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REVIEW

Inhibitory kappa B kinases as targets for pharmacological regulation

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The inhibitory kappa B kinases (IKKs) are well recognized as key regulators of the nuclear factor kappa B (NF-κB) cascade and as such represent a point of convergence for many extracellular agents that activate this pathway. The IKKs generally serve to transduce pro-inflammatory and growth stimulating signals that contribute to major cellular processes but also play a key role in the pathogenesis of a number of human diseases. Therefore, the catalytic IKKs represent attractive targets for intervention with small molecule kinase inhibitors. IKK isoforms are assembled as variable multi-subunit IKK complexes that regulate not only NF-κB dimers, but also protein substrates out-with this cascade. Consequently, close consideration of how these individual complexes transduce extracellular signals and more importantly what impact small molecule inhibitors of the IKKs have on functional outcomes are demanded. A number of adenosine triphosphate (ATP)-competitive IKKβ-selective inhibitors have been developed but have demonstrated a lack of activity against IKKα. A number of these chemicals have also exhibited detrimental outcomes such as cellular toxicity and immuno-suppression. The impact of small molecule inhibitors of IKK catalytic activity will therefore be reappraised, examining the advantages and potential disadvantages to this type of intervention strategy in the treatment of diseases such as arthritis, intestinal inflammation and cancer. Furthermore, we will outline some emerging strategies, particularly the disruption of protein–protein interactions within the IKK complex, as an alternative route towards the development of novel pharmacological agents. Whether these alternatives may negate the limitations of ATP-competitive molecules and potentially avoid the issues of toxicity will be discussed.

Abbreviations

ABIN, A20-binding protein; ADP, adenosine diphosphate; ATM, ataxia telangiectasia mutated; ATP, adenosine triphosphate; βTrCP, β-transducin repeat-containing protein; Bcl2, B-cell leukaemia 2; Bcl-xL, B-cell lymphoma-extra large; CIA, collagen-induced arthritis; CIKS, connection to IKK and SAPK; COPD, chronic obstructive pulmonary disease; COX, cyclo-oxygenase; DMARD, disease-modifying anti-rheumatic drugs; DS, disulfiram; DSS, dextran sulphate sodium; HNSCC, head and neck squamous cell carcinoma; Hsps, heat-shock proteins; HTS, high-throughput screening; IAP, inhibitor of apoptosis; IBD, inflammatory bowel disease; IgG, immunoglobulin G; IKIP, IKK interacting protein; IKK, inhibitory kappa B kinase; IκB, inhibitory of kappa B; IL-1, interleukin-1; IRFs, interferon regulatory factors; JNK, c-Jun N-terminal kinase; KO, knockout; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; Mdm2, murine double minute 2; MEFs, murine embryonic fibroblasts; MEKK, MAP or ERK kinase kinase; MMP, matrix metalloproteinases; NAK, NF-κB-activating kinase; NBD, NEMO-binding domain; NEMO, NF-κB essential modulator; NIK, NF-κB-inducing kinase; NF-κB, nuclear factor kappa B; NLS, nuclear localization sequence; NSAIDs, non-steroidal anti-inflammatory drugs; PGE, prostaglandin E; PMA, phorbol myristoyl acetate; RA, rheumatoid arthritis; RANTES, regulated upon activation, normal T cell expressed and secreted; SAR, structure–activity relationship; SMRT, silencing mediator for retinoic acid and thyroid hormone receptor; SOD, superoxide dismutase; TNBS, trinitrobenzene sulphonic acid; TGF-β, transforming growth factor-β; TRAFs, TNF receptor-associated factors; TBK1, TANK-binding kinase 1; TLR, toll-like receptor; TNF-α, tumour necrosis factor-α; TRAMP, transgenic adenocarcinoma of mouse prostate; UV, ultraviolet

Introduction

In the early 1980s, Sen and Baltimore (1986) embarked on a study to identify nuclear factors required to regulate immunoglobulin G (IgG) gene expression in B cells. One of these bound specifically within the promoter of the Ig κ light chain and believing the factor to be B cell specific, called this nuclear factor bound to the κ site of B cells (NF-κB). This initial seminal work, and the subsequent realization of the ubiquitous nature of this transcription factor, has made NF-κB one of the most intensively studied signalling paradigms in the last 25 years. NF-κB has provided a model for the understanding of inducible membrane to nuclear signalling, in particular linking cytokine receptors to interior signalling platforms. In addition, because of its role in a number of disease conditions including arthritis, cancer, cardiovascular disorders and neurodegeneration, the NF-κB pathway has become a key therapeutic target for drug development.

NF-κB comprises the family of Rel proteins of which there are five members: p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1) and p52/p100 (NF-κB2). These transcription factors mediate signalling from the cell surface to regulate key genes involved in inflammation, cell division and immunity. The basic paradigm characterized in the early 1990s indicated that NF-κB isoforms reside in the cytosol, some as larger precursors, bound to a series of inhibitory proteins called the inhibitory kappa Bs (IκBs). Following stimulation, IκBα underwent phosphorylation, ubiquitination and proteolytic degradation to release NF-κB, allowing it to translocate to the nucleus where it bound to specific κB sites to regulate gene transcription (Figure 1). Identifying the enzyme(s) which mediated phosphorylation of the IκBs proved difficult and not achieved until sometime later through the isolation of the inhibitory kappa B kinases (IKKs), the major regulatory kinases within the pathway. This review focuses on the regulation and functions of these kinases, progress in designing drugs to inhibit

