalling Complex (DISC). Thus, high levels of FLIP prevent caspase-8 recruitment to the DISC. It has been reported an up-regulation of FLIP in many tumours which could explain the resistance to death receptor apoptosis [35-39]. Other proteins, TRAF2 and TRAF6, activated by TNF may also be targets of NFκB and may lead to activation of pro-survival signalling [40]. NFκB also induces the expression of the Inhibitors of Apoptosis (IAPs) [41,42] and some members of the anti-apoptotic Bcl-2 family [43,44]. The IAPs (c-IAP1, c-IAP2, and XIAP) suppress apoptosis through direct inhibition of effector caspases (caspases-3, -6, -7, and 9) [40,45], while the anti-apoptotic members of the bcl-2 family antagonize the function of the pro-apoptotic members A1/BFL1 and Bcl-XL [46].

Furthermore, NFκB may interfere with the transcriptional activity of p53. In healthy cells, the level of p53 remains typically low under the control of Mdm2, which is responsible for p53 ubiquitination leading to its rapid degradation [47]. In turn, synthesis of Mdm2 transcript is controlled by p53 [48], which defines the negative feedback. DNA damage activates the checkpoint proteins, which destabilize Mdm2 and trigger p53 phosphorylation elevating its stability and transcriptional activity [49]. This disturbs the homeostatic balance between Mdm2 and p53 leading to oscillations and/or rise of the p53 level. Activated p53 triggers transcription of groups of genes, products of which are responsible for cell cycle arrest and DNA repair and, if the last fails or takes
too long, for initiation of apoptosis. In tumour cells, NFkB inhibits p53-induced apoptosis, by up-regulating anti-apoptotic genes, and down-regulating p53 levels.

**B. NFkB and proliferation**

Several genes, such as TNF, IL-1β and IL-6, that mediate cell proliferation are under the transcriptional control of NFkB. Besides these growth factors, certain cell cycle regulatory proteins are also regulated by NFkB. In particular, NFkB promotes cell cycle progression, by regulating the expression of cyclins D1, D2, D3, cyclin E 50-53 and c-myc [54-56]. NFkB-induced cyclin D1 expression appears to be a key element in mammary gland development and breast carcinogenesis [57]. It was shown that growth factors like epithelial growth factor and platelet-derived growth factor induce proliferation of tumour cells through activation of NFkB [58]. It has been reported that proliferation of Hodgkin/Reed-Sternberg cells depends on activated NFkB [59,60]. As happens in apoptosis, NFkB exert a reciprocal regulation of cell proliferation by inducing inhibition or stimulation, depending on cell type. For example, NFkB activation can suppress the proliferation of keratinocytes 61 and c-Rel overexpression induces cell cycle arrest in HeLa cells 62. On the other hand, NFkB induces the expression of cell adhesion molecules (ICAM-1, E-selectin), and proteins involved in invasion (matrix metallo-proteinases). However, generally, in tumour cells NFkB induces cell proliferation and the expression of angiogenic factors.

**C. NFkB and angiogenesis**

Metastasis of cancer cells is a complex process involving multiple steps, including invasion, angiogenesis, trafficking of cancer cells through blood vessels, extravasations, organ specific homing, and growth. Proteins like matrix metalloproteinase 2 (MMP2), MMP9 and serine protease urokinase-type plasminogen activator (uPA) which play an important role in tumour invasion and metastasis, are under the transcription control of NFkB. Indeed, it has been demonstrated that NFkB blockade induces down-regulation of pro-metastatic MMP-9, uPA, and heparanase and reciprocal up-regulation of anti-metastatic TIMP-1 and -2 and PAI 2 [63]. Furthermore, NFkB regulates the expression of intracellular adhesion molecule 1 and vascular cell adhesion molecule 1 that are associated with tumour metastasis [64]. Tumour angiogenesis is regulated by chemokines (monocyte chemo-attractant protein-1, IL-8) and growth factors (TNF, VEGF) produced by macrophages, neutrophils and other inflammatory cells [65]. The production of these angiogenic factors has been shown to be regulated by NFkB activation [66,67]. It has been demonstrated that NFkB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4 [68]. Huang et al reported that blockade of NFkB signalling also inhibits angiogenesis of human ovarian cancer cells by suppressing expression of VEGF and IL-8 [69]. Cyclooxygenase 2, which is up-regulated in more aggressive forms of colorectal cancer, is known to be transcriptionally activated by NFkB and promote angiogenesis [70].

**D. NFkB and chemo-resistance**

Tumours with constitutive NFkB activation usually show increased resistance to chemotherapy [71]. It has been suggested that NFkB may induce the expression of the multidrug resistance P-glycoprotein, involved in the development of resistance to chemotherapy drugs in many cancers [72]. In some tumours, cells exposed to radiation or certain chemotherapeutic drugs show enhanced activation of NFkB [71]. On the other hand, inhibition of NFkB improves the apoptotic response to radiation therapy [71,73]. For instance, it has been found that inhibition of NFkB activation confers sensitivity to TNF-α by impairment of cell cycle progression in six human malignant glioma cell lines [73]. Inhibitors of NFkB activation can block the neoplastic transformation response. Indeed, inhibition of NFkB through adenoviral delivery of a modified form of IκB, a specific inhibitor of NFkB, has been reported to sensitize chemo-resistant tumours to the apoptotic potential of TNF-α and to the chemotherapeutic compound CPT-11, resulting in tumour regression [74].

**VI. INFLAMMATION AND CANCER**

A link between inflammation and cancer has been suspected for many years. While acute inflammation is a part of the defence response, chronic inflammation can mediate several diseases, including cardiovascular diseases, cancer, diabetes, arthritis, autoimmune diseases [75]. Since NFkB becomes activated in response to inflammatory stimuli and its constitutive activation has been associated with cancer, NFkB represents the link between these two processes. Indeed, several pro-inflammatory gene products have been associated to tumour genesis and they are all under the transcription control of NFkB. In particular, TNF, interleukins, chemokines, COX-2, 5-LOX, and MMP-9 have all a key role in cancer development [75,76].

