Available online at www.sciencedirect.com
ScienceDirect
Cytokine & Growth Factor Reviews 19 (2008) 157–165
Cytokine Growth Factor Reviews
www.elsevier.com/locate/cytogfr

Mini review

IκB kinase β (IKKβ/IKK2/IKBKB)—A key molecule in signaling to the transcription factor NF-κB

Johannes A. Schmid [a,b,1,*], Andreas Birbach [b,1]

[a] Center for Biomolecular Medicine and Pharmacology, Medical University Vienna, Austria
[b] Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria

Abstract

IKKβ/IKBKB (IκB kinase beta), also designated as IKK2, was named after its function of phosphorylating IκB molecules, the inhibitors of NF-κB transcription factors. The kinase activity of IKKβ targets two adjacent serine residues of IκB leading to ubiquitination and proteasomal degradation of the inhibitor, followed by release and activation of NF-κB. Many signaling pathways that activate NF-κB converge at the level of IKKβ. Examples of stimuli leading to IKKβ and subsequent NF-κB activation include inflammatory cytokines (IL-1, TNFα), endotoxins (lipopolysaccharide), viral infection and double strand RNA as well as physical signals such as UV-irradiation.

Transcription factors of the NF-κB protein family have a great variety of functions in regulating the immune system, cellular differentiation, survival and proliferation.

NF-κB is an essential factor in acute as well as chronic inflammation, a pathological state which is either cause or co-factor in a great variety of diseases. Moreover, recent data suggest that many variants of cancer are characterized by elevated constitutive activity of NF-κB, which can act as a survival factor for malignant cells by its predominantly anti-apoptotic function. Given the tight regulation of NF-κB by IκB molecules and the central role of IKKβ in phosphorylation and degradation of the inhibitor, IKKβ is a very promising target for pharmaceutical substances aiming at interfering with NF-κB activation.
© 2008 Elsevier Ltd. All rights reserved.

Keywords: NF-κB; Ubiquitination; Signaling cascades; Kinase inhibitors

1. Introduction

The currently best-documented function of IKKβ is to activate members of the NF-κB transcription factor family via the so-called classical (or canonical) pathway by phosphorylation of IκB inhibitors [1–3]. NF-κB transcription factors play an important role in the balance between cell survival and apoptosis and are involved in the regulation of cell proliferation and development or differentiation of various cell types [4,5]. Changes in activity and/or regulation of IKKβ and NF-κB are found in many diseases associated with chronic or acute inflammation and more recently it became evident that NF-κB exhibits higher constitutive activity or aberrant regulation in various forms of cancer [4,6–12].

The functional entities of NF-κB transcription factors are homo- or heterodimers of members of this gene/protein family, consisting of the proteins NF-κB1 (p50 and the precursor p105), NF-κB2 (p52 and the precursor p100), RelA (p65), RelB and c-Rel. All these molecules contain a homologous DNA-binding domain (the Rel homology domain); however, just three of them (RelA/p65; RelB and c-Rel) contain a transactivation domain. Transcriptionally active NF-κB dimers contain one of these three factors. In non-activated cells NF-κB dimers are associated with molecules of

Abbreviations: IκB, inhibitor of NF-κB; IKKa, IκB kinase a; IKKβ, IκB kinase β; IL-1, interleukin-1; NEMO, NF-κB essential modulator; NF-κB, nuclear factor-kappa B; TCR, T cell receptor; TNFα, tumor necrosis factor alpha.

* Corresponding author at: Ludwig Boltzmann Institute for Cancer Research, Medical University Vienna, Waehringerstr. 13A, A1090 Vienna, Austria. Tel.: +43 1 4277 64111; fax: +43 1 4277 9641.
E-mail addresses: Johannes.Schmid@meduniwien.ac.at (J.A. Schmid), Andreas.Birbach@lbicr.lbg.ac.at (A. Birbach).
[1] These authors contributed equally to the manuscript.

1359-6101/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cytogfr.2008.01.006

the IκB protein family (mainly IκBα, IκBβ and IκBε), which inhibit NF-κB binding to DNA [13] and shift the steady state localization to the cytosol [4]. This model is supported by structural data of IκB/NF-κB complexes [14,15]. The precursor forms of p52 and p50 (p100 and p105, respectively) contain pro-domains, which are homologous to IκB molecules and fulfill the same inhibitory role. Both NF-κB and IκBα molecules shuttle between cytosol and nucleus [16–18], but in the presence of IκBα the predominant steady state localization of the complex is in the cytosol.