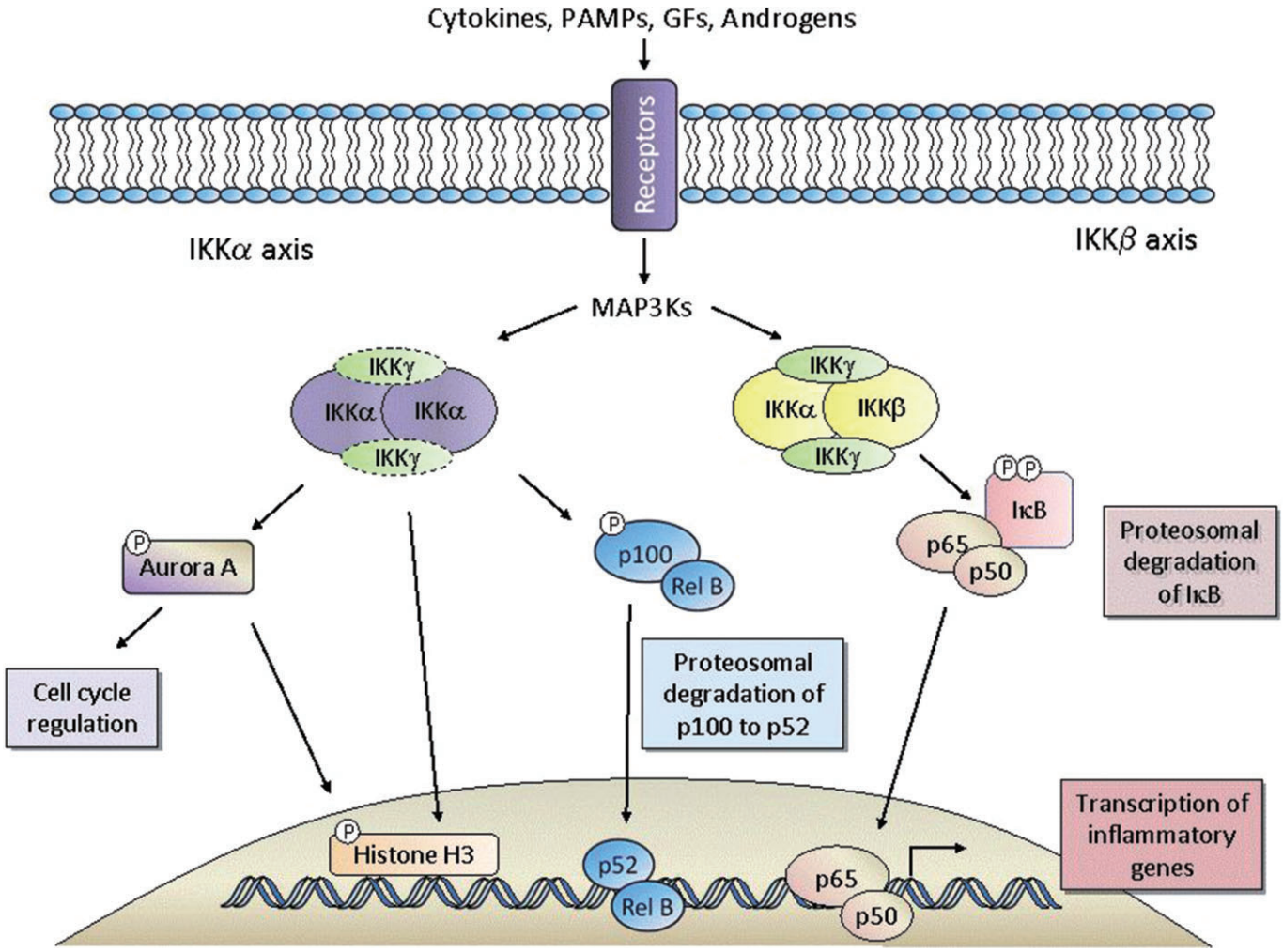

Figure 1

Schematic representation of IKKα and IKKβ-mediated cell signalling encompassing the canonical NF-κB cascade (NEMO dependent —), non-canonical NF-κB cascade (NEMO dependent/independent ---) and substrates outwith these pathways. A variety of extracellular agents; pro-inflammatory cytokines, pathogen-associated molecular profiles (PAMPs), growth factors (GFs) and hormones, in a variety of cell types, display the ability to engage these diverse signalling events leading to gene transcription and chromatin modification that impact on inflammatory responses, cell cycle progression and cell growth/apoptosis.

their activities and the use of these drugs pharmacologically in models of disease.

The role of the IKKs in the regulation of the NF-κB pathway

The IKKs are a series of four enzymes, three of which were initially purified as part of a high-molecular-weight, multi-subunit kinase of approximately 700–900 kDa (Chen *et al.*, 1996). IKKα and IKKβ are the catalytic subunits and share 52% overall sequence homology, with 64% identity across their catalytic domains (Woronicz *et al.*, 1997). IKKγ or NF-κB essential modulator (NEMO) is a 48 kDa non-catalytic protein that plays a scaffolding/regulatory role and is required for kinase function (Rothwarf *et al.*, 1998; Yamaoka *et al.*, 1998). Although IKKβ is the isoform that appears to bind with higher affinity to NEMO, the predominant and most active form of the complex contains one molecule each of IKKα and IKKβ bound to two molecules of IKKγ (Huynh *et al.*, 2000; Miller and Zandi, 2001; May *et al.*, 2002; Rushe *et al.*, 2008). A third IKK known as IKK-i/ε and an IKK-related kinase known as TANK-binding kinase 1 (TBK1)/NF-κB-activating kinase (NAK)/tumour necrosis factor-α (TNF-α) receptor-associated factor 2-associated kinase (T2K) have been isolated but do not function as NF-κB inducing kinases, although have been suggested as potential modulators of p65 transactivation (Buss *et al.*, 2004; Adli and Baldwin, 2006; Wietek *et al.*, 2006). While TBK1 is expressed constitutively and is activated in response to agonist stimulation (Tojima *et al.*, 2000), IKK-i/ε is an inducible enzyme and, once expressed, is constitutively active (Shimada *et al.*, 1999). These enzymes predominantly regulate members of the family of interferon regulatory factors (IRFs) that contribute to specific gene transcription events such as cytokine production and suppression in the context of infection and immunity (Chau *et al.*, 2008). TBK1 has also been implicated in angiogenesis (Korherr *et al.*, 2006; Czabanka *et al.*, 2008), and IKK-i in the constitutive activation of gene transcription in a number of cancer cell lines (Eddy *et al.*, 2005; Adli and Baldwin, 2006).

Activation of the IKK isoforms α and β regulate two divergent NF-κB signalling pathways, the classical canonical pathway and the non-canonical pathway. Each cascade relies on different IKKs for maximal activation, and it is these differences that give rise to the potential for therapeutic intervention and the development of isoform selective inhibitors. The canonical pathway is activated by pro-inflammatory stimuli such as TNF-α, interleukin-1 (IL-1) and lipopolysaccharide (LPS) through the toll-like receptors (TLRs) (Figure 1). This results in the recruitment of a number of well-described adaptor molecules identified as the TNF receptor-associated factors (TRAFs). This facilitates the recruitment of key enzymes such as MAP or ERK kinase kinase 3 (MEKK3) and transforming growth factor-β (TGF-β)-activated kinase 1 (TAK1) which specifically activate IKKβ through phosphorylation of amino residues Ser 177 and Ser 181 within the activation loop (Yang *et al.*, 2001; Schmidt *et al.*, 2003). Activated IKKβ then phosphorylates Ser 32 and Ser 36 of IκBα (or Ser 19 and Ser 23 of IκBβ) (Brown *et al.*, 1995; DiDonato *et al.*, 1996), leading to polyubiquitination and subsequent degradation of IκB by the proteasome. As IκB is usually bound to NF-κB, the nuclear localization sequence (NLS) within the Rel homology domain of NF-κB is masked and as such is retained predominantly in the cytoplasm (there is some evidence for shuttling of NF-κB : IκBα complexes) (Malek *et al.*, 2001). The removal of IκB therefore results in the 'unmasking' of the NLS to induce nuclear translocation of NF-κB and binding to consensus binding sites within promoter regions of specific genes. IKKβ can also mediate phosphorylation of NF-κB p65 at Ser 536 to initiate transactivation leading to increased transcriptional activation following DNA binding (Sakurai *et al.*, 1999).

In parallel to this paradigm of IKK-mediated NF-κB activation, it is also recognized that NF-κB complexes can be mobilized in an atypical, IKK-independent manner, reliant on alternative mechanisms of regulation. The phosphorylation of IκBα at Tyr42 has been reported in response to a variety of stimuli such as treatment with hydrogen peroxide, the tyrosine-phosphatase inhibitor pervanadate, exposure to nerve growth factor and in response to hypoxia and reperfusion (reviewed in Perkins, 2006; Perkins and Gilmore, 2006). Alternative casein kinase-II (CK2)-mediated phosphorylation of IκBα in its C-terminal PEST domain may also lead to NF-κB activation, for example, exposure of cells to ultraviolet (UV) light, or the expression of the erbB2 oncogene in breast cancer cells (reviewed in Perkins, 2006). Therefore, through differential phosphorylation of IκBs, additional mechanisms of activation of NF-κB subunits can be achieved.

The identification of the IKK complex allowed the role of IKKβ within the canonical pathway to be defined very quickly. Studies using dominant-negative mutants of IKKβ and IKKβ knockout (KO) mice confirmed the requirement for this isoform in nuclear translocation and expression of key NF-κB genes such as those involved in inflammation, apoptosis and cell survival (Li *et al.*, 1999; Tanaka *et al.*, 1999). In fact, the inhibition of IKKβ in a variety of cells has now identified clearly the key role this isoform has in regulating survival of cells and protecting against cellular apoptosis (Mustapha *et al.*, 2000; Wullaert *et al.*, 2011). However, defining the role of IKKα in regulation of NF-κB dependent transcription was more challenging. Although inhibition or gene deletion of IKKα revealed no positive regulatory role in IκBα degradation and subsequent NF-κB translocation, no other clear mechanistic function was apparent. In fact, initial studies implicated IKKα as a potential negative regulator of IKKβ and IKK complex catalytic activity (O'Mahony *et al.*, 2000; Lawrence *et al.*, 2005; Li *et al.*, 2005). Nevertheless, studies showed that IKKα KO mice have a phenotype distinct from those of the IKKβ KO, which indicated a unique cellular function for IKKα (Takeda *et al.*, 1999; Hu *et al.*, 2001).