**A. Role of cytokines in cancer**

Several inflammatory interleukins, including IL-1, IL-6, IL-8, and IL-18, are associated to tumour genesis.
Secretion of IL-1α promotes growth of cervical carcinoma [77] while autocrine production of interleukin IL-1β promotes growth and confers chemoresistance in pancreatic carcinoma cell lines [78]. IL-1β secretion into the tumour milieu also induces several angiogenic factors from tumour and stromal cells that promotes tumour growth through an increase of neovascularization in lung carcinoma growth in vivo [79]. IL-6 acts as a paracrine growth factor for multiple myeloma, non-Hodgkin’s lymphoma, bladder cancer, colorectal cancer, and renal cell carcinoma (RCC) [80-84]. Several evidences underline the key role of TNF-α as mediator of inflammation and cancer [85,86]. Although initially thought to be a product only of macrophages, many malignant tumours are characterized by a constitutive production of TNF-α from the tumour microenvironment and its presence often associates with poor prognosis. Indeed, TNF-α is produced by a wide variety of tumour cells, including those of B cell lymphoma [87], cutaneous T cell lymphoma [88], megakaryoblastic leukaemia [89], adult T cell leukaemia [90], AML [91], CLL [92], ALL [93], breast carcinoma [94], colon carcinoma, lung carcinoma, squamous cell carcinoma, pancreatic cancer [95], ovarian carcinoma [96]. As TNF-α receptors are expressed on both epithelial and stromal cells, TNF-α can directly facilitate cancer development by regulating the proliferation and survival of neoplastic cells; alternatively it can also act on endothelial cells and other inflammatory cells present at the tumour microenvironment [97]. Tumour stromal cells, including macrophages, dendritic cells and fibroblasts, release several inflammatory cytokines, such as TNF-α, IL-1 and IL-6, which attract and recruit more inflammatory cells to the tumour microenvironment to further enhance the proliferation and survival of tumour cells. TNF-α is involved in all steps of tumour genesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis. Indeed, several reports indicate that TNF-α induces cellular transformation, proliferation, and tumour promotion [86,98-101]. First, TNF-α induces tumour initiation and promotion and enhances tumour cell proliferation. All these actions are mediated by the activation of NFκB. Indeed, in mouse epidermal JB6 cells, TNF-α treatment increases NFκB activity in a dose dependent manner and TNF-α induced NFκB activation is essential for neoplastic transformation of these cells [102]. TNF-α also promotes tumour cell survival by inducing genes coding for NFκB dependent anti-apoptotic molecules [103]. In addition TNF-α not only acts as an autocrine growth factor but also induces the expression of other growth factors such as amphiregulin, EGFR and TGF-α, leading to increased tumour proliferation. For instance, in cervical cells TNF-α induces amphiregulin, which stimulates cell proliferation [77], whereas in pancreatic cells TNF-α induces the expression of epidermal growth factor receptor (EGFR) and transforming growth factor (TGF-α), which mediates proliferation. Finally TNF-α enhances tumour angiogenesis through different angiogenic factors such as IL-8 and VEGF, and also is a critical regulator of VEGF and jagged-1 expression via a JNK- and AP-1- dependent pathway [104].

It has been demonstrated that tumour necrosis factor (TNF-α) has a therapeutic role when expressed locally by the cells of the immune system [105]. The anti-cancer actions of TNF can be due to direct effects on tumour cells and/or indirect effects on host stroma, and many of these effects are potentiated by IFN-γ. Vascular damage is widely accepted as a mechanism of its anti-tumour effects. In a breast cancer xenograft model, locally injected human TNF resulted in growth inhibition of established tumours. However macroscopic necrosis was observed in these mice when systemic rat IFN-γ, which has no activity alone, was also given. Within 4 h of administration of this cytokine combination, platelet adherence to tumour cells was observed, followed by destruction of the tumour vasculature. Both necrosis and apoptosis of tumour cells was demonstrated and there was up-regulation of mRNA for a range of stromal cytokines and adhesion molecules [106].

TNF-α produced by tumours can act as an endogenous tumour promoter [98]. Komori’s group reported that human TNF-α is 1000 times more effective than the chemical tumour promoters okadaic acid and 12-O-tetradecanoylphorbol-13-acetate in inducing cancer [107]. In most of these cells, TNF-α acts as an autocrine growth factor, however in some cell types TNF-α induces the expression of other growth factors, which mediate proliferation of tumours. TNF-α has been reported to induce angiogenic factor up-regulation in malignant glioma cells [108] which in turn promotes angiogenesis and tumour progression. TNF-α could enhance invasiveness of some carcinomas or stimulate epithelial wound healing in vivo [109] and it has been even reported to mediate macrophage-induced angiogenesis [110].

B. Role of chemokines in cancer

The chemokines are soluble, small proteins that bind to their associate G-protein coupled receptors (GPCRs) to elicit a cellular response [111]. Tumour cells secrete and respond to chemokines, which in turn facilitate cancer growth through means of increased angiogenesis, inflammation, endothelial cell recruitment and cell migration. Furthermore, chemokines regulate the recruitment and trafficking of leukocytes to sites of inflammation. Chemokines are grouped into four classes based on the positions of key cysteine residues: C, CC, CXC, and CX3C. Different classes of chemokines recognize different subset of cells, expressing the corresponding receptor [112] (Table 1).
Tumour cells also release soluble mediators such as VEGF-A (vascular endothelial growth factor-A), TGF-β and TNF-α that act on myeloid and endothelial cells and induce the expression of non-classical chemokines such as the S100 chemokine. Interestingly, S100 chemokines are implicated in targeting of the tumour cells to the pre-metastatic sites rather than the metastatic sites [113,114]. Evidence from murine models and human cancers suggests that CC chemokines are major determinants of macrophage and lymphocyte infiltration in melanoma, ovarian carcinoma, breast, and cervix, and in sarcomas and gliomas [115]. The chemokines elaborated from the tumour and the stromal cells bind to the chemokine receptors present on these cells. The two chemokine receptor–chemokine pairs that are involved commonly in many tumour are CXCR4–CXCL12 and CCR7–CCL21 [112]. Chemokine receptors CXCR4 and CCR7 are highly expressed in human breast cancer cells, malignant breast tumours, and metastasis [116]. Their respective ligands CXCL12/ SDF-1α and CCL21/6ckine are highly expressed in organs representing the first destinations of breast cancer metastasis. In breast cancer cells, signalling through CXCR4 or CCR7 mediates actin polymerization and pseudopodia formation and subsequently induces chemotactic and invasive responses. In vivo, neutralizing the interactions of CXCL12/CXCR4 significantly impairs metastasis of breast cancer cells to regional lymph nodes and lung. Malignant melanomas show high expression levels of CCR10 in addition to CXCR4 and CCR7. Thus chemokines and their receptors have a critical role in determining the metastatic destination of tumour cells. Melanoma growth stimulatory activity/growth-regulated protein (MGSA/GRO), is a CXC chemokine constitutively expressed in melanoma tumours and is associated with constitutive NFκB activity [117]. Ovarian cancers express CXCR4 chemokine receptors [118]. Its ligand, CXCL12 (stromal cell-derived factor 1), in ovarian cancer cells stimulates cell migration and invasion through extracellular matrix, as well as DNA synthesis and EGFR transactivation [119,120].

