NF-κB: arresting a major culprit in cancer

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Nuclear factor-κB (NF-κB) has long been known to play a central role in the immune system by regulating the expression of key genes. Moreover, activation of this transcription factor helps a wide variety of cell types survive damage induced by pro-apoptotic stimuli. Because of its crucial role in the regulation of pro-inflammatory genes, NF-κB is a promising target for the discovery of anti-inflammatory drugs. More recently, NF-κB has also emerged as a major culprit in a variety of human cancers mainly because of its ability to protect transformed cells from apoptosis. The pharmaceutical industry should, therefore, seriously consider testing inhibitors of NF-κB, identified as part of their anti-inflammatory drug discovery programs, in combination with other chemotherapeutic drugs in models of cancer.

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▼ The nuclear factor-κB (NF-κB) family of transcription factors has an almost ubiquitous role in the signal transduction, as well as the expression, of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α). As a result, inhibition of signal transduction proteins in the pathways leading to activation of NF-κB is now widely recognized as a valid strategy to combat inflammatory disease. Many pharmaceutical and biotechnology companies have drug discovery programs aimed at NF-κB, and have been investing heavily in the discovery of proteins that regulate the transcription factor. More recently, it has become obvious that inhibition of NF-κB activity is not only desirable in the treatment of inflammation but also in cancer therapy. In a recent review, Michael Karin even described NF-κB as a major culprit in cancer [1]. This should not be surprising because there is a link between inflammation and cancer that was first suggested by Galen (AD 129–ca AD 216) and later demonstrated by Virchow [2].

The link between inflammation and cancer

In 1863, Rudolf Virchow noted the presence of leukocytes in malignant tissues and proposed that this reflected an origin of cancer at sites of chronic inflammation. Since then, it has been recognized that many chronic inflammatory conditions, such as inflammatory bowel disease, predispose to cancer. Examination of the inflammatory microenvironment of neoplastic tissues has supported the hypothesis of inflammation as a cofactor in oncogenesis for a variety of cancers [3,4].

The leukocytes that infiltrate the microenvironment of tumours might contribute to tumour growth and spread, as well as to the systemic immunosuppression associated with cancer, rather than to fighting the disease. This seems paradoxical but can be explained by the findings that natural killer cells are rare in the tumour microenvironment, that tumour-associated leukocytes release pro-inflammatory cytokines [most importantly TNF-α, interleukin-1 (IL-1) and interleukin-6 (IL-6)], which contribute to malignant progression, and that leakage of these cytokines into the immune system could lead to desensitization of circulating leukocytes. TNF-α, IL-1 and IL-6 can act as growth and survival factors in a variety of malignancies and can stimulate production of angiogenic factors [5].

TNF-α can be found in ovarian, breast, prostate, bladder and colorectal cancer as well as in lymphomas and leukaemias. The systemic release of TNF-α is known to contribute to the severity of non-Hodgkins lymphoma and high circulating TNF-α levels are also associated with poor prognosis in certain leukaemias [6,7]. When chronically produced, TNF-α is thought to act as a tumour promoter that contributes to the tissue remodelling that is necessary for tumour growth and metastasis [8]. Evidence for a role for TNF-α in tumour formation comes from TNF-α knockout mice, which are resistant to skin carcinogenesis, and from human ovarian cancer mouse models in which TNF-α promotes the adhesion of free-floating ascitic cancer cells to the peritoneum and solid-tumour formation

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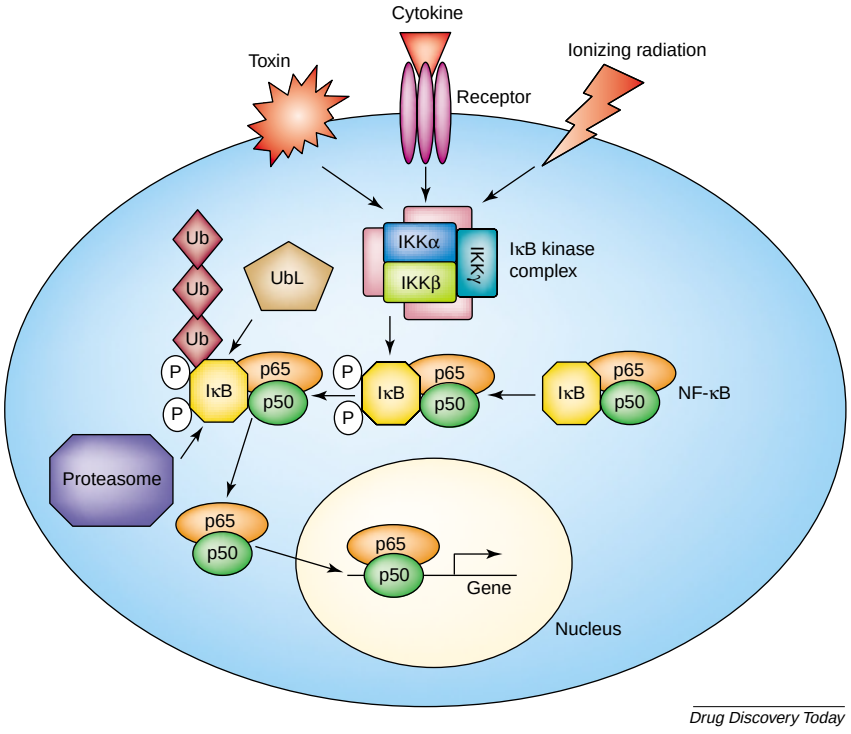