These KO studies and a closer investigation of NF-κB isoforms have identified IKKα as a key component in the alternative, non-canonical pathway. In this model, albeit demonstrated in very few systems (Matsushima *et al.*, 2001; Dejardin *et al.*, 2002), NF-κB precursors of higher molecular weight (e.g. p100) are processed to generate other lower-molecular-weight NF-κB isoforms (e.g. p52). Another MEKK distinct from MEKK3, namely NFκB-inducing kinase (NIK), first phosphorylates IKKα on Ser 176 (Ling *et al.*, 1998). IKKα then, in turn, phosphorylates p100 at multiple sites (serines 99, 108, 115, 123 and 872) (Xiao *et al.*, 2004) which target it for ubiquitination and proteolytical processing to p52. In support of this model, IKKα$^{-/-}$ B cells show defective process-

ing of p100, while the constitutive cleavage of p105 is unchanged (Senftleben *et al.*, 2001). The nuclear translocation of p52 : RelB heterodimers results in the transcription of genes involved in B cell maturation and lymphoid organogenesis. The composition of NF-κB dimers in this system, which is distinct from p65, ultimately leads to a different pattern of gene transcription which is cell-type specific.

More recently, IKKα has also been shown to participate in the canonical pathway via a novel nuclear mechanism. After TNF-α stimulation, IKKα translocates to the nucleus where it plays a role in modulating gene expression through the phosphorylation of histone H3 (Anest *et al.*, 2003; Yamamoto *et al.*, 2003). In addition, IKKα has been shown to modulate NF-κB gene expression by regulating silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) derepression (Hoberg *et al.*, 2004), influence cell cycle progression via phosphorylation of Aurora A (Prajapati *et al.*, 2006), and regulate mammary gland development (Cao *et al.*, 2001) and angiogenesis (Agarwal *et al.*, 2004). Interestingly, expression of a kinase inactive IKKα mutant in IKKα$^{-/-}$ murine embryonic fibroblasts (MEFs) rescued expression of a subset of NF-κB genes, suggesting that the catalytic activity of IKKα is not always essential (Massa *et al.*, 2005). Further evidence for this has been provided from studies that show IKKα to be essential for keratinocyte differentiation, independent of its kinase activity (Hu *et al.*, 2001; Sil *et al.*, 2004).

Thus, despite also functioning within the canonical NF-κB pathway, IKKα can nevertheless initiate a distinct pattern of gene expression overlapping with, but distinct to, that induced by IKKβ. This again presents IKKα as a potential target for drug development. However, despite the opportunity to develop isoform selective inhibitors, IKKβ has thus far proven to be the more tractable target.

The development of novel small molecule inhibitors of the IKKs

Based on early studies that identified IKKβ as the key driver of classical NF-κB signalling, large pharmaceutical companies have developed diverse, large-scale, high-throughput screening (HTS) programmes encompassing hit-to-lead development and characterization of structure–activity relationships (SAR), all in the absence of resolved crystal structures for IKKα and β. This has led to a number of chemical entities of relatively low molecular weight with drug-like features that commonly function as IKKβ selective inhibitors (see Table 1). The majority of these compounds perform as adenosine triphosphate (ATP)-competitive molecules or alternatively possess allosteric action to limit IKK activities (see Table 1). Furthermore, a number of these molecules have been pursued *in vivo* in animal models of disease. In short, full characterization of mechanism of action related to potency *in vitro*, efficacy in cell-based experiments and animal studies have been requisite to their potential movement from preclinical analysis to patient trials, although, to date, use of these inhibitors clinically has yet to be reported.

Well-recognized and thoroughly studied IKK inhibitors, depicted in Figure 2, include Bayer 'Compound A' (1), PS-1145 (2) and related ML120B (3) based on a β-carboline scaffold, thiophene carboximides such as SC-514 (4) and TPCA1 (5), diarylbenzamides (6), hydroxybenzamide compounds [e.g. IMD-03; (7)] and the more recently developed GlaxoSmithKline (GSK) classes of alkoxyisoquinoline (8) and azaindoles (9). Interestingly, both Bayer 'Compound A' and TPCA1 also display activity against IKKα albeit with 100-fold and 10-fold selectivity, respectively, toward IKKβ (see Table 1). The Bristol Myers Squibb (BMS) compound BMS-345541 (10) also displays action against both IKKα and IKKβ, displaying 10-fold selectivity towards IKKβ (see Table 1). Interestingly however, it possesses very different pharmacology. BMS-345541 acts as an allosteric inhibitor of both IKKα and IKKβ (see Table 1), binding to IKKβ is in a non-mutually exclusive manner with respect to adenosine diphosphate (ADP), while binding to IKKα has a secondary influence on the active site and therefore effects ATP binding (Burke *et al.*, 2003).

Collectively, these inhibitors, perhaps expectedly, block numerous agonist-stimulated gene transcription events linked to IKKβ-NF-κB activation. This includes regulated upon activation, normal T cell expressed and secreted (RANTES) protein and monocyte chemoattractant proteins (MCPs) in synoviocytes and matrix metalloproteinases (MMPs) in chondrocytes which are related to cellular changes and disease onset in arthritis/joint destruction (see Section Arthritis), the production of cytokines such as TNF-α and ILs 1, 6 and 8 in monocytic cells which are linked to inflammation (see Section Intestinal Inflammation) and the modulation of cell cycle regulators in tumour development (see Section Cancer). However, only a subset of these inhibitors has been utilized in studies *in vivo*, although the introduction of these molecules into ever-diversifying models is ongoing (see Table 2). Of particular note is that molecules such as BMS-345541, ML120B and TPCA1 have been efficacious in rodent models of arthritis. Both BMS-345541 and ML120B have been reported to display desirable pharmacokinetics in mice and rats with good oral bioavailability; oral administration dose dependently inhibited both cellular inflammation and associated disease incidence and severity (McIntyre *et al.*, 2003). In contrast TPCA1 was administered intra-peritoneally due to poor oral bioavailability, but also resulted in a reduction in disease severity (Podolin *et al.*, 2005). SC-514 also displayed poor oral bioavailability (Kishore *et al.*, 2003), yet upon intra-peritoneal administration was able to reduce LPS-stimulated TNF-α production.

Aside from HTS strategies with synthetic small molecules, it is also well recognized that natural products may represent a route towards novel pharmacological agents that target the IKKs. For example, wedelolactone [Figure 2, (11)] has been suggested to act as an irreversible inhibitor of both IKKα and IKKβ (Kobori *et al.*, 2003). Another compound, noraristeromycin, has been shown to be a selective inhibitor of IKKα *in vitro* (Asamitsu *et al.*, 2008); however, the assay conditions employed in the study did not exclude an additional effect upon IKKβ. Indeed, apart from a few key examples, the characterization of natural products as IKK inhibitors has been limited, tending to stop short of a proper elucidation of the mechanism of inhibition within the IKK/NF-κB pathway. Nevertheless, the diversity of compounds, both synthetic or natural, and the constituent chemical scaffolds that have been reported to inhibit components of the NF-κB pathway, including but not limited to the IKKs, continue to grow (Bremner and Heinrich, 2002; Gupta *et al.*, 2010a,b; Luqman and Pezzuto, 2010). The Gilmore laboratory (Boston

Table 1

Chemical scaffolds, potency and selectivity of IKK inhibitor molecules characterized in cell-based studies and animal models *in vivo*