## C. Role of matrix metallo-proteinases in cancer

NFκB regulates several dependent-matrix metallo-proteinases (MMPs), which are correlated with malignant prognosis of various cancer types including colorectal, breast, and bladder cancers [121]. Indeed, the analyses on the human MMP-9 gene promoter revealed that NFκB is one of major transcription factors responsible for its induction [121]. MMPs are key modulators of many biological processes during pathophysiological events, such as skeletal formation, angiogenesis, cellular migration, inflammation, wound healing, and cancer [122]. By means of in vivo selection, transcriptomic analysis, functional verification and clinical validation, Minn et al have identified a set of genes comprising MMPs, that marks and mediates breast cancer metastasis to the lungs. In particular, MMP-2 acts mainly as virulence gene that may allow tumours to aggressively invade, colonize and grow in the lungs without markedly contributing to primary tumour growth, whereas MMP-1, determine metastatic potential of breast cancer to produce lung metastases [123]. MMP-7 also promotes cancer invasion by proteolytic cleavage of the extracellular matrix substrates and activates other MMPs, such as proMMP-2 and proMMP-9, to facilitate tumour invasion [124]. It has been demonstrated that transgenic mice lacking MMP-9 show reduced keratinocyte hyper proliferation at all neoplastic stages and a decreased incidence of invasive tumours [125]. Yet those carcinomas that do arise in the absence of MMP-9 show a greater loss of keratinocyte differentiation, indicative of a more aggressive and higher grade tumour [125].

## VII. NFκB INHIBITION AND CANCER THERAPY

It is known that a sustained, constitutive activation of NFκB contributes to malignant progression and therapeutic resistance in most of the major forms of human cancer, such as human lymphomas [60], carcinomas of the breast [126], prostate [127], lung [128], colon [129], pancreas [130], thyroid [131], head and neck [132] and cervix [133]. Thus, the modulation of NFκB activity could represent an useful therapeutic strategy for cancer, since NFκB inhibition promotes apoptotic events induced by chemotherapy, reduces the high proliferative rate that characterizes tumour cells and inhibits metastasis [134]. To date, different approaches have been developed to block NFκB in several conditions by regulating different steps in NFκB signalling pathway:

### A. IKK inhibition

A protein that disrupts the association of the IKK complex is used to prevent inflammatory bone destruction [135]. Similarly, the inhibition of IκBα phosphorylation

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Table 1. List of chemokines subfamilies and their specific target cell types

| CHEMOKINE SUBTYPE | TARGET CELL TYPES |
|-------------------|-------------------|
| C                 | B cells, T cells, natural killer cells, neutrophils |
| CC                | Dendritic cells, lymphocytes, macrophages, eosinophils, natural killer cells |
| CXC               | Neutrophils, lymphocytes, endothelial and epithelial cells |
| CXC               | Effector T cells |

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by the Bay 11-7082 compound, has been successfully used to prevent tumour growth and leukemic infiltration in a mouse model of adult T cell leukaemia [136]. The IKK inhibitors BAY 11-7082 and BAY 11-7085 also induce the apoptosis of colon cancer cells [137]. Some anti-inflammatory drugs and other substances such as curcumin, trans-resveratrol or parthenolide may inhibit NFκB by interfering with IKK activity [138-142]. Curcumin is a polyphenol derived from the plant Curcuma longa that exerts anti-oxidant, anti-inflammatory, anti-angiogenic and anti-tumoral activity. It was found to suppress COX-2 expression by inhibiting extracellular signal-regulated kinase (ERK) activity and NFκB in phorbol ester-induced mouse skin tumour genesis [143]. Non-steroidal anti-inflammatory agents (NSAIDs), including aspirin, have been shown to suppress NFκB activation by inhibiting IKK activation and IκBα degradation in tumour cells [144]. A small molecule inhibitor of IKK (PS-1145) was found to be selectively toxic for subtypes of diffuse large B-cell lymphoma cells that are associated with NFκB activation [2,145]. This compound was shown to lead to down-regulation of a set of NFκB-dependent genes [2].

B. IκB upregulation

The inhibition of NFκB activation by expression of a degradation, increased NFκB dependent apoptosis to stimuli such as TNFα [146]. Zhou et al. transfected the dominant-negative mutant inhibitor of NFκB (IκBm) into an acute lymphoblastic leukaemia (ALL) cell line with constitutive NFκB activation [147]. Overexpression of IκBm simultaneously down-regulates NFκB activation and sequesters p53 in the cytoplasm, thus enhancing NFκB-regulated apoptosis but blocking p53-dependent apoptosis [147]. We have demonstrated that in vitro, adenovirus mediated overexpression of the RH domain of GRK5 (AdGRK5-NT) in human tumour cells (KAT-4) induces IκB accumulation and inhibits NFκB transcriptional activity leading to apoptotic events [27]. In BALB/c nude mice harbouring KAT-4 induced neoplasias, intra-tumour delivery of AdGRK5-NT reduces in a dose-dependent fashion tumour growth, with the highest doses completely inhibiting it. This phenomenon is paralleled by a decrease of NFκB activity, an increase of IκB levels and apoptotic events [27]. To move towards a pharmacological setup, we synthesized the TAT-RH protein. In cultured KAT-4 cells, different dosages of TAT-RH reduced cell survival and increased apoptosis. In BALB/c mice, the anti-proliferative effects of TAT-RH appear to be dose-dependent and highest dose completely inhibits tumor growth [27] (fig 3).