Figure 1. Pathway of NF-κB activation. Cytokines such as TNF-α and environmental hazards such as ionizing radiation or toxins trigger the nuclear translocation of NF-κB via activation of the inhibitor-of-NF-κB (IκB) kinase complex (IKK). IKK phosphorylates IκB bound to NF-κB, which consists of a dimer of Rel family proteins such as p65 and p50. This phosphorylation is the signal for ubiquitination of IκB by a ubiquitin ligase (UbL). This marks IκB for degradation by the proteasome, which then results in the release of NF-κB. The transcription factor is now free to become translocated to the nucleus where it binds to specific DNA elements and activates the transcription of NF-κB-dependent genes [12]. Abbreviation: Ub, ubiquitin.

[9,10]. Pharmacological evidence for a link between inflammation and carcinogenesis comes from the observation that non-steroidal anti-inflammatory drugs might reduce the risk of human gastrointestinal cancers [11].

Evidence for a role for NF-κB in tumourigenesis

NF-κB is well established as a transcription factor that is activated during cellular defence mechanisms against damaging environmental conditions, such as hypoxia, ionizing radiation, and the fight against infectious microorganisms where pro-inflammatory cytokines play a major role. All of these stimuli activate a generic signal transduction pathway that induces the translocation of NF-κB to the nucleus (Fig. 1). Briefly, cytokines such as TNF-α and environmental hazards such as ionizing radiation or toxins trigger the nuclear translocation of NF-κB via activation of the inhibitor-of-NF-κB (IκB) kinase complex (IKK). IKK phosphorylates IκB bound to NF-κB, which consists of a dimer of Rel family proteins such as p65 and p50. This phosphorylation is the signal for ubiquitination of IκB (i.e. the attachment of a chain of the protein ubiquitin) by a ubiquitin ligase. This marks IκB for degradation by the proteasome, which then results in the release of NF-κB. The transcription factor is now free to become translocated to the nucleus where it binds to specific DNA elements and activates the transcription of NF-κB-dependent genes [12].

The earliest evidence of a role for NF-κB in cell transformation comes from the finding that the avian reticuloendotheliosis virus strain-T oncogene product, v-Rel, is derived from the NF-κB protein c-Rel [13]. More recently, it has become evident that this transcription factor also plays a crucial role in certain types of human cancer where chromosomal alterations of NF-κB family genes have frequently been found [14,15]. The study of NF-κB in cell transformation has been pioneered by Albert S. Baldwin and his team (Lineberger Comprehensive Cancer Centre, University of North Carolina, NC, USA). In 1997, this group published two articles showing that the oncogenic Ras protein found in many common human malignancies depends on the activation of NF-κB for cell transformation and the suppression of apoptosis [16,17]. One year later, the group reported that this phenomenon was not unique to oncogenic Ras, but that Bcr–Abl, the transforming fusion protein in chronic myelogenous leukaemia (CML) also required NF-κB for cell transformation [18]. In 1999, the group was able to show how NF-κB controls cell growth by enhancing the transcription of cyclin D1 [19]. These discoveries clearly established that NF-κB promotes tumourigenesis by suppression of apoptosis and stimulation of cell proliferation.

Since the publication of this ground-breaking work, there have been many articles in the biomedical literature demonstrating the involvement of increased NF-κB activity, which is typical of many human tumours, in the pathogenesis of cancer [20,21]. For example, Arthur Pardee and his co-workers have identified EGF-induced NF-κB activation as a major pathway of cell-cycle progression in highly malignant oestrogen-receptor-negative breast cancer and have proposed inhibitors of NF-κB activation as potential therapeutic agents [22,23].

Further evidence for a role for NF-κB in human carcinogenesis comes from the observation that a variety of cancer chemopreventive agents including soybean isoflavone [24], *trans*-resveratrol, a phytoalexin found at high concentrations in grapes [25], curcumin and capsaicin [26] target this transcription factor.

The long history of TNF-α in tumour necrosis and the treatment of cancer

In the 1890s, before aseptic surgery was introduced, a New York surgeon, William B. Coley, noticed that some cancer patients who survived severe operating room infections showed complete and lasting remission of their tumour in contrast to other patients who did not get infected. Coley learned that miracle recoveries from cancer under similar circumstances were not unknown in medical folklore and he suspected that the infection provoked a massive immune response that occasionally destroyed remaining cancer cells. Coley went on to use bacterial toxins for cancer therapy with remarkable success [27–29]. Responses were most frequently seen when the toxin preparations were administered repeatedly into, or close to, the tumour and in sufficient quantities to induce systemic reactions such as fevers and chills. In 270 of 1200 recorded cases treated with these toxins, complete and lasting tumour regression was observed. This therapy was successful mostly in sarcomas and some other types of cancer derived from tissues of mesodermal origin. Regression was achieved even in cases of extensive, advanced metastatic disease. By the 1930s, however, the variability of the toxin preparations in terms of efficacy and the rise of radiotherapy had led to the abandonment of this treatment. Nevertheless, experimental studies continued in animal models and, in 1975, TNF-α was isolated from the serum of endotoxin-treated mice [30] and shown to cause regression, and sometimes cure, of a range of murine tumours and human tumour xenografts.