Inhibitor molecule	Chemical name/Scaffold	IC_{50s} IKKα vs. IKKβ	Cell-based studies (Refs.)	*In vivo* analysis (Refs.)
Bayer 'Compound A'	2-Amino-3-cyano-4-alkyl-6-(2-hydroxyphenyl)pyridine	135 nM vs. 2 nM	B/T-lymphocytes, HEKs, PBMCs (Murata *et al.*, 2003; 2004a,b)	pulmonary inflammation (Ziegelbauer *et al.*, 2005)
BMS-345541 [Related cmpds.]	(4(2′-aminoethyl)amino-1, 8-dimethylimidazo(1,2-a)quinoxaline)	4000 nM vs. 300 nM [N. D. vs. 10-60 nM]	THP-1 monocytes (Burke *et al.*, 2003); HUVECs (MacMaster *et al.*, 2003); THP-1 monocytes (Beaulieu *et al.*, 2007)	LPS-induced TNFα prod^{n} (Burke *et al.*, 2003); CIA (McIntyre *et al.*, 2003); colitis model (MacMaster *et al.*, 2003); cardiac graft rejection (Townsend *et al.*, 2004)
GSK-'benzamide-8h'	2-Amino-3,5-diarylbenzamide	316 vs. 100 nM	PBMCs (Christopher *et al.*, 2007)	Not determined
GSK-'azaindole-7'	4-phenyl-7-azaindoles	39.8 nM vs. 31.6 nM	A549 cells (Liddle *et al.*, 2009)	Not determined
GSK-'isoquinoline-21'	6-Aryl-7-alkoxyisoquinoline	>25 μM vs. 100 nM	PBMCs(Christopher *et al.*, 2009)	Not determined
IMD-0354	N-(3,5-bis(trifluoromethyl)phenyl)-5-chloro-2-hydroxybenzamide	N. D./250 nM	mast cells (Tanaka *et al.*, 2005)	MIR (Onai *et al.*, 2004; 2007); OLIR (Kamon *et al.*, 2004); colitis model (Hayakawa *et al.*, 2009); asthma model (Ogawa *et al.*, 2011); allergic inflammation (Sugita *et al.*, 2009)
ML120B	*N*-(6-chloro-7-methoxy-9*H*-β-carbolin-8-yl)-2-methyl-nicotinamide β-carboline	>100 000 nM vs. 45 nM	HASMCs (Catley *et al.*, 2006); synoviocytes, chondrocytes, mast cells (Wen *et al.*, 2006)	arthritis model (Schopf *et al.*, 2006)
PS-1145	*N*-(6-chloro-9*H*-β-carbolin-8-yl) nicotinamide β-carboline	>100 000 nM vs. 100 nM	HeLa cells (Castro *et al.*, 2003)	LPS-induced TNFα prod^{n} (Castro *et al.*, 2003)
SC-514 [Related cmpds.]	4-Amino-[2′,3′-bithiophene]-5-carboxamide	>200 000 nM vs. 2.7 μM [N. D. vs.333 nM]	synovial fibroblasts (Kishore *et al.*, 2003); RASMCs (Gomez *et al.*, 2005); synovial fibroblasts (Bonafoux *et al.*, 2005)	Rat model of inflammation (Kishore *et al.*, 2003)
TPCA1	2-[(aminocarbonyl)amino]-5-(4-fluorophenyl)-3-thiophenecarboxamide	400 nM vs. 18 nM	PBMCs (Baxter *et al.*, 2004); PBMCs (Podolin *et al.*, 2005)	CIA (Podolin *et al.*, 2005)
Wedelolactone	7-Methoxy-5,11,12-trihydroxy-coumestan	<10 μM vs. <10 μM	BALB/c fibroblasts, HeLa cells, murine splenocytes (Kobori *et al.*, 2004)	Not determined

ND, none detected.

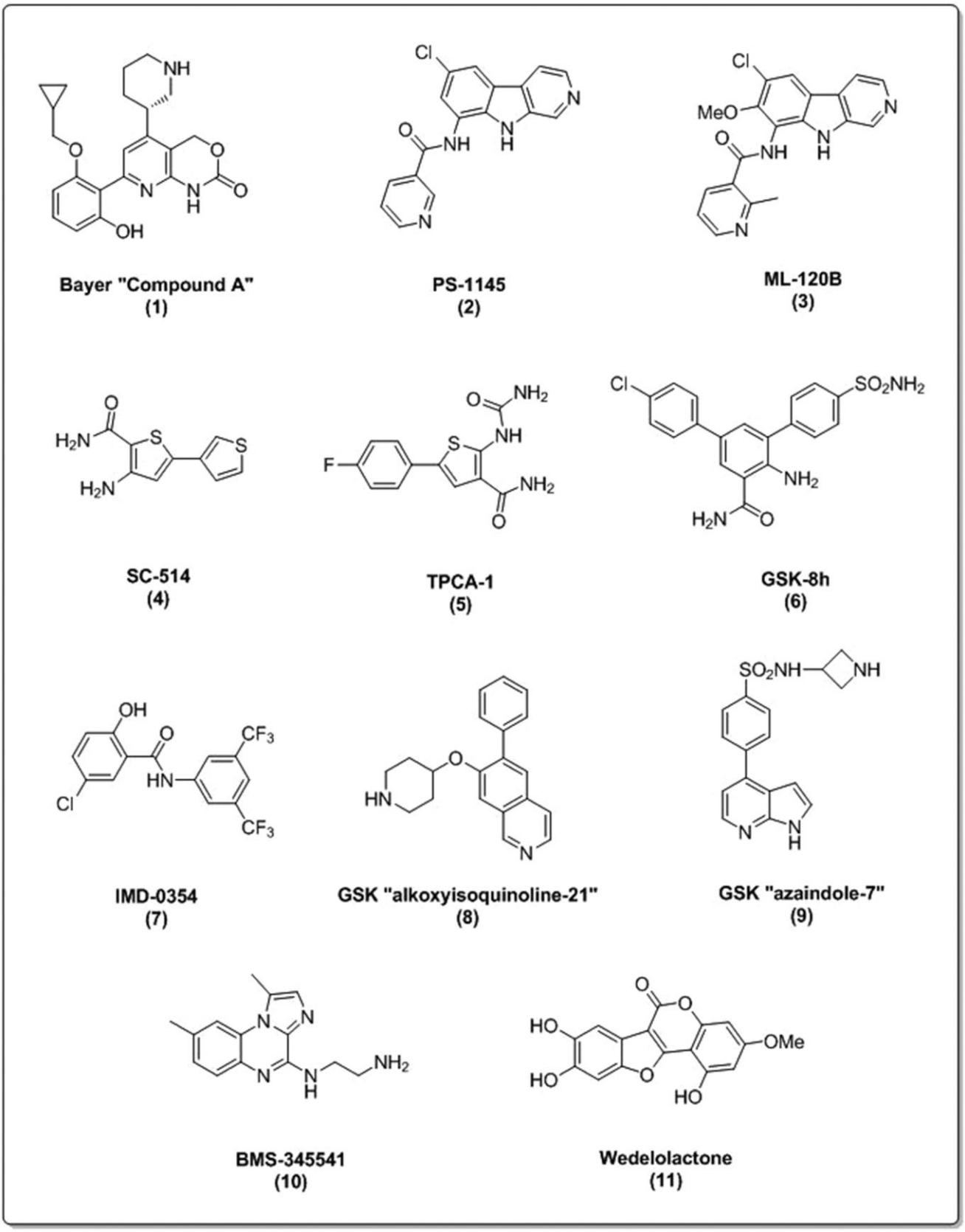

Figure 2
Chemical structures of IKK inhibitors.