For full activation NF-κB has to be released from IκB, which is achieved by degradation of the inhibitor (or the inhibitory pro-domains of p100 and p105) by 26S proteasomes. Signal-induced proteasomal degradation of IκB or inhibitory pro-domains requires prior polyubiquitination, which is carried out by $SCF^{\beta TrCP}$-type E3 ligases [19–21]. This in turn is triggered by phosphorylation of IκB on two adjacent serine residues (Ser32/Ser36 in IκBα) in the N-terminal signal-response domain. Two kinases were reported to be capable of phosphorylating these residues: IKKα (IκB kinase α, IKBKA, IKK1) and IKKβ (IKBKB, IKK2, Fig. 1) [22–25]. IKKα and IKKβ form heterodimers and are found in a 700–900 kDa complex with a non-enzymatic accessory molecule named IKKγ [26] or NEMO (NF-κB essential modulator [27]). This complex, also known as IKK-signalosome, was postulated to contain two IKK-dimers and most likely a tetramer of IKKγ/NEMO [1]. IKKβ turned out to be the crucial kinase of the IKK-signalosome for activation of NF-κB by most inflammatory stimuli, which is considered the classical or canonical pathway of NF-κB activation. Signals activating this pathway comprise the inflammatory cytokines TNFα and IL-1, bacterial components like lipopolysaccharide (LPS) which activate Toll-like receptors (TLR) and signals that activate T-cell receptors (TCR) [2,4,5]. Interestingly, recent data indicate that IKKβ is dispensable for some pathways of canonical NF-κB activation, as it was observed that IKKα can activate NF-κB in response to IL-1, but not TNFα by forming functional IKK-complexes with NEMO even in the absence of IKKβ [28].

Despite tedious studies, the exact molecular mechanisms leading from the different stimuli to activation of the IKK-complex are not clear and there are most probably different modes of activating IKKβ. It was shown that various kinases (such as MEKK1, MEKK3, TAK1, NIK, NAK or PKC-theta, as reviewed in [4]) can activate IKKβ by phosphorylating serine residues within its activation loop (Ser177 and Ser181). Gene deletion experiments verified the importance of MEKK3 (mitogen-activated protein kinase/ERK kinase kinase-3) and TAK1 (TGFβ-activated kinase 1) in the canonical NF-κB activation pathway [29,30]. Besides activation by upstream kinases there is evidence that IKK molecules can also activate themselves by a transphosphorylation mechanism of homo- or heterodimers in a proximity-induced self-activation process [31,32]. While all these studies led to the identification of a variety of possible upstream signaling pathways, the mechanistic details of these signaling events are still not fully understood. In the last few years, it became increasingly clear that ubiquitination cascades upstream of IKK signalosomes play a pivotal role in signaling to effector molecules.

In this article, we want to highlight recent insights in the activation of IKKβ and NF-κB including specific ubiquitination events. We describe recently identified substrates of IKKβ which extend the role of IKKβ beyond the NF-κB signaling pathway. Furthermore, we report on pharmaceutical efforts to develop inhibitors or modulators of IKKβ, as these substances have an enormous potential of application in a great variety of diseases.

2. Signaling and function of IKKβ

2.1. Ubiquitination processes modulate signaling pathways converging on IKKβ

The current view of the canonical pathway of NF-κB activation is that specific and distinct ubiquitination processes are involved in the activation of the IKK-complex (Fig. 2) [3,33]. In fact, it was shown that the IκB kinase activity requires non-degradative ubiquitination even before the molecular identification of IKKs [34]. In general, binding of ligands such as IL-1 or TNFα to respective receptors induces association of adaptor molecules with cytosolic receptor domains. These adaptor molecules include RING finger-proteins of the TRAF family (TNF-receptor associated factors), with TRAF6 being essential for IL-1-mediated IKK activation and TRAF2 or TRAF5 as crucial factors in TNFα-induced signaling. Biochemical analysis of factors linking TRAF6 to IKK activation led to the identification of Ubc13/Uev1A as a dimeric E2 ubiquitin-conjugating enzyme complex and demonstrated that the RING domain of TRAF6 acts as E3 ligase catalyzing the formation of lysine-63-linked polyubiquitin chains [35]. In contrast to lysine-48-linked polyubiquitin chains, which