Phase I clinical trials of systemically administered TNF-α in advanced cancer began shortly after the gene for TNF-α was cloned in 1984 enabling the production of pure cytokine in sufficient quantities. When given intravenously, the major dose-limiting side effect was hypotension. Intramuscular or subcutaneous administration often resulted in dose-limiting local inflammation. Rigors followed by fever, chills, fatigue and headache were frequently observed, as were leukopenia, thrombocytopenia and anaemia. Phase II clinical trials of systemic TNF-α administration were subsequently conducted with uniformly negative results in advanced melanomas, pancreatic, breast, colon, stomach and kidney cancer. Only when given locally or locoregionally, such as by intratumoural injection or hepatic artery infusion to treat metastatic liver cancer, did TNF-α begin to live up to the expectations raised by preclinical studies, but responses were short-lived. However, the eradication of disease observed when TNF-α was used to treat ascitic xenografts of ovarian cancer in nude mice was paralleled in humans with refractory malignant ascites from a variety of adenocarcinomas, although this treatment was merely palliative.

More recently, impressive results resembling the observed necrosis of animal tumours have been reported in multicentre trials of isolated limb perfusion in localized melanomas, sarcomas and squamous cell carcinomas. High-dose TNF-α (3–4 mg, i.e. 20 times the maximal tolerated dose) was combined with interferon-γ, the cytotoxic drug melphalan and hyperthermia of the affected limb in a 90 min perfusion. Complete and sustained regression was achieved in 44 out of 49 patients with in-transit melanoma metastases, 11 out of 22 patients with soft tissue sarcomas, and all of three patients with carcinomas. TNF-α has since been licensed by the European Medicine Evaluation Agency (EMEA) for this application.

Novel strategies to extend the therapeutic use of TNF-α in the treatment of cancer are being developed, including isolated hypoxic hepatic perfusion using balloon catheters, the generation of TNF-α variants with reduced systemic toxicity and increased antitumour activity, liposomal delivery of TNF-α and the systemic application of low-dose TNF-α in combination with liposomal anti-tumour drug formulations [31,32]. After almost having been written off for cancer therapy, TNF-α has thus recently made a surprising comeback and in combination with more conventional therapies now appears likely to bring considerable benefit to cancer patients in the forseeable future.

Inhibition of NF-κB to induce apoptosis and inhibit multidrug resistance

Combination chemotherapy appears to be the anti-cancer strategy that is most likely to succeed in destroying tumours (rather than merely delaying their progression) because of the enormous genetic instability of tumour cells, and the fact that the vast majority of human cancers rely on more than one genetic alteration to achieve uncontrolled, invasive growth and the suppression of apoptosis. Although most tumours might indeed be clonal in origin, having arisen from the accumulation of a series of somatic mutations that started in a single cell (a paradigm that has recently been challenged by some researchers in the field [33]), the daughter cells of this original tumour cell are likely to suffer varying further genetic lesions that result in a highly heterogenous population of cells forming the tumour. Unless there exists one specific oncogenic lesion on which all of these cells depend for growth and survival

– a sort of Achilles' heel of the tumour cells – a single drug acting on a single target is unlikely to result in the destruction of the tumour.

Combination therapy

The increasing evidence of a role for NF-κB in the suppression of apoptosis and observations that this transcription factor is activated by several apoptosis-inducing drugs, as well as ionizing radiation, has inspired research groups around the world to investigate the potential for NF-κB inhibitors in combination with chemo- and radiotherapy. Baldwin and colleagues found that inducible chemoresistance to CPT-11 (irinotecan, a topoisomerase inhibitor) in human fibrosarcoma and colorectal cancer cell lines, as well as xenograft models and to gemcitabine in non-small lung cancer cell lines, is overcome by transient inhibition of NF-κB through adenovirus-mediated expression of a degradation-resistant mutant of IκB [34–36]. Treatment with a combination of CPT-11 and the proteasome inhibitor PS-341, which inhibits IκB degradation, had the same effect in colorectal cancer cell lines and xenografts resulting in an increase in apoptosis from 10% to >80% and a 94% decrease in tumour size [37]. Similar observations were made by Japanese researchers when human lung adenocarcinoma cells were treated with a combination of paclitaxel and the proteasome inhibitor PS1 [38]. Proteasome inhibition by PS-341 or direct NF-κB inhibition by expression of degradation-resistant IκB was also shown to enhance radiosensitivity in the fibrosarcoma and the colorectal cancer model systems [39,40]. Furthermore, a German group recently published that inhibition of NF-κB by small-molecule inhibitors or degradation-resistant IκB sensitizes human pancreatic carcinoma cells to apoptosis induced by etoposide or doxorubicin [41].

p53 Status

The success of apoptosis induction, however, might depend on the p53 status of the tumour cells. Apoptosis induction by several chemotherapeutic compounds appears to depend on functional p53 [42,43] and p53 has been shown to require NF-κB for the induction of apoptosis [44]. Thus, NF-κB could also have pro-apoptotic effects depending on the context of its activation. This might limit the use of such combination therapy to compounds that do not have an absolute requirement for the presence of functional p53, and to tumours in which p53 has become defective (which appears to be the majority), but certainly does not invalidate this approach as demonstrated by the results described here.