Table 2

In vivo effect of cell-permeable peptides targeting the NBD

Peptide	Model	Effect	Reference
Drosophila antennapedia (ANT)-NBD *(drqikiwfqnrrmkwkk*-TALDWSWLQTE)	Mouse model of ear oedema Mouse model of peritonitis	Reduced ear oedema Inhibition of polymorphonuclear cell migration and decreased NO accumulation in the peritoneum	May *et al.* (2000)
ANT-NBD *(drqikiwfqnrrmkwkk*-TALDWSWLQTE)	Cerulein-induced acute pancreatitis in a mouse model	Decreased pancreatic inflammation and associated lung haemorrhage. Inhibited neutrophil sequestration in both the pancreas and lungs.	Ethridge *et al.*, (2002)
ANT-NBD *(drqikiwfqnrrmkwkk*-TALDWSWLQT)	Experimental allergic encephalomyelitis (EAE) in an adoptively transferred mouse model	Inhibited p65 expression and mononuclear cell invasion in the spinal cord. Reduced clinical symptoms of EAE and inhibited progression of the disease	Dasgupta *et al.* (2004)
TAT-NBD (ygrkkrrqrrrg-TTLDWSWLQME)	Mouse model of inflammatory arthritis	Inhibited IKK enzyme activity and NF-κB DNA binding. Inhibited osteoclast formation in the joints and reduced bone erosion	Dai *et al.* (2004)
ANT-NBD *(drqikiwfqnrrmkwkk*-TALDWSWLQTE)	Mouse model of collagen induced rheumatoid arthritis	Inhibited NF-κB DNA binding. Reduced inflammation, cartilage destruction and bone erosion. Inhibited osteoclast formation in the joints.	Jimi *et al.* (2004)
ANT-NBD *(drqikiwfqnrrmkwkk*-TALDWSWLQT)	Carrageenan-induced mouse paw oedema	Reduced NF-κB DNA binding, reduced expression of NF-κB target genes and inhibited oedema formation in a dose-dependent manner	di Meglio *et al.* (2005)
TAT-NBD (ygrkkrrqrrrg-TTLDWSWLQME)	Sodium taurocholate-induced pancreatitis in a rat model	Significantly reduced tissue damage due to a decrease in fatty tissue and parenchymal cell necrosis	Long *et al.* (2009)

University, MA, USA) has compiled extensive listings of similar and related molecules that intervene in IKK-NF-κB signalling/transcription (see www.nf-kb.org) as a resource to aid in the further development of IKK inhibitors.

Targeting IKK inhibition in disease

The well-recognized role of NF-κB in underpinning cellular inflammation has implicated the IKKs as important intermediates involved in a number of disease conditions including asthma, atherosclerosis, neurodegeneration, rheumatoid arthritis (RA) and inflammatory bowel disease (IBD) (Grivennikov *et al.*, 2010). Ideally, the inhibition of NF-κB would therefore be a worthwhile therapeutic strategy; unfortunately, however, there are caveats. NF-κB proteins play a pivotal role in normal physiological functions such as innate immunity and cell survival. Inhibition of NF-κB could therefore have a catastrophic effect upon susceptibility to infection and normal development. Hence, targeting the pathway in some conditions is feasible, but not without problems. In the succeeding discussion, we focus on RA and IBD, as inflammatory conditions in which drug development has the potential to make the greatest immediate impact, and also on cancer, a multifaceted condition in which the IKKs may be targetable.

Arthritis

In RA, up-regulation of IKK activity, predominantly IKKβ, is well recognized (Han *et al.*, 1998). Studies in cultured synovial cells, demonstrating enhanced IKK and NF-κB (Bannai *et al.*, 1996; Roshak *et al.*, 1996; Yamasaki *et al.*, 2001), correlate with studies in human arthritic joints (Danning *et al.*, 2000; Carlsen *et al.*, 2002; Benito *et al.*, 2004) and in animals with experimentally induced arthritis (Tsao *et al.*, 1997; Han *et al.*, 1998). Increased NF-κB is associated with many aspects of disease progression; autoreactive T-cells, B-cell antibody production and macrophage activation may all be increased. Correspondingly, genetic deletion of key IKK-regulated NF-κB subunits, for example p50, can prevent many of these features and consequently stop the development of either collagen- or BSA-induced arthritis (Campbell *et al.*, 2000). Increased IKK/NF-κB activity is also associated with enhanced adhesion molecule expression in the joint synovium, recruitment of lymphocytes, production of inflammatory mediators such as IL-1β and TNF-α, cartilage destruction and pannus formation (Han *et al.*, 1998). Infection with catalytically inactive, dominant-negative variants of IKKβ profoundly inhibited these parameters *in vitro* (Andreakos *et al.*, 2003) and ameliorated adjuvant-induced arthritis *in vivo* (Tak *et al.*, 1999).

Given the potential of NF-κB and IKKβ as therapeutic targets in inflammatory and autoimmune disorders, synthetic

strategies have focussed on the development of highly selective, potent inhibitors of IKKβ. As outlined above, BMS-345541, TPCA1 and ML120B have shown some success in reducing NF-κB activation as well as joint destruction in different animal models (Table 1). In a murine collagen-induced arthritis (CIA) model, the administration of BMS-345541, when initiated in parallel with collagen immunization, reduced arthritic severity and the expression of IL-1β in the joint (McIntyre *et al.*, 2003). When tissue sections from the joints of these rodents were examined histologically, the efficacy of IKKβ inhibitors in this setting was illustrated with a significant reduction in inflammation and cartilage destruction. In the CIA model, TPCA1 has also reduced disease progression in a similar manner to BMS-345541. This inhibitor delayed both onsets and reduced the severity of disease, with an associated decrease in NF-κB p65 nuclear localization and DNA binding. Additionally, administration of TPCA1 resulted in a reduction in the expression levels of NF-κB-regulated cytokines, highlighting the role of IKKβ in NF-κB activation (Podolin *et al.*, 2005). In a related study, oral administration of ML120B to rats in an adjuvant-induced arthritis model resulted in significantly reduced inflammation and bone destruction (Schopf *et al.*, 2006). The use of peptide disruptors of the IKKβ–NEMO interaction have also been an alternative treatment for arthritis (see Section Alternative strategies to target the IKK complex: protein–protein interactions and their disruption & Table 2). These peptides resulted in a reduction in the inflammation of the arthritic joints of rodents, observed as reduced local bone erosion, and the blockade of osteoclastogenesis (Dai *et al.*, 2004; Jimi *et al.*, 2004).

Current therapeutic approaches to RA have been influenced by the intractable nature of the disease. This has necessitated the use of a wide variety of drugs including non-steroidal anti-inflammatory drugs (NSAIDs), steroids, disease-modifying anti-rheumatic drugs (DMARDs) and biologics. While no IKKβ inhibitors have thus far reached the clinic, many of the currently used pharmacological interventions may ascribe their actions at least in part to IKK inhibition. Kopp and colleagues discovered that the cyclooxygenase (COX) inhibitors aspirin and certain salicylates blocked NF-κB activation by inhibiting IKKβ activity (Kopp and Ghosh, 1994). These drugs were shown to function by blocking the ATP binding site (Yin *et al.*, 1998). Further studies examining sulindac, ibuprofen and flurbiprofen demonstrated that they display anti-inflammatory and anti-proliferative effects independently of COX activity and prostaglandin E (PGE) synthesis and, at high doses, inhibit NF-κB by decreasing IKKβ kinase activity (Tegeder *et al.*, 2001). The DMARD sulphasalazine, has been shown to inhibit NF-κB activation induced by TNF-α, LPS or phorbol myristoyl acetate (PMA) (Wahl *et al.*, 1998). This inhibition was associated with suppression of IκBα degradation and phosphorylation, suggesting inhibition of IKKβ. The NSAID mesalamine, which is an aminosalycilate derivative, displayed anti-inflammatory properties and prevented IL-1-mediated stimulation of p65 phosphorylation without inhibiting IκBα degradation (Egan *et al.*, 1999). Steroids, while not inhibiting IKK directly, act at least in part to increase expression of IκBα, thereby retaining NF-κB in the cytoplasm (Auphan *et al.*, 1995; De Bosscher *et al.*, 1997; Thiele *et al.*, 1999). Furthermore, in the murine CIA model, it was found that while the 'gold standard' treatment for RA, methotrexate, was not effective after therapeutic administration, the IKKβ inhibitor BMS-345541 displayed similar anti-inflammatory efficacy to glucocorticoids (McIntyre *et al.*, 2003), suggesting that specific IKKβ inhibitors may be a useful alternative to currently used therapeutics.