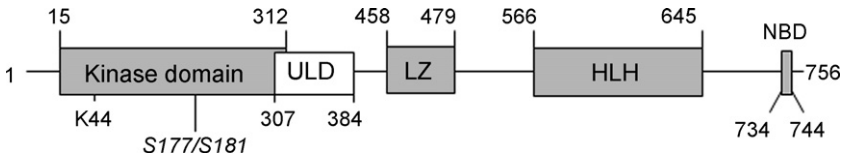

Fig. 1. Scheme of the IKKβ domain structure. ULD, ubiquitin-like domain; LZ, leucine zipper; HLH, helix–loop–helix domain; NBD, NEMO-binding domain. Numbering of domain borders differ slightly between different references. The catalytic active site lysine residue (K44) is indicated and the two serine-residues (S177/S181) of the activation loop, which are phosphorylated upon activation, are shown in *italics*.

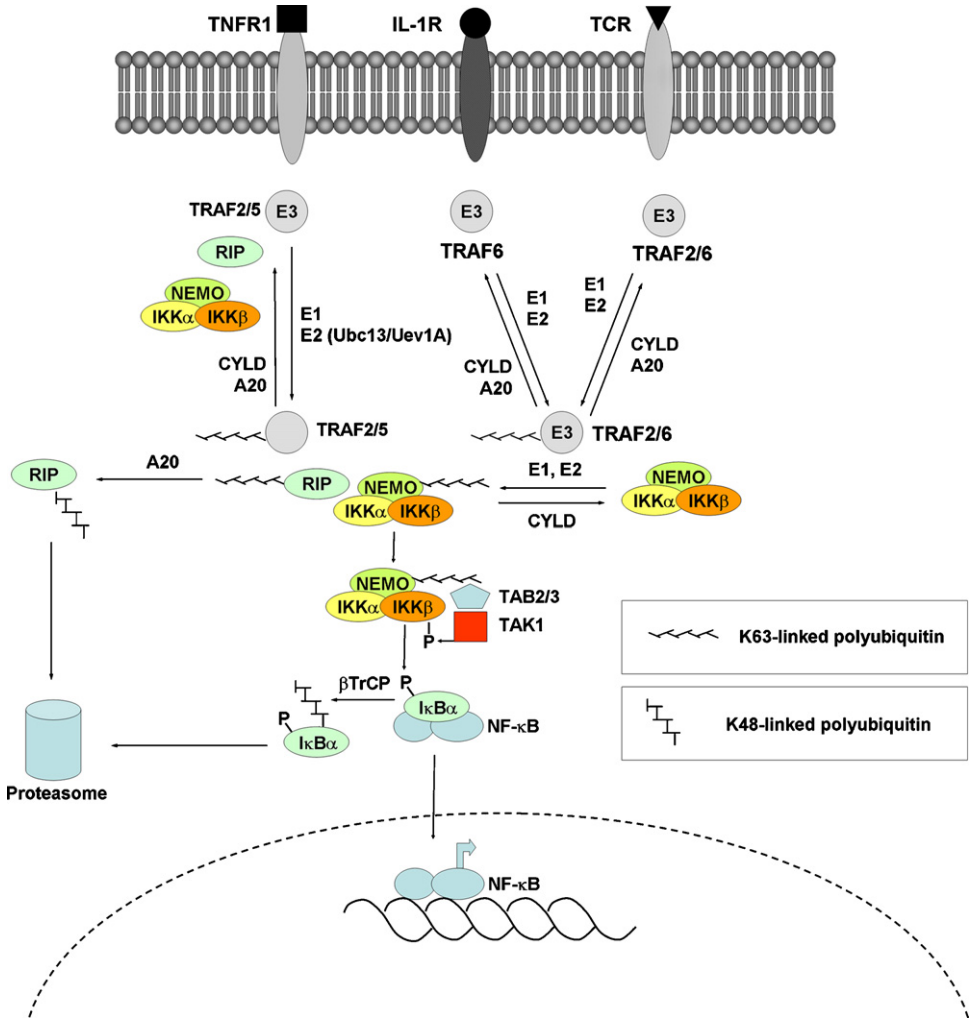

Fig. 2. Signaling pathways leading to activation of IKKβ and ubiquitination processes involved therein. Binding of ligands to the respective receptors leads to association of TRAF molecules, which then act as E3 ligases to catalyze the formation of K63-linked polyubiquitin chains on themselves as well as on RIP and NEMO. These polyubiquitin chains create a binding platform for TAB2 or TAB3 together with TAK1, which activates IKKβ by phosphorylation. Activated IKKβ phosphorylates IκB molecules, triggering their K48-linked polyubiquitination via βTrCP followed by proteasomal degradation of IκB and release of active NF-κB. Deubiquitinating enzymes such as CYLD or A20 counteract the signaling cascade by cleaving K63-polyubiquitin chains. A20 can also catalyze K48-linked, degradative polyubiquitination of RIP.