Drug resistance

Because the expression of multidrug resistance P-glycoproteins appears to be NF-κB-dependent [45,46], inhibitors of this transcription factor used in cancer chemotherapy could be predicted to have the additional desirable effect of helping to prevent or overcome multidrug resistance. CML patients taking Gleevec, the recently approved Bcr–Abl protein tyrosine-kinase inhibitor, might also benefit from taking an additional drug that inhibits NF-κB because Bcr–Abl depends on this transcription factor for suppression of apoptosis and because it has recently been found that patients frequently develop resistance to Gleevec as the disease progresses [18,47,48].

TNF-α family members and cachexia

The promising results with TNF-α as an anti-cancer therapeutic agent in high-dose localized perfusion treatment, and the finding that both the pro-inflammatory and the anti-apoptotic effects of this cytokine are dependent on NF-κB activation, suggest that it might be possible to reduce the high TNF-α concentrations so far required to induce cell death in tumour cells. This should alleviate the severe side effects of systemic TNF-α treatment by co-administration with an NF-κB inhibitor. In late-stage cancer patients, the wasting syndrome known as cancer cachexia often results in death before the tumour and its metastases have actually destroyed vital organs. High serum-levels of TNF-α are thought to have a role in the muscle wasting associated with cachexia [49]. Moreover, inhibition of NF-κB has been shown to inhibit cachexia in a mouse tumour model [50]. Baldwin's team recently discovered that TNF-α stimulates NF-κB-induced loss of the mRNA that encodes the myogenic transcription factor MyoD, which causes muscle decay in mice [51]. Treatment with NF-κB inhibitors might, therefore, improve the quality of life and increase the survival times of late-stage cancer patients. Judging from Coley's findings more than a century ago, and from the results of Baldwin and co-workers more recently, NF-κB inhibition in these patients might even result in tumour regression because of increased tumour cell apoptosis induced by the high systemic levels of TNF-α.

Combination of TRAIL with NF-κB inhibitors

The recently identified TNF-α family member, TNF-α-related apoptosis inducing ligand (TRAIL), has been found to elicit apoptosis in a variety of human cancer cell lines and xenografts independent of p53 status, but not in normal cell lines and is, therefore, being investigated in clinical trials as a potential anti-cancer agent [52–54]. Most breast cancer cell lines have shown resistance to TRAIL-induced apoptosis. This resistance has recently been overcome by the combination of TRAIL with inhibition of NF-κB, which is activated by TRAIL in these cell lines [55]. This finding suggests that combination therapy with an NF-κB inhibitor

could increase the clinical efficacy of TRAIL.

Combination of TNF-α with matrix metalloproteinase inhibitors

A recent publication suggests that using suitable combination therapy, the systemic use of TNF-α as an anti-cancer agent might in future become possible. The authors showed that in a mouse model of TNF-α-induced lethal hepatitis, the inhibition of matrix metalloproteinases (MMPs) using batimastat enabled the animals to tolerate TNF-α treatment, which, in combination with this MMP inhibitor, resulted in complete cancer regression in tumour-bearing mice without causing lethality [56]. TNF-α induces MMP gene expression through the activation of NF-κB [57,58].

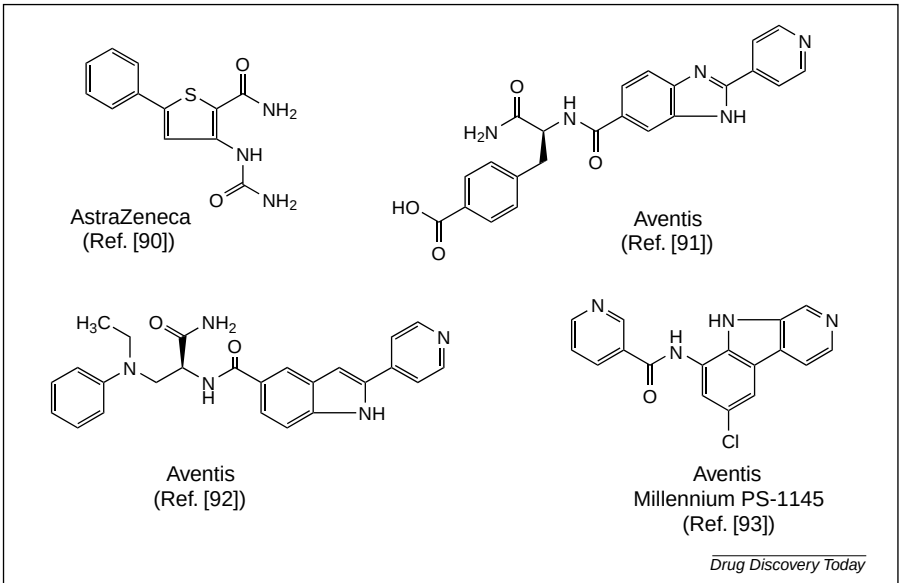

Figure 2. Structures of IKK inhibitors in preclinical development disclosed in patent applications (publication number in brackets; Source: http://www.DailyDrugNews.com).