C. Proteasome inhibition

Another way to approach NFκB inhibition is to target the process of proteasome degradation. Proteasome inhibitors prevent NFκB activation by blocking the degradation of IκBαs, NFκB1/p105 or NFκB2/p100. A successful strategy is using a proteasome inhibitor, Bortezomib or PS-341, to treat patients with refractory or resistant multiple myeloma [148]. Bortezomib is a dipeptidyl boronic acid that specifically inhibits 26S proteasome, the principal regulator of intracellular protein degradation like IκB. The treatment with this compound alone or in combination with other drugs, inhibits proliferation and induces apoptosis in several solid tumours [149-153], and is currently approved for treatment of multiple myeloma [152]. The importance of NFκB in multiple myeloma is suggested from its involvement downstream of CD40, the TNF receptor family member that is expressed in a variety of B-cell malignancies and which is associated with multiple myeloma homing. Consistent with this, monoclonal antibodies to CD40 block CD40L-induced NFκB activation as well as IL-6 and VEGF secretion in cultures of multiple myeloma cells and bone marrow-derived stromal cells. Other haematological malignancies are susceptible to NFκB inhibition. Proteasome inhibition blocks cell growth and induces apoptosis in adult T-cell leukaemia, an NFκB-relevant malignancy, correlated with stabilized IκBα and inhibited NFκB [2,153].

All these approaches open new fields for the management of NFκB-associated diseases like cancer. Clinical trials are being performed with some of the above described drugs and more other compounds that are able to block NFκB activity but the most significant clinical data comes from studies with the protease inhibitor bortezomib.
VIII. CONCLUSIONS AND FUTURE PROSPECTS

It is now well established that NFκB has a key role in carcinogenesis and that the inhibition of NFκB is a promising strategy for cancer therapy. Therefore, an increasing number of compounds able to block NFκB activity at different stages of its signalling pathway have been tested. Most of these drugs have given promising results in preclinical models of tumour (pancreas, lung, colon, ovarian and breast cancer), but failed in the clinical efficacy. Actually, the only pharmacological inhibitors of NFκB approved for clinical use are proteasome inhibitors for treatment of multiple myeloma or adult T-cell leukaemia, for whose pathogenesis it has been clearly demonstrated the key role of NFκB. The difficulty to find an efficient drug for cancer treatment is due to the fact that these drugs are able to block not only the oncogenic activity of NFκB but also its physiological roles in immunity, inflammation and cellular homeostasis. Moreover, the treatment is not specifically targeted on tumour cells thus affecting also healthy cells. Finally, these drugs induce many highly toxic side effects. In the future, new drugs might be designed that should be more specific in their function, in order to avoid affecting the induction of genes that are required for immunity, and in cell targeting, in order to protect normal cells from death.

REFERENCES

[1] D Hanahan, RA Weinberg. The hallmarks of cancer. Cell. Jan 7 2000;100(1):57-70.
[2] HJ Kim, N Hawke, AS Baldwin. NF-kappaB and IKK as therapeutic targets in cancer. Cell Death Differ. May 2006;13(5):738-747.
[3] J Downward. PI 3-kinase, Akt and cell survival. Semin Cell Dev Biol. Apr 2004;15(2):177-182.
[4] DS Latchman. Transcription factors: an overview. Int J Biochem Cell Biol. Dec 1997;29(12):1305-1312.
[5] MJ Vaquerizas, SK Kummerfeld, SA Teichmann, NM Luscombe. A census of human transcription factors: function, expression and evolution. Nat Rev Genet. Apr 2009;10(4):252-263.
[6] GJ Narlikar, HY Fan, RE Kingston. Cooperation between complexes that regulate chromatin structure and transcription. Cell. Feb 22 2002;108(4):475-487.
[7] JL Xu, CK Glass, MG Rosenfeld. Coactivator and corepressor complexes in nuclear receptor function. Curr Opin Genet Dev. Apr 1999;9(2):140-147.
[8] DJ Engelkamp, V van Heyningen. Transcription factors in disease. Curr Opin Genet Dev. Jun 1996;6(3):334-342.
[9] DW Nebert. Transcription factors and cancer: an overview. Toxicology. Dec 27 2002;181-182:131-141.
[10] S Ghosh, M Karin. Missing pieces in the NF-kappaB puzzle. Cell. Apr 2002;109 Suppl:S81-96.
[11] AS Baldwin. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol. 1996;14:649-683.
[12] A Lin, M Karin. NF-kappab in cancer: a marked target. Semin Cancer Biol. Apr 2003;13(2):107-114.
[13] M Karin, Y Ben-Neriah. Phosphorylation meets ubiquitination: the control of NF-κB activity. Annu Rev Immunol. 2000;18:621-663.
[14] ZW Li, W Chu, Y Hu, et al. The IKKbeta subunit of IkappaB kinase (IKK) is essential for nuclear factor kappaB activation and prevention of apoptosis. J Exp Med. Jun 7 1999;189(11):1839-1845.
[15] Q Li, D Van Antwerp, F Mercurio, KF Lee, IM Verma. Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. Science. Apr 9 1999;284(5412):321-325.
[16] M Tanaka, ME Fuentes, K Yamaguchi, et al. Embryonic lethality, liver degeneration, and impaired NF-kappa B activation in IKK-beta-deficient mice. Immunity. Apr 1999;10(4):421-429.
[17] U Senftleben, ZW Li, V Baud, M Karin. IKKbeta is essential for protecting T cells from TNFalpha-induced apoptosis. Immunity. Mar 2001;14(3):217-230.
[18] NJ Solan, H Miyoshi, EM Carmona, GD Bren, CV Paya. RelB cellular regulation and transcriptional activity are regulated by p100. J Biol Chem. Jan 11 2002;277(2):1405-1418.
[19] M Karin, Y Cao, FR Greten, ZW Li. NF-kappaB in cancer: from innocent bystander to major culprit. Nat Rev Cancer. Apr 2002;2(4):301-310.
[20] B Rayet, C Gelinus. Aberrant rel/nfkb genes and activity in human cancer. Oncogene. Nov 22 1999;18(49):6938-6947.
[21] A Garg, BB Aggarwal. Nuclear transcription factor-kappaB as a target for cancer drug development. Leukemia. Jun 2002;16(6):1053-1068.
[22] CH Lee, YT Jeon, SH Kim, YS Song. NF-kappaB as a potential molecular target for cancer therapy. Biofactors. 2007;29(1):19-35.
[23] TD Gilmore. Multiple mutations contribute to the oncogenicity of the retroviral oncoprotein v-Rel. Oncogene. Nov 22 1999;18(49):6925-6937.
[24] G Xiao, ME Cvijic, A Fong, et al. Retroviral oncoprotein Tax induces processing of NF-kappaB/p100 in T cells: evidence for the involvement of IKKalpha. EMBO J. Dec 3 2001;20(23):6805-6815.
[25] G Mosialos. The role of Rel/NF-kappa B proteins in viral oncogenesis and the regulation of viral transcription. Semin Cancer Biol. Apr 1997;8(2):121-129.
[26] Y Bours, M Bentires-Alj, AC Hellin, et al. Nuclear factor-kappaB, cancer, and apoptosis. Biochem Pharmacol. Oct 15 2000;60(8):1085-1089.
[27] D Sorrentino, A Campanile, G Santulli, et al. A new synthetic protein, TAT-RH, inhibits tumor growth through the regulation of NFkappaB activity. Mol Cancer. 2009;8:97.
[28] D Sorrentino, G Santulli, A Fusco, A Anastasio, B Trimmer, G Iaccarino. Intracardiac injection of AdGK5-NT reduces left ventricular hypertrophy by inhibiting NF-kappab-dependent hypertrophic gene expression. Hypertension. Oct;56(4):696-704.
[29] K Puszyński, R Bertolusso, T Lipniacki. Crosstalk between p53 and nuclear factor-B systems: pro- and anti-apoptotic functions of NF-B. *IET Syst Biol*. Sep 2009;3(5):356-367.