lead to proteasomal degradation, K63-linked polyubiquitins appear to serve as a platform for association of molecules with ubiquitin-binding domains and the subsequent activation of signaling molecules. For IL-1-mediated signaling, it was shown that TRAF6 catalyzes K63-linked autoubiquitination, as well as K63-linked polyubiquitination of NEMO, which leads to recruitment of the kinase TAK1 together with the ubiquitin-binding proteins TAB1 and TAB2 [36]. This results in activation of TAK1 which phosphorylates and activates IKKβ. TAB2 or the homologous TAB3 have been identified as the essential ubiquitin-binding factors for association with K63-linked polyubiquitin chains of TRAF6. The molecular mechanism by which binding of TABs to autoubiquinated TRAF6 leads to activation of TAK1 is still not clear, but it is supposed that it might facilitate oligomerization and self-activation of TAK1 by transphosphorylation [37].

For the TNFα-induced signaling pathway, it is assumed that TRAF2 and TRAF5 catalyze auto- and transubiquitination as well as K63-polyubiquitination of RIP1 (receptor-interacting protein-1) and IKKγ/NEMO. This induces binding and activation of TAK1 mediated by TAB2/3, followed by activation of IKKβ by phosphorylation of serines 177 and 181 in the activation loop. The crucial role of TAK1 for both the TNFα- and the IL-1-mediated NF-κB activation has recently been confirmed in a conditional knockout system [29].

Given the essential role of ubiquitination and ubiquitin-binding proteins, it is important to note that IKKβ itself contains a ubiquitin-like domain (ULD, see Fig. 1), which was shown to be essential for activation of the kinase [38]. Interestingly, this domain cannot be replaced with the ubiquitin sequence and it does not contain lysines at positions equivalent to K48 or K63 of ubiquitin, indicating

that it is not a target of polyubiquitination. However, it might have a role in binding of co-factors such as TAB2/3.

In line with the model that ubiquitination processes are essential in signaling to IKKβ, deubiquitinating enzymes have been shown to be involved in termination and feedback inhibition of the IKK-mediated NF-κB activation. The known NF-κB inhibitory protein A20 [39,40] and the protein CYLD (cylindomatosis protein [41,42]) have been identified as deubiquitinating enzymes (DUBs) in the NF-κB pathway. CYLD cleaves K63-polyubiquitin chains of TRAF2 and NEMO, while the inhibitory action of A20 involves two concerted mechanisms: one domain of A20 cleaves the activating K63-polyubiquitin chains of RIP1 and a second domain catalyzes K48-linked polyubiquitination of RIP1 leading to its proteasomal degradation.

2.2. Substrates of IKKβ

IKKβ is activated by phosphorylation of S177 and S181 and catalyzes not only the phosphorylation of two crucial serine residues in IκB molecules, but also autophosphorylation on C-terminal serine residues. This is assumed to lead to self-inactivation representing a negative feedback loop [43], thereby being important for the termination of IKK activity in addition to phosphatases like PP2A or PP2Cβ [22,44]. Besides IκB and IKKβ itself, there are other molecules of the NF-κB pathway phosphorylated by IKKβ. One of them is RelA/p65, which is targeted by IKKβ at serine 536 [45,46]. Phosphorylation of this residue is important for full transcriptional activity of RelA/p65 and thus represents an enhancement of NF-κB activity. In the case of the NF-κB family member p105, phosphorylation by IKKβ leads to polyubiquitination and degradation similar to IκB [47]. As p105 is not only involved in the NF-κB pathway, but also binding effector molecules of other signaling pathways (e.g. TPL2/COT, a component of the MAPK pathway), its degradation can lead to liberation of these molecules and activation of the respective pathway [47,48]. Another substrate of IKKβ related to NF-κB activation is Bcl10 (B-cell lymphoma protein 10), a molecule involved in IKKβ activation by TCR signaling. In this case, IKKβ has a dual role: it is required for formation of the Carma1/Bcl10/MALT1 complex essential for TCR-mediated NF-κB activation, and it also attenuates the signaling by phosphorylating Bcl10 [49].