Small-molecule inhibitors of NF-κB

IKK inhibitors

Most of the large pharmaceutical companies and several biotech companies are running programs to discover and develop small-molecule inhibitors of NF-κB. The most popular target in the signal transduction pathway leading to NF-κB activation (Fig. 1) appears to be IKK [59]. As explained previously, this serine/threonine protein kinase marks IκB for degradation by the proteasome. However, there are also companies that have identified inhibitors of the proteasome itself, which, not surprisingly, have an effect on NF-κB activation. In Fig. 2, the disclosed structures of IKK inhibitors are shown and Table 1 lists their source and properties.

GSK-3β and other kinases

IκB phosphorylation and degradation are not the only events in the activation of NF-κB that can potentially be targeted by small-molecule inhibitors. It is becoming increasingly clear that there are other protein kinases downstream of IKK that phosphorylate NF-κB itself and that are required for maximum activity of the transcription factor. Some of these kinases, such as the protein tyrosine kinases involved in the regulation of NF-κB [60], still remain to be identified. Recently, however, it has been shown by knockout analysis that glycogen synthase kinase-3β (GSK-3β), better known for its function in the regulation of blood-glucose levels and its role in the β-catenin/Wnt signaling pathway, is also necessary for the activation of NF-κB by TNF-α [61]. It has, however, not yet been published whether GSK-3β, which appears to regulate NF-κB DNA binding, can phosphorylate the transcription factor directly or whether the kinase activates other proteins that enhance NF-κB DNA binding. Because the specific inhibition of GSK-3β can result in the stabilization of β-catenin and might consequently lead to tumour formation [62], this kinase has, until recently, generally not been regarded as a drug target with great potential. With the recent determination of the structure of GSK-3β [63,64] and the analysis of regulation of the catalytic activity of this protein kinase, the situation now appears to be different.

Results from Sir Philip Cohen's group (MRC Protein Phosphorylation Unit, University of Dundee, UK) suggest that inhibition of GSK-3β does not inevitably have to lead to β-catenin stabilization because it might be possible to find compounds that inhibit the phosphorylation of some GSK-3β targets but not others [65]. This could be because some substrates, such as glycogen synthase but apparently not axin, are only recognized by GSK-3β if they are 'primed', that is, already carry a phosphate residue on a specific amino acid that is recognized by GSK-3β and, at the same time, orientates the substrate relative to the kinase in a way that allows efficient phosphorylation on GSK-3β target sites. The 'priming phosphate' binding pocket of GSK-3β also recognizes serine residue 9 within the N-terminal pseudosubstrate inhibitory domain of GSK-3β after it has been phosphorylated by Akt, MAPKAP-K1 (MAP kinase-activated protein kinase 1) or $p70^{S6K}$, resulting in inhibition of phosphorylation of all substrates by GSK-3β.