[30] S Fulda, KM Debean. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*. Aug 7 2006;25(34):4798-4811.

[31] K Cain, SB Bratton, C Langlais, et al. Apaf-1 oligomerizes into biologically active approximately 700-kDa and inactive approximately 1.4-MDa apoptosis complexes. *J Biol Chem*. Mar 3 2000;275(9):6067-6070.

[32] CY Wang, DC Guttridge, MW Mayo, AS Baldwin. NF-kappaB induces expression of the Bcl-2 homologue A1/Bcl-1 to preferentially suppress chemotherapy-induced apoptosis. *Mol Cell Biol*. Sep 1999;19(9):5923-5929.

[33] S Kreutz, D Siegmund, P Scheurich, H Wajant. NF-kappaB inducers upregulate cFLIP, a cycloheximide-sensitive inhibitor of death receptor signaling. *Mol Cell Biol*. Jun 2001;21(12):3964-3973.

[34] O Micheau, S Lens, O Gaide, K Alevizopoulos, J Tschopp. NF-kappaB signals induce the expression of c-FLIP. *Mol Cell Biol*. Aug 2001;21(16):5299-5305.

[35] RR Bullani, B Haard, I Viard-Leveugle, et al. Selective expression of FLIP in malignant melanocytic skin lesions. *J Invest Dermatol*. Aug 2001;117(2):360-364.

[36] TS Griffith, WA Chiu, GC Jackson, DH Lynch, MZ Kubin. Intracellular regulation of TRAIL-induced apoptosis in melanoma cells. *J Immunol*. Sep 15 1998;161(6):2833-2840.

[37] M Irisarri, J Plumas, T Bonnefoix, et al. Resistance to CD95-mediated apoptosis through constitutive c-FLIP expression in a non-Hodgkin's lymphoma B cell line. *Leukemia*. Dec 2000;14(12):2149-2158.

[38] M Imler, M Thome, M Hahne, et al. Inhibition of death receptor signals by cellular FLIP. *Nature*. Jul 10 1997;388(6638):190-195.

[39] DJ Panka, T Mano, T Suhara, K Walsh, JW Mier. Phosphatidylinositol 3-kinase/Akt activity regulates c-FLIP expression in tumor cells. *J Biol Chem*. Mar 9 2001;276(10):6893-6896.

[40] CY Wang, MW Mayo, RG Korneluk, DV Goeddel, AS Baldwin. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. *Science*. Sep 11 1998;281(5383):1680-1683.

[41] R Takahashi, Q Deveraux, I Tamm, et al. A single BIR domain of XIAP sufficient for inhibiting caspases. *J Biol Chem*. Apr 3 1998;273(14):7787-7790.

[42] QL Deveraux, N Roy, HR Stennicke, et al. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *EMBO J*. Apr 15 1998;17(8):2215-2223.

[43] QM Chen, VC Tu. Apoptosis and heart failure: mechanisms and therapeutic implications. *Am J Cardiovasc Drugs*. 2002;2(1):43-57.

[44] JU Lee, R Hosotani, M Wada, et al. Role of Bel-2 family proteins (Bax, Bel-2 and Bel-X) on cellular susceptibility to radiation in pancreatic cancer cells. *Eur J Cancer*. Sep 1999;35(9):1374-1380.

[45] ZL Chu, TA McKinsey, L Liu, JJ Gentry, MH Malim, DW Ballard. Suppression of tumor necrosis factor-induced cell death by inhibitor of apoptosis c-IAP2 is under NF-kappaB control. *Proc Natl Acad Sci U S A*. Sep 16 1997;94(19):10057-10062.

[46] EY Lin, A Orlofsky, BS Berger, MB Prystowsky. Characterization of A1, a novel hemopoietic-specific early-response gene with sequence similarity to bcl-2. *J Immunol*. Aug 15 1993;151(4):1979-1988.

[47] Y Haupt, R Maya, A Kazaz, M Oren. Mdm2 promotes the rapid degradation of p53. *Nature*. May 15 1997;387(6630):296-299.

[48] Y Barak, T Juven, R Haffner, Oren M. mdm2 expression is induced by wild type p53 activity. *EMBO J*. Feb 1993;12(2):461-468.

[49] B Vogelstein, D Lane, AJ Levine. Surfing the p53 network. *Nature*. Nov 16 2000;408(6810):307-310.

[50] DC Guttridge, C Albanese, JY Reuther, RG Pestell, Baldwin AS, Jr. NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol*. Aug 1999;19(8):5785-5799.

[51] M Hinz, D Krappmann, A Eichten, A Heder, C Scheideereit, M Strauss. NF-kappaB function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition. *Mol Cell Biol*. Apr 1999;19(4):2690-2698.

[52] M Hinz, P Loser, S Mathas, D Krappmann, B Dorken, C Scheideereit. Constitutive NF-kappaB maintains high expression of a characteristic gene network, including CD40, CD86, and a set of antiapoptotic genes in Hodgkin/Reed-Sternberg cells. *Blood*. May 1 2001;97(9):2798-2807.