Intestinal inflammation

Several studies have also described excessive or inappropriate NF-κB activation in intestinal inflammation, in both Crohn's disease and ulcerative colitis and in animal models of IBD (Neurath *et al.*, 1996; Rogler *et al.*, 1998; Schreiber *et al.*, 1998). Both colonic mucosal biopsies and lamina propria mononuclear cells from patients with IBD have been shown to exhibit increased levels of p65 in the nucleus as compared to healthy controls (Ellis *et al.*, 1998; Schreiber *et al.*, 1998). Initial studies using animal models demonstrated that administration of antisense oligonucleotides directed against p65 were able to abrogate established trinitrobenzene sulphonic acid (TNBS)-induced colitis in mice (Neurath *et al.*, 1996). This was further confirmed by studies using curcumin to inhibit NF-κB in various mouse models of colitis (Jobin *et al.*, 1999; Sugimoto *et al.*, 2002). At the level of IKKβ, treatment of mice with BMS-345541, in a dextran sulphate sodium (DSS)-induced animal model of colitis, countered weight loss and changes in tissue characteristics of the colon, particularly thickening and shortening (MacMaster *et al.*, 2003), which may be representative of IBD-related clinical outcomes. Additionally, an inhibitory peptide directed against the NEMO-binding domain (NBD) of IKKβ was shown to reduce inflammation in TNBS- and DSS-induced colitis and in spontaneous colitis developed in IL-10 deficient mice (Dave *et al.*, 2007; Shibata *et al.*, 2007).

In the clinical setting, targeting intestinal inflammation has generally taken the same approach as that described for arthritis, with the use of DMARDs and steroids. In an intestinal epithelial cell model, sufasalazine and lefunomide have been shown to inhibit NF-κB activation by preventing the phosphorylation of IκBα (Wahl *et al.*, 1998), and by blocking functionally important post-translational modifications of the p65 subunit (Egan *et al.*, 1999), suggesting the potential for inhibition of IKK to be a feature of their mode of action. Steroids have also been proven to be highly effective in the treatment of active IBD, an effect presumed to be mediated, at least in part, by preventing migration of activated NF-κB into the cell nucleus and subsequent binding to DNA (Auphan *et al.*, 1995; Scheinman *et al.*, 1995). Other treatments for IBD (and arthritis) have centred on the use of anti-cytokine biological agents such as infliximab (chimeric anti-human TNF-α monoclonal antibody), adalimumab (recombinant anti-human TNF-α monoclonal antibody) and anakinra (recombinant form of human IL-1 receptor antagonist), which target cytokines whose expression is regulated by NF-κB but which can also themselves activate the NF-κB pathway (Wahl *et al.*, 1998; Podolsky, 2002). Thus, taken together, these studies would suggest that IKK inhibitors could be excellent new therapies for the treatment of both arthritis and IBD, equally as efficacious as current therapeutics (McIntyre *et al.*, 2003; Podolin *et al.*, 2005), without the damaging side effects associated with some of these compounds (Auphan *et al.*, 1995; Scheinman *et al.*, 1995), more

specific in their mode of action than the DMARDs and less likely to encounter resistance in some patient cohorts. However, there are potential problems with cellular and systemic toxicity which need to be addressed. In targeting IKKβ particularly, there may be the potential for significant cell death/apoptosis of normal epithelial and cardiac cells respectively (Mustapha *et al.*, 2000; Wullaert *et al.*, 2011). Effective treatment of inflammatory disorders will require maintaining a delicate balance between suppressing inflammation and interfering with normal cellular functions. Treatments aimed at inhibiting components of the NF-κB pathway in a tissue- or cell-specific manner may have better therapeutic efficacy and reduce systemic toxicity.

Cancer

The early observation that NF-κB could be activated by cellular oncoproteins such as Ras, as well as by a number of viral oncoproteins, suggested that the NF-κB cascade could contribute to the cellular transformation that underpins tumour development (Gilmore, 1999; Valentine *et al.*, 2010). Indeed, a large body of work has identified two features of cellular IKK activation that relates to a potential role in cancer; expression of NF-κB-dependent cell survival genes which play an important anti-apoptotic role and, secondly, a NF-κB-independent role in cell cycle progression. More recently, an additional aspect has emerged, with relevance to NF-κB; a chronic inflammatory state may be of significance in increasing the propensity for tumourigenesis *in vivo* (Tysnes, 2010). This feature is relevant in colorectal, pancreatic and lung cancers (Charalambous *et al.*, 2003; Katoh, 2007; Yang *et al.*, 2007).

Outwith the context of chronic inflammatory state, hyperactivation of the NF-κB pathway has been reported in multiple tumour cell lines and tissue samples. For example, nuclear p65 phosphorylation has been shown to contribute to the malignant phenotype of head and neck cancer, regulating epithelial to mesenchymal transition (Arun *et al.*, 2009) and leading to less favourable clinical outcomes (Chung *et al.*, 2006). Similarly, nuclear NF-κB in histological sections of prostate adenocarciomas was found to be a prognostic marker for disease relapse (Domingo-Domenech *et al.*, 2005). NF-κB-associated genes are also over-expressed in basal breast cancer cells, a highly proliferative hormone-insensitive cell type associated with poor disease prognosis (Bertucci *et al.*, 2009). In tissue samples from patients with pancreatic cancer, components of the non-canonical pathway, p52 and RelB, have been reported to co-localize in the nuclear compartment. Associated cellular studies have suggested that this may be a consequence of upstream NIK stabilization which leads to constitutive p100 phosphorylation (Wharry *et al.*, 2009).

The constitutive activation of the IKKs themselves have also been observed in various tumour-derived cell lines (Romieu-Mourez *et al.*, 2001; Gasparian *et al.*, 2002; Charalambous *et al.*, 2003; Olsen *et al.*, 2004). To date, however, there is very little evidence to indicate that directly inheritable or somatic mutations in IKK genes underpin their overactivity. In multiple myeloma, mutations in genes encoding regulatory components of the non-canonical cascade, but not the IKKs, have been identified (Keats *et al.*, 2007), indicating that the activity of the IKKs is enhanced indirectly (Romieu-Mourez *et al.*, 2001). Irrespective of this fact, increased cellular IKKs, in particular IKKβ, seem to be a key to enhanced survival. The IKKβ/NF-κB axis encodes numerous survival genes such as superoxide dismutase (SOD), which prevents the formation of damaging agents such as hydrogen peroxide, which in turn limits the activities of pro-apoptotic pathways such as c-Jun N-terminal kinase (JNK). NF-κB also regulates the expression of pro-survival genes such as inhibitors of apoptosis (IAPs) and B-cell lymphoma-extra large (Bcl-xL) (Pahl, 1999). Indeed, in numerous studies, expression of dominant-negative IKKβ or treatment with IKKβ inhibitors promotes death *per se*, enhances cell death in response to agents such as TNF-α or increases the sensitivity of cancer cells to apoptosis inducing cancer drugs (Romieu-Mourez *et al.*, 2001). This model of NF-κB-mediated cellular survival underpins the immediate potential for therapeutic targeting of the IKKβ/NF-κB pathway in this disease (see succeeding discussion) (Escarcega *et al.*, 2007; Lee and Hung, 2008).