Table 1. Inhibitors of protein kinases of the NF-κB pathway in development

Compound names	Chemical class	Source	Properties
IKK inhibitors			
Unknown	Heterocyclic carboxamides	AstraZeneca [91]	Unknown
Unknown	Substituted benzimidazoles	Aventis [92]	IC_{50} = 7 μM Not active against PKA, PKC and CKII at 100 mM
Unknown	Substituted indoles	Aventis [93]	IC_{50} = 1 μM Not active against PKA, PKC and CKII at 100 mM
PS-1145	β-Carbolines	Aventis/ Millennium [94]	IC_{50} = 125 nM Delayed graft rejection in mouse heterotopic cardiac transplant model at 25 mg kg^{-1} day^{-1} Treatment of multiple myeloma cells with 10 mM for 90 min blocked TNF-α-induced IκB phosphorylation and NF-κB activation Inhibited proliferation and stimulated TNF-α-induced apoptosis
SPC0023579	Unknown	Celgene/Serono	IC_{50} = 10 mM for (ICAM-1) expression
SPC839/ AS602868 (AS2868)	Unknown		IC_{50} = 60 nM, IC_{50} = 1 mM for ICAM-1 expression Inhibition of lipopolysaccharide-stimulated TNF-α production with an ED_{50} of 30 mg kg^{-1} p.o. Active in a mouse collagen-induced arthritis model at oral doses of 15 or 30 mg kg^{-1} day^{-1}
NVPIKK004	Unknown	Novartis	ATP-competitive. Higher selectivity for IKK-1 over IKK-2 and for IKK over p38 and JNK Inhibit IκB degradation in cells as well as lipopolysaccharide stimulated TNF-α release in rats and thioglycollate-induced peritonitis in mice
NVPIKK005	Unknown		
GSK-3 inhibitors			
SB410111 SB495052 SB517955 SB216763 SB415286	Maleimides	GlaxoSmithKline [95,96]	*In vitro* IC_{50} ranging from 11–80 nM, respectively. SB216763 and SB415286 stimulate glycogen synthesis in human liver cells and expression of β-catenin regulated genes
Unknown	Diamino-1,2,4-triazole-carboxylic acid derivatives and 2,5-dihydro-1H-pyrrole-2,5-dione derivatives	GlaxoSmithKline [97,98]	Unknown
Unknown	Diaminothiazoles	NovoNordisk [99]	IC_{50} below 5 μM
CT98014 CT98023 CT99021	Bicyclic compounds, pyrazine derivatives, pyrimidine- or pyridine derivatives, and purine derivatives	Chiron [100–103]	Reduced glucose levels in mouse and rat models of diabetes at 16–48 mg kg^{-1} once or twice daily before glucose challenge. 30 mg kg^{-1} CT98023 given orally to 9–10-week-old Zucker diabetic fatty rats (ZDF) resulted in a twofold increase in liver but not muscle glycogen CT98014 IC_{50} = 50–100 nM for activation of glucose synthase in CHO-IR cells incubated for 30 min. Enhanced insulin-stimulated glucose transport in isolated skeletal muscle of ZDF rats
Unknown	2-amino-3-(alkyl)-pyrimidone derivatives	Sanofi/Mitsubishi [104]	Unknown
Unknown	1H-imidazol-4-amine derivatives	Pfizer [105]	Unknown
Unknown	3-indolyl-4-phenyl-1H-pyrrole-2,5-dione derivatives	Roche [106]	Also inhibits cyclin-dependent kinases 2 and 5

Sources: http://www.dailydrugnews.com, the Investigational Drugs database (http://www.iddb.com), and http://www.esp@cenet.com. Abbreviation: CHO-IR, Chinese hamster ovary cells overexpressing the insulin receptor.

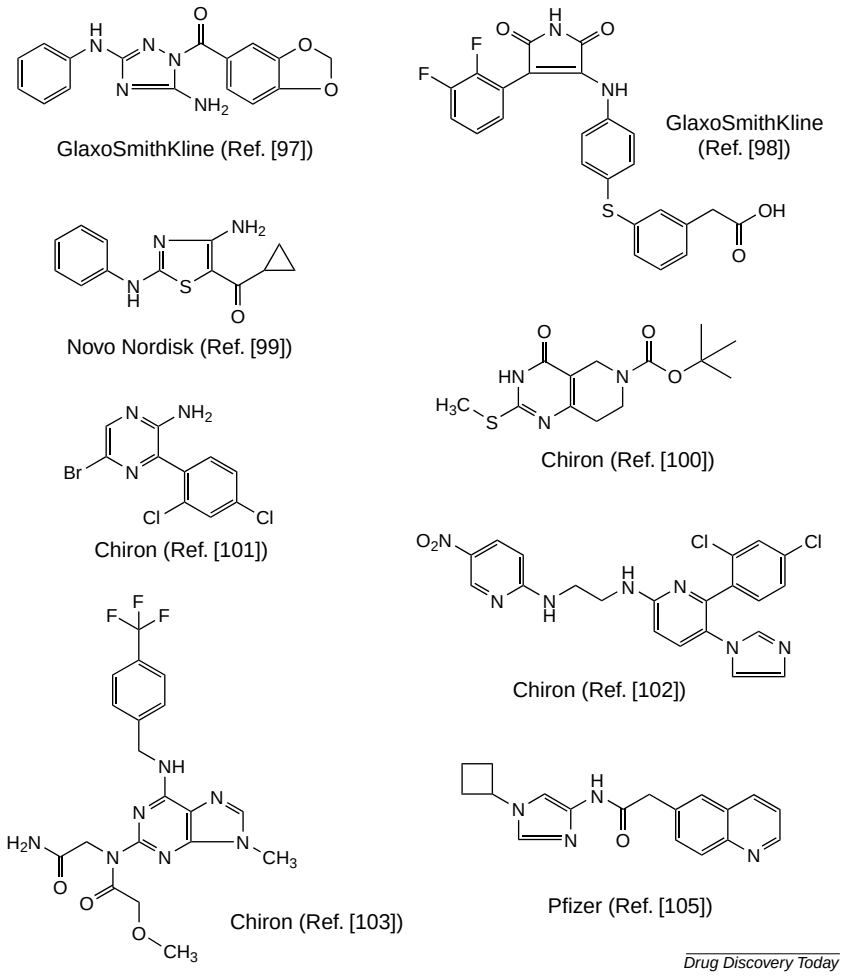

Figure 3. Structures of GSK-3 inhibitors in preclinical development disclosed in patent applications (publication number in brackets; Source: http://www.DailyDrugNews.com).