[53] CY Hsia, S Cheng, AM Owyang, SF Dowdy, HC Liou. c-Rel regulation of the cell cycle in primary mouse B lymphocytes. *Int Immunol*. Aug 2002;14(8):905-916.

[54] P Chen, V Castranova, X Shi. New insights into the role of nuclear factor-kappaB in cell growth regulation. *Am J Pathol*. Aug 2001;159(2):387-397.

[55] MP Duyao, DJ Kessler, DB Spicer, et al. Transactivation of the c-myc promoter by human T cell leukemia virus type 1 tax is mediated by NF kappa B. *J Biol Chem*. Aug 15 1992;267(23):16288-16291.

[56] NJ Thornburg, R Pathamathan, N Raab-Traub. Activation of nuclear factor-kappaB p50 homodimer/Bcl-3 complexes in nasopharyngeal carcinoma. *Cancer Res*. Dec 1 2003;63(23):8293-8301.

[57] Q Yu, Y Geng, P Sicsinski. Specific protection against breast cancers by cyclin D1 ablation. *Nature*. Jun 28 2001;411(6841):1017-1021.

[58] T Jiang, B Grabner, Y Zhu, et al. CARMA3 is crucial for EGFR-Induced activation of NF-kappaB and tumor progression. *Cancer Res*. Mar 15;71(6):2183-2192.

[59] S Mathas, M Hinz, I Anagnostopoulou, et al. aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF-kappaB. *EMBO J*. Aug 1 2002;21(15):4104-4113.

[60] RC Bargou, F Emmerich, D Krappmann, et al. Constitutive nuclear factor-kappaB-RelA activation is required for proliferation and survival of Hodgkin's
disease tumor cells. J Clin Invest. Dec 15 1997;100(12):2961-2969.

[61] K Hinata, AM Gervin, Y Jennifer Zhang, PA Khavari. Divergent gene regulation and growth effects by NF-kappa B in epithelial and mesenchymal cells of human skin. Oncogene. Apr 3 2003;22(13):1955-1964.

[62] J Bash, WX Zong, C Gelinis. c-Rel arrests the proliferation of HeLa cells and affects critical regulators of the G1/S-phase transition. Mol Cell Biol. Nov 1997;17(11):6526-6536.

[63] VB Andela, EM Schwarz, JR O'Keefe, RN Rosier. Tumor metastasis and the reciprocal regulation of prometastatic and antimetastatic factors by nuclear factor kappaB. Cancer Res. Dec 1 2000;60(23):6557-6562.

[64] JP Johnson. Cell adhesion molecules in the development and progression of malignant melanoma. Cancer Metastasis Rev. 1999;18(3):345-357.

[65] T Loeh, B Michalski, U Mazurek, M Granicka. [Vascular endothelial growth factor (VEGF) and its role in neoplastic processes]. Postepy Hig Med Dosw. 2001;55(2):257-274.

[66] D Chilov, E Kukk, S Taiera, et al. Genomic organization of human and mouse genes for vascular endothelial growth factor. C. J Biol Chem. Oct 3 1997;272(40):25176-25183.

[67] H Huang, A DeGuzman, CD Bucana, IJ Fidler. Nuclear factor-kappaB activity correlates with growth, angiogenesis, and metastasis of human melanoma cells in nude mice. Clin Cancer Res. Jun 2000;6(6):2573-2581.

[68] G Helbig, KW Christopherson, P Bhat-Nakshatri, et al. NF-kappaB promotes breast cancer cell migration and metastasis by inducing the expression of the chemokine receptor CXCR4. J Biol Chem. Jun 13 2003;278(24):21631-21638.

[69] S Huang, JB Robinson, A Deguzman, CD Bucana, IJ Fidler. 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. Oct 1 2000;60(19):5334-5339.

[70] M Tsuji, S Kawano, S Tsuji, H Sawaoka, M Hori, RN Dubois. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell. May 29 1998;93(5):705-716.

[71] BB Aggarwal, S Shishodia, SK Sandur, MK Pandey, G Sethi. Inflammation and cancer: how hot is the link? Biochem Pharmacol. Nov 30 2006;72(11):1605-1621.

[72] F Thevenod F, Friedmann JM, Katsen AD, Hauser IA. Up-regulation of multidrug resistance P-glycoprotein via nuclear factor-kappaB activation protects kidney proximal tubule cells from cadmium- and reactive oxygen species-induced apoptosis. J Biol Chem. Jan 21 2000;275(3):1887-1896.

[73] G Otsuka, T Nagaya, K Saito, M Mizuno, J Yoshida, H Seo. Inhibition of nuclear factor-kappaB activation confers sensitivity to tumor necrosis factor-alpha by impairment of cell cycle progression in human glioma cells. Cancer Res. Sep 1 1999;59(17):4446-4452.

[74] CY Wang, JC Cusack, R Liu, AS Baldwin. Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-kappaB. Nat Med. Apr 1999;5(4):412-417.

[75] BB Aggarwal. Nuclear factor-kappaB: the enemy within. Cancer Cell. Sep 2004;6(3):203-208.

[76] Shishodia S, Aggarwal BB. Nuclear factor-kappaB: a friend or a foe in cancer? Biochem Pharmacol. Sep 15 2004;68(6):1071-1080.

[77] Woodworth CD, McMullin E, Iglésias M, Plowman GD. Interleukin 1 alpha and tumor necrosis factor alpha stimulate autocrine amphiregulin expression and proliferation of human papillomavirus-immortalized and carcinoma-derived cervical epithelial cells. Proc Natl Acad Sci U S A. Mar 28 1995;92(7):2840-2844.

[78] A Arit, J Vorndamm, S Muerkoster, et al. Autocrine production of interleukin 1beta confers constitutive nuclear factor kappaB activity and chemoresistance in pancreatic carcinoma cell lines. Cancer Res. Feb 1 2002;62(3):910-916.

[79] Y Saijo, M Tanaka, M Miki, et al. Proinflammatory cytokine IL-1 beta promotes tumor growth of Lewis lung carcinoma by induction of angiogenic factors: in vivo analysis of tumor-stromal interaction. J Immunol. Jul 1 2002;169(1):469-475.

[80] B Kleijn, XG Zhang, M Jourdan, et al. Paracrine rather than autocrine regulation of myeloma-cell growth and differentiation by interleukin-6. Blood. Feb 1989;73(2):517-526.