In addition to the canonical pathway of NF-κB activation, there are also atypical pathways leading to IKKβ and NF-κB activation. For instance, genotoxic stress (e.g. UV-irradiation) induces DNA double strand breaks leading to activation of the kinase ATM (ataxia telangiectasia mutated), which phosphorylates a nuclear, sumoylated fraction of IKKγ/NEMO. This phosphorylation leads to replacement of the SUMO-1 modification by a mono-ubiquitination, which in turn triggers nuclear export of a NEMO–ATM complex. In the cytosol this complex then activates IKKβ in collaboration with the IKK-associated molecule ELKS [50].

Besides the well-documented role of IKKβ in the activation of NF-κB it could also be shown to phosphorylate other substrates than IκB or NF-κB molecules. One of these substrates is the Forkhead transcription factor FOXO3a [51], a tumor suppressor protein capable of inducing cell cycle arrest or apoptosis. IKKβ-mediated phosphorylation of FOXO3a results in its translocation to the cytoplasm and degradation. Another non-IκB substrate of IKKβ is 14-3-3β, which binds mRNAs containing AU-rich elements (ARE) in cooperation with the protein tristetraproline (TTP), leading to mRNA destabilization. Upon IKKβ-mediated phosphorylation of 14-3-3β, the complex with TTP is released and the corresponding mRNAs (including those coding for cytokines such as TNFα) are stabilized, providing a positive feedback loop for the NF-κB pathway [52]. Other newly identified substrates for IKKβ are insulin receptor substrate 1 (IRS1), where IKKβ-mediated phosphorylation is thought to inhibit tyrosine phosphorylation and impair insulin signaling [53], and the related protein DOK1 (docking protein 1), a tyrosine kinase substrate that promotes cell migration [54]. Given these recent new insights, it has to be expected that additional targets for IKKβ will be found in the future. Aligning the known IKK2 substrates in the region of their phosphorylated serine residue reveals a conserved core consensus site and additional conserved amino acids, about 15 amino acids N- and 11 amino acids C-terminal of the core region (Fig. 3).

```
                1                                                  50
IκBα    (1) -----WAMEGPRDGLKKERLLDDRHDSGLDSMKDEEYEQMVKELQEIRLE
IκBβ    (1) --------MAGVACLGKAADADEWCDSGLGSLGPDAAAPGGPGLGAEL--
IκBε    (1) -------PGPVKEPQEKEDADGERADSTYGSSSLTYTLSLLGGPEAE---
IKK2    (1) IVLQQGEQRLIHKIIDLGYAKELDQGSLCTSFVGTLQYLAPELLEQQKYT
p65     (1) -PDPAPAPLGAPGLPNGLLSGDEDFSSIADMDF-SALLSQISS-------
p105    (1) AHSLPLSPASTRQQIDELRDSDSVCDSGVETSFRKLSFTESLTSGASLLT
14-3-3  (1) -----ATQPESKVFYLKMKGDYFRYLSEVASGDNKQTTVSNSQQAYQEAF
Bcl10   (1) ---LEHLKGLKCSSCEPFPDGATNNLSRSNSDESNFSEKLRASTVMYHPE
DOK1    (1) --------LAPKPQGPAFPEPGTATGSGIKSHNSALYSQVQKSGASGS--
FOXO3a  (1) ---ECDMESIIRSELMDADGLDFNFDSLISTQNVVGLNVGNFTGAKQASS
IRS-1   (1) SVPLRRHHLNNPPPSQVGLTRRSRTESITATSPASMVGGKPGSFRVRASS
Consensus(1)            K         D   DS I S           S
```

Fig. 3. IKKβ substrates and phosphorylation sites. Domains surrounding known phosphorylation sites of IKKβ substrates have been aligned with VectorNTI™ software (Invitrogen Inc.). The highly conserved phosphorylated serine residues are shaded in yellow.