The reason for this is that the pseudosubstrate domain bound to the priming-phosphate binding pocket prevents access to the catalytic site of GSK-3β. Therefore, blocking priming-phosphate binding to GSK-3β by a small molecule might be a strategy to specifically inhibit glycogen synthase phosphorylation without affecting the phosphorylation of axin, thus circumventing the potential problem of promoting tumorigenesis. Recently published results suggest that *in vivo*, in contrast to *in vitro*, phosphorylation of β-catenin by GSK-3β also requires priming. The authors, therefore, dispute the validity of this strategy [66].

GlaxoSmithKline (Greenford, UK), Novo Nordisk (Bagsvaerd, Denmark), Chiron (Emeryville, CA, USA), Vertex (Cambridge, MA, USA), Roche (Basel, Switzerland) and Pfizer (Sandwich, UK) have already identified small-molecule inhibitors of GSK-3β as potential drugs for diabetes and neurodegenerative disorders such as Alzheimer's disease (Fig. 3 and Table 1), in which phosphorylation events mediated by GSK-3β appear to have a causative effect, as well as affective disorders such as manic depression in which lithium, an inhibitor of GSK-3β, has long been used as a therapeutic agent. It seems likely that these compounds might also show efficacy in models of TNF-α-induced inflammation and in combination with TNF-α in tumour models.

Other kinases downstream of IKK capable of inducing NF-κB transcriptional activity are casein kinase II [67], protein kinase A [68], protein kinase Cζ [69] and T2K [70]. Even IKK itself has recently been shown not only to phosphorylate IκB, but also the p65 subunit of NF-κB resulting in enhanced transactivation. This indicates that the regulatory role of this kinase in the NF-κB signal transduction pathway is rather more complex than initially envisioned [71,72].

Natural products

Apart from the small molecules recently identified by the pharmaceutical and biotech industry in screens for inhibitors of NF-κB, there are several natural products and other compounds, some of them already well established on the market, which have not been discovered as NF-κB inhibitors but have later been found to inhibit this transcription factor. Most prominently, these are aspirin (acetyl salicylic acid), sodium salicylate and sulfasalazine {5-[4-(2-pyridylsulfamoyl)phenylazo]salicylic acid}. These anti-inflammatory compounds have been shown to act as ATP-competitive inhibitors of IKK *in vitro* and *in vivo* [73,74], albeit only at micromolar concentrations. Whereas sulfasalazine was found to inhibit both IKKα and IKKβ, aspirin and sodium salicylate were reported to specifically inhibit IKKβ. This has since been challenged, as has the idea that IKK is their cellular target in the inhibition of cytokine-induced NF-κB activation [75].

Two other well known non-steroidal anti-inflammatory drugs that are now known to inhibit NF-κB are ibuprofen and sulindac. Both compounds have been found to reduce cellular IKK activity [76,77]. Moreover, sulindac was reported to inhibit *in vitro* IKKβ, but not IKKα, protein kinase activity, suggesting that it might be possible to find

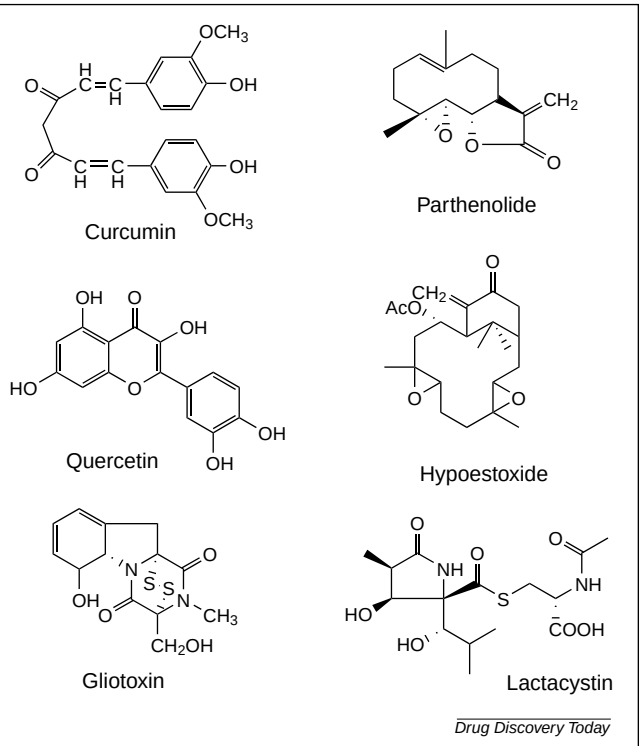

Figure 4. Selected natural product NF-κB inhibitors of plant and microbial origin.

isoform-specific inhibitors of IKK. Even thalidomide, withdrawn as a sedative for causing an epidemic of birth defects in the 1950s but later revived because of its anti-inflammatory effect in erythema nodosum leprosum, is an inhibitor of cellular IKK activity [78], and immunomodulatory derivatives have now entered clinical trials for cancer and a variety of inflammatory diseases [79].