More recently, it has emerged that independent of NF-κB, the IKKs may regulate cell cycle progression directly. IKKα has been shown to phosphorylate the mitotic kinase Aurora A on threonine 288 which is a key site for kinase activity (Prajapati *et al.*, 2006). Knockdown of IKKα by siRNA impairs cell cycle progression in HeLa cells, and IKKα has been shown to regulate the M-phase of the cell cycle (Prajapati *et al.*, 2006). Chk1 has been shown to associate with and phosphorylate IKKα during S-phase in human osteosarcoma U2OS cells, inhibiting the ability of IKKα to phosphorylate p100 (Barre and Perkins, 2007). In MCF7 cells, IKKα has been shown to regulate S-phase following oestrogen stimulation (Tu *et al.*, 2006), further outlining the role of IKKα in the checkpoint control machinery of cell cycle progression. Recent studies have indicated that IKKα is a part of the TGFβ-Smad2/3 signalling pathway exerting control over the cell cycle in keratinocyte differentiation (Descargues *et al.*, 2008). Since it is well recognized that Smad2/3-mediated cell cycle control is integrated with p53 function, it would not be surprising to suggest that p53 phosphorylation be mediated by IKKα. Experimental evidence however suggests that it is in fact IKKβ that phosphorylates p53 at serines 362 and 366 which leads to p53 ubiquitination and degradation by β-transducin repeat-containing protein (βTrCP) in a murine double minute 2 (Mdm2)-independent manner. This suggests that inhibition/blocking of IKKβ and/or βTrCP could result in the stabilization of p53 and enable it to retain its tumour suppressor function (Xia *et al.*, 2009). The involvement of the individual IKKs in the cell cycle suggests that selective inhibition of these kinases may be effective in halting cell division, and therefore, in part, tumour progression.

Despite having a role in cellular survival, more recent evidence implicates the IKKs in the chronic inflammation associated with cancer development. In a transgenic adenocarcinoma of mouse prostate (TRAMP) model of prostate cancer (Gingrich *et al.*, 1996), mice expressing an inactive mutant form of IKKα ($IKK\alpha^{AA/AA}$/TRAMP) developed fewer distant-site metastases in areas such as the liver, lung, pelvic or renal lymph nodes (Luo *et al.*, 2007) and also displayed a delayed onset of cancer of the prostate in comparison to the WT/TRAMP mice, and a corresponding increase in survival. In this instance, tumour progression was associated with an IKKα-mediated inflammatory response involving infiltration

of lymphocytes which promoted metastasis and secondary tumours (Luo *et al.*, 2007). This pro-inflammatory input for IKKα in cancer has been further supported by experiments which demonstrated that androgen ablation caused infiltration of regressing androgen-dependent tumours in which IKK was driving the production of inflammatory cytokines to enhance hormone-free tumour survival (Ammirante *et al.*, 2010). Independent studies observing over-expression of p65, as a function of IKKβ, suggest that this isoform plays a role in the early stages of prostate cancer, an effect likely to be linked to cell survival (Sweeney *et al.*, 2004). Therefore, IKKα inhibition may represent a strategy to combat late-stage prostate cancer at least in part by regulating the inflammatory environment, while targeting IKKβ may be more relevant to early stages of the disease. Other studies are required to confirm if similar complimentary roles apply to different types of cancer. For example, while IKKβ is thought to play a role in melanoma, IKKα is proposed to protect cells against the development of UV-B-induced skin cancer (Luo *et al.*, 2007; Descargues *et al.*, 2008; Bettermann *et al.*, 2010).

To date, IKK inhibitors have not been used therapeutically in the clinic. Nevertheless, the functional roles of IKKs in cell survival and cell cycle progression make drugs of this type promising anticancer therapies, particularly for use in combination with chemo- and radiotherapeutics. It is well documented that UV and ionizing radiation, used commonly in cancer therapy, activate the NF-κB pathway (Li and Karin, 1998) and, as for a number of genotoxic stresses, is induced via an IKKβ-dependent mechanism (Janssens and Tschopp, 2006). This relies on the nuclear translocation of NEMO scaffolding protein, its sumoylation and subsequent phosphorylation by the checkpoint kinase ataxia telangiectasia mutated (ATM) (Wu *et al.*, 2006). With de-sumoylation and ubiquitylation of NEMO, nuclear export of a NEMO-ATM complex results in activation of the IKK complex, principally IKKβ (Wu *et al.*, 2006). Whether this mechanism of regulation is activated in response to all forms of radiation in all cell types remains unclear. Furthermore, whether NF-κB activation in response to UV and ionizing radiation is predominantly IKKβ dependent, to the exclusion of IKKα-mediated regulation, is as yet unexplored, although the non-canonical pathway has been implicated in mediating survival of endometrial carcinoma cells post-treatment with ionizing radiation (Wu *et al.*, 2011).

Therefore, to gain further benefit from these approaches, compromised IKK/NF-κB signalling may be required to offset stress-induced survival mechanisms involving NF-κB. This approach could also be extended to chemotherapeutics, where NF-κB has been associated with chemoresistance. Many drug-resistant cancer cell lines have been shown to have high levels of NF-κB-DNA binding activity, and inhibition of NF-κB has been shown to improve the efficacy of some current chemotherapeutics. For example, the targeted inhibition of NF-κB in combination with the chemotherapy drug doxorubicin was shown to enhance the level of apoptosis (Bednarski *et al.*, 2009; Guo *et al.*, 2010). Over-expression of p50 and p65 has been shown in MCF7 breast cancer cells, which correlated directly with resistance to 2′,2′-difluorodeoxycytidine/gemcitabine, a pyrimidine analogue also known as gemcitabine (Guo *et al.*, 2010). Targeted inhibition of the IKKs, as regulators of p65, may therefore offer a therapeutic advantage when used in combination with gemcitabine in this setting. In salivary gland cancer cells, 5-fluorouracil (5-FU) (also a pyrimidine analogue) induces apoptosis through suppression of NF-κB and inhibition of IKK (Azuma *et al.*, 2001). However, in colorectal cancer (CRC) cells, NF-κB activity is reported to increase following 5-Fu treatment. The authors went further to demonstrate that this was mediated through activation of IKK in RKO cells and specifically required IKKβ (Fukuyama *et al.*, 2007). In turn, disulfiram (DS)-mediated inhibition of NF-κB in the CRC setting enhanced the cytotoxic effects of 5-Fu. This evidence may lend weight to the notion of IKK/NF-κB inhibition as a means to decrease the expression of pro-survival genes which may help to combat chemoresistance. Therefore, collectively, the clinical treatments for cancer described above and their modes of action in cellular settings have further illustrated the potential of NF-κB to be one of a number of cellular targets for anti-cancer therapies.

A number of synthetic compounds and natural products that target NF-κB, distinct from the IKKs, have been assessed for their anti-cancer potential at the cellular level and are presently in clinical trials (Sethi *et al.*, 2009). Some have progressed to the clinic; one of these, the proteosomal inhibitor Bortezomib (Velcade), has been found to be effective in patients presenting with head and neck squamous cell carcinoma (HNSCC) (Chen *et al.*, 2008), multiple myeloma (Field-Smith *et al.*, 2006) and mantle cell lymphoma (Alinari *et al.*, 2009), acting at least in part by inhibiting NF-κB translocation/activation (Chiao *et al.*, 2002; Singh *et al.*, 2007). Indeed, the promising data emerging on the use of NF-κB inhibitors to combat chemoresistance (Syrovets *et al.*, 2005; Tapia *et al.*, 2007; Schôn *et al.*, 2008; Gao *et al.*, 2010) point to a therapeutic direction in which the addition of IKK inhibition may improve current treatment strategies. Similar approaches could be applied to the use of radiopharmaceuticals and existing radiotherapeutics. This awaits experimental outcomes from current ongoing studies.