[81] N Voorzanger, R Touitou, E Garcia, et al. Interleukin (IL)-10 and IL-6 are produced in vivo by non-Hodgkin's lymphoma cells and act as cooperative growth factors. Cancer Res. Dec 1 1996;56(23):5499-5505.

[82] M Okamoto, H Kawamata, K Kawai, R Oyasu. Enhancement of transformation in vitro of a nonmalignant rat urothelial cell line by interleukin 6. Cancer Res. Oct 15 1995;55(20):4581-4585.

[83] S Landi, V Moreno, L Gioia-Patricola, et al. Association of common polymorphisms in inflammatory genes interleukin (IL)-6, IL-8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. Cancer Res. Jul 1 2003;63(13):3560-3566.

[84] LS Angelo, M Talpaz, R Kurzrock. Autocrine interleukin-6 production in renal cell carcinoma: evidence for the involvement of p53. Cancer Res. Feb 1 2002;62(3):932-940.

[85] G Sethi, B Sung, BB Aggarwal. TNF: a master switch for inflammation to cancer. Front Biosci. 2008;13:5094-5107.

[86] J Falkwijk, Tumour necrosis factor and cancer. Nat Rev Cancer. May 2009;9(5):361-371.

[87] W Digela, M Stefan, W Schoniger, et al. Tumor necrosis factor induces proliferation of neoplastic B cells from chronic lymphocytic leukemia. Blood. Apr 1989;73(5):1242-1246.

[88] DK Giri, BB Aggarwal. Constitutive activation of NF-kappaB causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. J Biol Chem. May 29 1998;273(22):14008-14014.
[89] RY Liu, C Fan, S Mitchell, Q Chen, J Wu, KS Zuckerman. The role of type I and type II tumor necrosis factor (TNF) receptors in the ability of TNF-alpha to transduce a proliferative signal in the human megakaryoblastic leukemia cell line Mo7e. *Cancer Res.* May 15 1998;58(10):2217-2223.

[90] K Tsukasaki, CW Miller, T Kubota, et al. Tumor necrosis factor alpha polymorphism associated with increased susceptibility to development of adult T-cell leukemia/lymphoma in human T-lymphotropic virus type I carriers. *Cancer Res.* May 1 2001;61(9):3770-3774.

[91] G Stifter, S Heiss, G Gastl, A Tzankov, R Stauder. Over-expression of tumor necrosis factor-alpha in bone marrow biopsies from patients with myelodysplastic syndromes: relationship to anemia and prognosis. *Eur J Haematol.* Dec 2005;75(6):485-491.

[92] AS Duncombe, HE Heslop, M Turner, et al. Tumor necrosis factor mediates autocrine growth inhibition in a chronic leukemia. *J Immunol.* Dec 1 1989;143(11):3828-3834.

[93] O Elbaz, LA Mahmoud. Tumor necrosis factor and human acute leukemia. *Leuk Lymphoma.* Jan 1994;12(3-4):191-195.

[94] R Montesano, P Soulie, JA Eble, F Carrozziino. Tumor necrosis factor alpha confers an invasive, transformed phenotype on mammary epithelial cells. *J Cell Sci.* Aug 1 2005;118(Pt 15):3487-3500.

[95] JW Schmiegel, C Roeder, J Schmielau, U Rodeck, H Kalthoff. Tumor necrosis factor alpha induces the expression of transforming growth factor alpha and the epidermal growth factor receptor in human pancreatic cancer cells. *Proc Natl Acad Sci U S A.* Feb 1 1993;90(3):863-867.

[96] H Takeyama, N Wakamiya, C O'Hara, et al. Tumor necrosis factor expression by human ovarian carcinoma in vivo. *Cancer Res.* Aug 15 1991;51(16):4476-4480.

[97] Y Wu, BP Zhou. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. *Br J Cancer.* Feb 16;102(4):639-644.

[98] F Balkwill. Tumor necrosis factor or tumor promoting factor? *Cytokine Growth Factor Rev.* Apr 2002;13(2):135-141.

[99] F Balkwill, A Mantovani. Inflammation and cancer: back to Virchow? *Lancet.* Feb 17 2001;357(9255):539-545.

[100] F Balkwill, LM Coussens. Cancer: an inflammatory link. *Nature.* Sep 3 2004;431(7007):405-406.

[101] A Mantovani. Cancer: inflammation by remote control. *Nature.* Jun 9 2005;435(7043):752-753.

[102] TC Hsu, R Nair, P Tsulian, et al. Transformation nonresponsive cells owe their resistance to lack of p65/nuclear factor-kappaB activation. *Cancer Res.* May 15 2001;61(10):4160-4168.

[103] S Shishodia, BB Aggarwal. Nuclear factor-kappaB activation mediates cellular transformation, proliferation, invasion angiogenesis and metastasis of cancer. *Cancer Treat Res.* 2004;119:139-173.

[104] DA Johnston, B Dong, CC Hughes. TNF induction of jagged-1 in endothelial cells is NFkappaB-dependent. *Gene.* Apr 15 2009;435(1-2):36-44.

[105] BB Aggarwal. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol.* Sep 2003;3(9):745-756.

[106] S de Kossodo, R Moore, S Gschmeissner, N East, C Upton, FR Balkwill. Changes in endogenous cytokines, adhesion molecules and platelets during cytokine-induced tumour necrosis. *Br J Cancer.* Nov 1995;72(5):1165-1172.

[107] A Komori, J Yatsunami, M Suganuma, et al. Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. *Cancer Res.* May 1 1993;53(9):1982-1985.

[108] LB Nabors, E Suswam, Y Huang, X Yang, MJ Johnson, PH King. Tumor necrosis factor alpha induces angiogenic factor up-regulation in malignant glioma cells: a role for RNA stabilization and HuR. *Cancer Res.* Jul 15 2003;63(14):4181-4187.

[109] YS Yoshida, M Ono, T Shono, et al. Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. *Mol Cell Biol.* Jul 1997;17(7):4015-4023.

[110] SJ Leibovich, PJ Polverini, HM Shepard, DM Wiseman, V Shively, N Nusseir. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. *Nature.* Oct 15-21 1987;329(6140):630-632.

[111] A Rot, UH von Andrian. Chemokines in innate and adaptive host defense: basic chemokine grammar for immune cells. *Annu Rev Immunol.* 2004;22:891-928.