2.3. Insights from knockout and transgene mouse models

While many different signaling molecules involved in activation or regulation of IKKβ have been identified and studied by different cell cultures experiments *in vitro*, it soon became clear that physiological studies *in vivo* using knockout or transgene mouse models are required to fully elucidate the complex signaling network involved. Signaling molecules postulated to be essential for NF-κB activation based on *in vitro* studies quite often turned out to be redundant and did not show the expected phenotype in knockout mice. In some cases, unexpected or tissue-specific effects could be observed (as reviewed in [55]).

Classical knockout mice with deletion of IKKβ die at an embryonic stage (around E13) due to TNF-mediated apoptosis of hepatocytes [56]. This phenotype is very similar to that of RelA/p65 knockout mice [57], supporting the importance of IKKβ for RelA/p65 activation downstream of TNFα signaling. Embryonic lethality can be rescued by additional deletion of TNF-receptor 1 supporting the notion that IKKβ and RelA/p65 counteract the pro-apoptotic effect of TNFα in a sensitive phase of liver development [56].

Conditional knockout models of IKKβ demonstrated the importance of this kinase in protecting macrophages [58], osteoclasts [59] or gut epithelium [60] from apoptosis triggered by Toll-like receptor signals. In contrast to the anti-apoptotic role of IKKβ in these as well as many other cell types, it seems to have a pro-apoptotic role in particular cells such as neurons [61]. Cell-type specific differences are also observed for the role of IKKβ in inflammation. While the kinase is essential for inflammatory signaling pathways in many cell types it was reported to have an anti-inflammatory role in keratinocytes [62]. Hepatocyte-specific deletion of IKK2 revealed that membrane-bound TNF elicits a different cellular response than soluble TNF. Mice lacking IKKβ in hepatocytes were still protected from apoptosis triggered by soluble TNFα, while they were sensitive to treatment with Concanavalin A, an agent which triggers a membrane-bound TNF response [63]. This conditional knockout also helped in detailing the mechanism of liver regeneration after partial hepatectomy. It was shown that liver regeneration depends on NF-κB and IKKβ-mediated production of TNFα by non-parenchymal liver cells (mainly Kupffer cells representing liver macrophages) but not on IKKβ and NF-κB activation in hepatocytes themselves [63,64].

Conditional knockout of IKKβ in other cells strengthened the notion that the function of IKKβ varies significantly with the cell type. In macrophages it is important for synthesis of inflammatory cytokines [65], in osteoclasts it is involved in inflammation-induced bone loss [59], while it acts as an anti-inflammatory molecule in keratinocytes [62].

Conditional IKKβ knockout models also revealed a role of IKKβ in the development of obesity-induced insulin resistance. These studies demonstrated that IKKβ dependent production of inflammatory molecules by myeloid cells is required for the onset of type II diabetes [66]. B-cell specific deletion of IKKβ results in a reduction of follicular and marginal zone B-cells supporting the role in B-cell development [67], and deletion of IKKβ in the T-cell lineage prevents the development of NKT cells, as well as CD4+/CD25+ regulatory T-cells [68]. In addition to conditional knockouts of IKKβ, transgenic mice expressing constitutively active IKKβ have been generated. These contain a mutant of IKKβ in which S177 and S181 are replaced by glutamic acids ($IKK\beta^{SS/EE}$) mimicking the negative charge of phosphorylated serines in the activation loop. Tissue-specific expression of this mutant in skeletal muscle results in muscle wasting [69], indicating a role of IKKβ in cachexia and muscle atrophy. Targeted expression of this constitutively active IKKβ variant in the T-cell lineage confirmed that IKKβ-dependent NF-κB activation promotes thymocyte survival at a specific stage of T-cell development [70].

2.4. Efforts to develop inhibitors of IKKβ to block NF-κB activation

Following the elucidation of the key role of IKKβ in inflammation and its importance in oncogenesis, pharmaceutical companies have undertaken a great effort in developing specific inhibitors. Generally, we may divide IKKβ inhibitors into two groups: one, known drugs which have been shown to at least partly own their effectiveness to inhibiting IKK activity, and two, novel specific inhibitors, which often show strong inhibition at low concentrations but have yet to undergo clinical testing.

The first group includes the well-known anti-inflammatory agents aspirin, sodium salicylate and sulindac, which have been shown to influence several signal transduction pathways [71,72]. Their inhibition of IKKβ activity likely contributes to their anti-inflammatory and anti-oncogenic activities, but the relative roles of different pathways are difficult to determine.