Alternative strategies to target the IKK complex: protein–protein interactions and their disruption

The apparent problems associated with directly inhibiting IKK activity, in particular IKKβ, suggest that targeting protein–protein interactions may be an alternative strategy for pharmacological intervention. This strategy relies upon identifying defined areas, regions or domains of proteins that are critical for the regulation and/or (de)activation of proposed target proteins. The site of interaction between IKKα/β and NEMO, which exists as a hydrophobic pocket, contains a central conserved hexapeptide sequence, LDWSWL, that is termed the NEMO-binding domain (NBD). This domain was initially predicted by means of hydropathy plots (May *et al.*, 2000) but was more recently detailed in an IKKα/β peptide-truncated NEMO co-crystal structure (Rushe *et al.*, 2008). Experiments have been conducted with peptides derived from the surrounding primary amino acid sequence (11- or 12-mers), fused to membrane transduction sequences. These peptides were observed to inhibit the NF-κB pathway across a

wide range of systems (Table 2), presumably by disruption of the IKKα/β-NEMO interaction.

Studies *in vitro* using cell-permeable peptides have also shown effective inhibition of NF-κB signalling. For example, treating human monocyte-derived dendritic cells with the NBD peptide arrested the cells in an immature state despite stimulation with LPS (Tas *et al.*, 2005). Similarly, use of the NBD peptide in human melanoma cultures inhibited NF-κB-DNA binding and also induced apoptosis via the activation of caspase 3 (Ianaro *et al.*, 2009). Most recently, two separate studies have shown that inhibition of the IKK complex by the NBD peptides *in vivo* (see Table 2) is an effective approach to the treatment of inflammatory diseases in which bone resorption plays a substantial pathological role (Dai *et al.*, 2004; Jimi *et al.*, 2004). The NBD peptide only blocks the induction of NF-κB activity in response to pro-inflammatory stimuli and does not inhibit basal activity. This helps limit possible side effects, for example, undesired apoptosis (Kucharczak *et al.*, 2003). The use of peptide-based disruptors of protein–protein interactions is not confined to IKKα/β-NEMO interactions. Other examples of the use of peptides for the inhibition of the NF-κB pathway include a cell-permeable peptide targeted to the NLS of p50, which was found to inhibit LPS-stimulated nuclear translocation of NF-κB complexes (Lin *et al.*, 1995). There was some evidence to suggest that this peptide also inhibited the nuclear translocation of other transcription factors, so a similar peptide targeting the RelB:p52 dimer (which did not have similar off-target effects) may be a better alternative (Torgerson *et al.*, 1998; Xu *et al.*, 2008). Interestingly, peptides targeting the IKK-binding domain of NEMO have thus far been proven to have little effect on NF-κB activity (Marienfeld *et al.*, 2006). However, peptides designed to block NEMO oligomerization have been shown to inhibit NF-κB-dependent gene expression and increase apoptosis in a number of studies (Agou *et al.*, 2004; Carvalho *et al.*, 2007; Wyler *et al.*, 2007).

As shown above, cell-permeable peptides targeting the IKK complex can efficiently inhibit NF-κB signalling, although there are several challenges. Other chemical entities that inhibit the IKKα/β-NBD-NEMO interactions *in vitro* have also recently been identified (Gotoh *et al.*, 2010). The chemical structure of these 'disruptors' remains undisclosed, and as such, it is unknown whether these 'compounds' represent low-molecular-weight entities or are peptide-based. The progression of any peptide-based disruptors of protein–protein interactions into drug-like molecules brings the significant challenges of 'in-building' the appropriate pharmacology and desired drug-like characteristics. This will likely require the development of peptidomimetics into small drug-like molecules that work as efficiently, if not better, than the original peptide. One technique that is proving to be a popular tool in the development of this approach is virtual screening of proteins. For example, the structural determination of human Mdm2 bound to a 15 residue peptide of p53 has led to the development of a number of small non-peptidic inhibitors (Shangary and Wang, 2009). Structure-based drug design has also been used to improve the druggability of a small molecule directed at the interaction between B-cell leukaemia 2 (Bcl2)/Bcl-xl (van Montfort and Workman, 2009). A similar strategy could therefore be applied to the interactions between the IKKs within the IKKs complex, relevant to both the canonical and non-canonical axes.

Summary and future perspectives

Within the NF-κB field and in the study of the IKKs, there remain the key challenges of understanding fully the functional roles of the individual kinase isoforms. This has in part driven the quest for IKK-selective inhibitors and has been based primarily on the development of ATP-competitive agents that are more easily identified in HTS. Unfortunately, ATP mimetics have a number of limitations (Garber, 2006). Despite being of low molecular weight, being orally bioavailable and able to inhibit target proteins, they can still 'hit' other kinases to generate 'off-target'/side effects (Garber, 2006). In the cancer setting, for example, it has also been observed that the strategy of using ATP-competitive inhibitors may be flawed as tumours, and kinases within them, develop mutations in the ATP-binding pocket that interferes with drug binding, negates its effects and leads to resistance; whether this is relevant to the IKKs across a number of pathophysiological settings remains to be determined. To avoid these issues, alternative strategies to targeting aberrant kinase activity are now emerging as the kinase field embarks on identifying agents that bind to, and inhibit, kinases in novel ways. Substrate-competitive inhibitors particularly are now being developed to this end (e.g. Bogoyevitch and Arthur, 2008; Licht-Murava *et al.*, 2011) and are providing promising leads. In time, this approach will likely be applied to the IKKs.

So, to date, despite the limitations of ATP-competitive molecules, significant advances have been made in developing inhibitors of the IKKs, particularly those that target and inhibit the intrinsic catalytic activity of IKKβ (see Section The development of novel small molecule inhibitors of the IKKs). This has been achieved again through the pursuit of HTS; however, these strategies have not delivered parallel inhibitors of IKKα. The synthesis and optimization of highly selective inhibitors of IKKα remains one of the key challenges in developing a fuller understanding of the functional role(s) of IKKα in both physiological and pathophysiological settings.

The further development of IKKα selective inhibitors (ATP competitive or otherwise), NBD-based novel IKK complex 'disruptors' and the refinement and reappraisal of existing IKKβ inhibitors will undoubtedly be supported by the availability of solved crystal structures for the IKK catalytic subunits. Significantly, Xu *et al.* (2011) have very recently reported the solved crystal structure for Xenopus-IKKβ in complex with an inhibitor, at a resolution of 3.6 Å, to identify a trimodular architecture. It may be that further successful crystallization of human IKKα and/or IKKβ requires association with similar inhibitor molecules, with IKKγ/NEMO or other interacting proteins, for example, heat-shock proteins (Hsps) (Chen *et al.*, 2002; Broemer *et al.*, 2004; Mohan *et al.*, 2009), connection to IKK and SAPK (CIKS) (Mauro *et al.*, 2003), IKK interacting protein (IKIP) (Hofer-Warbinek *et al.*, 2004), A20 (Zhang *et al.*, 2000; Zetoune *et al.*, 2001) and A20-binding protein (ABIN) (Mauro *et al.*, 2006). Further IKK-focussed proteomic experiments will likely inform on the

status of differing IKK complexes in cells. The complexity of these interactions may initially limit the progress of drug design, but in the long run could give rise to the opportunity for selectivity in the disruption of distinct interactions within the different protein complexes, for example, IKKα/Aurora A or IKKβ/ABIN, which lead to different functional outcomes.

Since their discovery in the late 1990s, the IKKs remain a potentially useful therapeutic strategy for the treatment of numerous conditions. Not only in RA, IBD and cancer but also in chronic obstructive pulmonary disease (COPD) (Shuto *et al.*, 2001), asthma (Yang *et al.*, 1998), ischaemia reperfusion (Herrmann, 2005), atherosclerosis (Saxena *et al.*, 1994; Reddy *et al.*, 2002) diabetes (Arkan *et al.*, 2005) and transplant rejection (Townsend *et al.*, 2004). Further development of IKK inhibitors or disruptors of protein–protein interactions could make an important contribution to the future alleviation of such diseases and conditions.

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Conflict of interest

The authors have no conflicts of interest with respect to the manuscript and its potential publication.

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