[112] D Raman, PJ Baughler, YM Thu, A Richmond. Role of chemokines in tumor growth. *Cancer Lett.* Oct 28 2007;256(2):137-165.

[113] S Hiratsuka, A Watanabe, H Aburatani, Y Maru. Tumour-mediated upregulation of chemotraffactors and recruitment of myeloid cells predetermines lung metastasis. *Nat Cell Biol.* Dec 2006;8(12):1369-1375.

[114] HS Rafii, D Lyden. S100 chemokines mediate bookmarking of premetastatic niches. *Nat Cell Biol.* Dec 2006;8(12):1321-1323.

[115] H Kulbe, NR Levinson, F Balkwill, JL Wilson. The chemokine network in cancer--much more than directing cell movement. *Int J Dev Biol.* 2004;48(5-6):489-496.

[116] A Muller, B Homey, H Soto, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature.* Mar 1 2001;410(6824):50-56.

[117] D Wang, A Richmond. Nuclear factor-kappa B activation by the CXC chemokine melanoma growth-stimulatory activity/growth-regulated protein involves the MEKK1/p38 mitogen-activated protein kinase pathway. *J Biol Chem.* Feb 2 2001;276(5):3650-3659.

[118] CJ Scotton, JL Wilson, D Milliken, G Stamp, FR Balkwill. Epithelial cancer cell migration: a role for chemokine receptors? *Cancer Res.* Jul 1 2001;61(13):4961-4965.

[119] P Porcile, A Bajetto, S Barbero, P Pirani, G Schettini. CXCR4 activation induces epidermal growth factor receptor transactivation in an ovarian cancer cell line. *Ann N Y Acad Sci.* Dec 2004;1030:162-169.
vessels? Am J Clin Pathol. Dec 2001;116 Suppl:S97-107.
[135]E Jimi, K Aoki, H Saito, et al. Selective inhibition of NF-kappa B blocks osteoclastogenesis and prevents inflammatory bone destruction in vivo. Nat Med. Jun 2004;10(6):617-624.
[136]MZ Dewan, K Terashima, M Tsuruishi, et al. Rapid tumor formation of human T-cell leukemia virus type 1-infected cell lines in novel NOD-SCID/gamma(null) mice: suppression by an inhibitor against NF-kappa B. J Virol. May 2003;77(9):5286-5294.
[137]CL Scaife, J Kuang, JC Willis, et al. Nuclear factor kappaB inhibitors induce adhesion-dependent colon cancer apoptosis: implications for metastasis. Cancer Res. Dec 1 2002;62(23):6870-6878.
[138]Y Yamamoto, RB Gaynor. Role of the NF-kappaB pathway in the pathogenesis of human disease states. Curr Mol Med. Jul 2001;1(3):287-296.
[139]Y Yamamoto, MJ Yin, KM Lin, RB Gaynor. Sulindac inhibits activation of the NF-kappaB pathway. J Biol Chem. Sep 17 1999;274(38):27307-27314.
[140]MJ Yin, Y Yamamoto, RB Gaynor. The anti-inflammatory agents aspirin and salicylate inhibit the activity of IkappaB kinase-beta. Nature. Nov 5 1998;396(6706):77-80.
[141]M Holmes-McNary, AS Baldwin. Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res. Jul 1 2000;60(13):3477-3483.
[142]J Keifer, DC Guttridge, BP Ashburner, AS Baldwin. Inhibition of NF-kappa B activity by thalidomide through suppression of IkappaB kinase activity. J Biol Chem. Jun 22 2001;276(25):22382-22387.
[143]KS Chun, YS Keum, SS Han, YS Song, SH Kim, YJ Surh. Curcumin inhibits phorbol ester-induced expression of cyclooxygenase-2 in mouse skin through suppression of extracellular signal-regulated kinase activity and NF-kappa B activation. Carcinogenesis. Sep 2003;24(9):1515-1524.
[144]Y Takada, A Bhardwaj, P Potdar, BB Aggarwal. Nonsteroidal anti-inflammatory agents differ in their ability to suppress NF-kappaB activation, inhibition of expression of cyclooxygenase-2 and cyclin D1, and abrogation of tumor cell proliferation. Oncogene. Dec 9 2004;23(57):9247-9258.
[145]LT Lam, RE Davis, J Pierce, et al. Small molecule inhibitors of IkappaB kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. Clin Cancer Res. Jan 1 2005;11(1):28-40.
[146]DJ Van Antwerp, SJ Martin, T Kafri, DR Green, IM Verma. Suppression of TNF-alpha-induced apoptosis by NF-kappaB. Science. Nov 1 1996;274(5288):787-789.
[147]M Zhou, L Gu, N Zhu, WG Woods, HW Findley. Transfection of a dominant-negative mutant NF-kB inhibitor (IkBm) represses p53-dependent apoptosis in acute lymphoblastic leukemia cells; interaction of IkBm and p53. Oncogene. Nov 6 2003;22(50):8137-8144.
[148] RC Kane, PF Bross, AT Farrell, RP Pazdur. Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist*. 2003;8(6):508-513.

[149] ML Lun, PL Zhang, PK Pellitteri, A Law, TL Kennedy, RE Brown. Nuclear factor-kappaB pathway as a therapeutic target in head and neck squamous cell carcinoma: pharmaceutical and molecular validation in human cell lines using Velcade and siRNA/NF-kappaB. *Ann Clin Lab Sci*. Summer 2005;35(3):251-258.

[150] PA Bunn. The potential role of proteasome inhibitors in the treatment of lung cancer. *Clin Cancer Res*. Jun 15 2004;10(12 Pt 2):4263s-4265s.

[151] JB Sunwoo, Z Chen, G Dong, et al. Novel proteasome inhibitor PS-341 inhibits activation of nuclear factor-kappa B, cell survival, tumor growth, and angiogenesis in squamous cell carcinoma. *Clin Cancer Res*. May 2001;7(5):1419-1428.

[152] PG Richardson, T Hideshima, C Mitsiades, K Anderson. Proteasome inhibition in hematologic malignancies. *Ann Med*. 2004;36(4):304-314.

[153] RNasr, ME El-Sabban, JA Karam, et al. Efficacy and mechanism of action of the proteasome inhibitor PS-341 in T-cell lymphomas and HTLV-1 associated adult T-cell leukemia/lymphoma. *Oncogene*. Jan 13 2005;24(3):419-430.