Several plant natural products other than aspirin have also been shown to inhibit IKK [80–83], such as curcumin, parthenolide, quercetin and, most recently, hypoestoxide (Fig. 4). Curcumin is an anti-tumour and anti-inflammatory polyphenol from tumeric (*Curcuma* spp.). It is not a specific inhibitor of this kinase but also inhibits other protein kinases such as the epidermal growth factor receptor [84]. Parthenolide is a sesquiterpene lactone from feverfew (*Tanacetum parthenium*). Quercetin is a flavonoid found in many plants. It is not a specific inhibitor of IKK because it has been reported to inhibit other kinases such as phosphatidylinositol 3-kinase [85]. Hypoestoxide is a novel diterpene IKK inhibitor from *Hypoestes rosea*, a member of the *Acanthaceae* family used in Nigerian folk medicine. This compound has been reported to inhibit the production of pro-inflammatory cytokines and nitric oxide in human cells, and paw edema and ear inflammation in mice after oral administration.

The fact that a variety of natural products and drugs with anti-inflammatory properties target IKK, suggesting that at least part of their activities result from inhibition of this enzyme, is intriguing and could be taken as proof-of-principle for IKK being a promising anti-inflammatory drug target in the NF-κB pathway. Because these compounds were not designed to be inhibitors of IKK, however, there is still considerable scope for the discovery of more specific and potent inhibitors of IKK in chemical libraries. In addition to plant natural product inhibitors of IKK, there are small molecules derived from bacterial or fungal sources that have been shown to inhibit IκB degradation by acting on the proteasome (Fig. 4). Lactacystin is a β-lactone from *Streptomyces lactacystinaeus* [86], and gliotoxin is a secondary metabolite of the fungus *Aspergillus fumigatus* [87,88]. It is not a specific proteasome inhibitor because it also inhibits farnesyl transferase [89].

With the exception of the Millennium (Cambridge, MA, USA) proteasome inhibitor, PS-519, which is based on lactacystin, none of the small-molecule inhibitors of NF-κB currently in development in the pharmaceutical and the biotech industry is derived from the natural products described here. This, however, does not mean that these molecules could not provide lead compounds for the synthesis of more potent and specific inhibitors of this transcription factor in the future. Moreover, such compounds might already exist in nature awaiting discovery. There are indeed many more natural or synthetic small molecules that have been shown to inhibit NF-κB. A large group of these are antioxidants such as caffeic acid phenethyl ester (CAPE) – an anti-carcinogenic, anti-inflammatory and immunomodulatory component of honeybee (*Apis mellifera*) propolis – and *N*-acetyl-L-cysteine. These compounds are not discussed here because an extensive review covering almost all known inhibitors of NF-κB was published in 1999 by Epinat and Gilmore [90].

What the future might hold for NF-κB inhibitors in cancer therapy

The signal transduction pathways leading to the activation of NF-κB are increasingly being recognized by biotech companies [e.g. Celgene Signal Research Division (Warren, NJ, USA), in collaboration with Serono (Geneva, Switzerland)] and pharmaceutical companies (e.g. Aventis; Strasbourg, France), as targets for the development of anti-inflammatory and anti-cancer drugs. Specific and potent inhibitors of the NF-κB activating kinase IKK have been identified by Celgene Signal Research Division, Millennium Pharmaceuticals, AstraZeneca (Macclesfield, UK) Aventis, and Novartis (Basel, Switzerland), but they are all still in the preclinical phase of development (Fig. 2 and Table 1).

The findings that, on the one hand, TNF-α is secreted locally in the environment of many tumours, can act as a tumour promoter and when reaching high serum concentrations is involved in cancer cachexia, and, on the other hand, is well known to destroy some tumours appears paradoxical. A probable explanation is that the cancer-promoting effect of TNF-α requires tumour cells to have acquired resistance to the apoptosis-promoting action of this cytokine. The upregulation of NF-κB seen in the course of progression of many tumours is likely to be an important mechanism by which cancer cells become resistant to TNF-α-induced apoptosis because it is by activation of this transcription factor that this cytokine sends an anti-apoptotic signal. A pro-apoptotic signal is sent by TNF-α via the activation of caspases. Inhibition of NF-κB in tumour cells could, therefore, turn cancer-promoting into cancer-killing TNF-α.

Academic research groups are increasingly beginning to realize the potential of their discoveries in the field of NF-κB research for anti-cancer drug discovery, as shown by the large number of filings for a patent by academic institutions that mention this application. For example, in a recent patent application (WO0147533) on the inhibition of GSK-3β, the authors (Hoeflich *et al.*) mention 'enhanced killing of tumour cells through the sensitizing action of GSK-3β inhibition, when administered with apoptosis-inducing ligands'. In another patent application (US6171786), researchers from the University of Illinois (Chicago, IL, USA) have asked for patent protection for methods for preventing multidrug resistance in cancer cells, which includes the inhibition of NF-κB.

It is, therefore, predictable that small anti-inflammatory molecules that inhibit NF-κB activity will be tested in future in models of human cancer either alone, or more probably in combination with established chemotherapeutic drugs, radiation therapy, or experimental biopharmaceuticals such as TRAIL. The inhibition of NF-κB might even revive TNF-α treatment as a more general anti-cancer therapy. Therefore, there is reason for hope that our increasing understanding of the link between inflammation and cancer will be translated into more effective combinations of drugs that are urgently needed by cancer patients.

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