The immunomodulatory drug thalidomide, which also exhibits anti-inflammatory and anti-angiogenic effects, is another example of an IκB kinase inhibitor [73]. Endogenous anti-inflammatory substances can also act by inhibiting IKKβ, as has been shown for cyclopentenone prostaglandins [74]. Arsenic trioxide, which has been used for a long time for the treatment of chronic myelogenous leukaemia and more recently against promyelocytic leukaemia [75], similarly inhibits NF-κB by mechanisms including the inhibition of IKKβ [76]. The inhibition by arsenite is one example for a drug blocking cysteine residue 179, which lies between the activating phosphorylation sites serine 177 and serine 181, but is also critical for control of IKK activity by redox reactions through *S*-glutathionylation [77].

Specific IKKβ inhibitors have been sought and found by a number of pharmaceutical companies. Celgene has

developed a series of quinazoline analogues including a molecule termed SPC 839, which inhibits IKKβ with an IC50 of 62 nM and has been shown to reduce paw oedema in a rat arthritis model [78]. This compound is one example for an ATP analog that shows specificity for IKKβ compared to other kinases, as demonstrated by an about 200-fold higher IC50 value for IKKα [79]. Other IKKβ inhibitors acting as ATP analogs are: β-carbolines, of which PS-1145 is being developed by Aventis and Millennium Pharmaceuticals [80], ML120B (Millennium Pharmaceuticals), a β-carboline demonstrated to be effective in a mouse apoptosis and a rat arthritis model, and SC-514, an aminothiophenecarboxamide [81–83]. Yet another mode of action is exemplified by BMS-345541 (Bristol-Myers-Squibb), which has been shown to inhibit IKK-β by binding at an allosteric site and was also successfully applied to *in vivo* inflammation models [84–86].

Several compounds pushed the limits with respect to effective inhibitory concentrations: some ureidocarboxamide thiophenes (AstraZeneca) show IC50 values as low as 18 nM and were also effective in a rat arthritis model [79]. Bayer has developed pyridooxazin derivatives with reported IC50 values of 2 nM [87]. CHS 828 (Leo Pharma), an antitumor agent undergoing clinical trials, was found to exert its actions through inhibition of IKK with an IC50 value of 8 nM [88]. Interestingly, all of the compounds mentioned have been shown to have IC50 values for IKKα which are several fold higher than for IKKβ and can thus be considered specific for the latter isoform at the right concentration.

In vivo testing of IKKβ inhibitors has not been restricted to inflammatory and cancer models. IMD-0354, a compound developed by the Institute of Medicinal Design (Japan), has been shown to ameliorate glucose intolerance in a mouse diabetes model [89].

Several other relevant small molecular inhibitors have been reviewed by Karin et al. [79].

Overall, there are more than 150 agents that have been shown to inhibit IκB kinases based on *in vitro* assays [90]. Among those are natural inhibitors, synthetic compounds and gene-based inhibitors. Genetic approaches to inhibit IκB kinases include the application of dominant-negative versions of IKKβ, which carry mutations in the kinase activation loop (S177A, S181A) or in the kinase domain (K44A) [23,24]. Moreover, antisense approaches have been developed with therapeutic aims [79].

In addition to efforts targeting IKKβ activity, strategies targeting the ubiquitination processes essential for activation of IKKβ have been developed. An example is the enforced expression of deubiquitinating enzymes such as CYLD using viral transduction techniques [91]. Moreover, small molecule inhibitors targeting the ubiquitination of IκBα have been described [92]. Future efforts may include screening for substances interfering with activating K63-linked polyubiquitination. However, the development of inhibitors targeting this reaction seems to be at an early stage, most likely because the underlying molecular mechanisms have just very recently been elucidated.

One concern with IKKβ inhibitors, as with NF-κB inhibitors in general, is their applicability to conditions like chronic inflammatory diseases. This is due to the well-described role of the IKKβ/NF-κB pathway in regulation of apoptosis and in immunological functions and potential side-effects (e.g. pro-apoptotic) of IKKβ inhibitors remain to be investigated in clinical trials. Moreover, IKKβ has been described to have other targets than IκB molecules (see above), and potential side-effects in clinical applications of inhibitors due to this heterogeneity remain to be described.

Altogether, this will determine the scope of IKKβ inhibitors with respect to diseases. Some inhibitors are already in clinical trials with reference to anti-cancer therapy (e.g. CHS 828), but the function NF-κB plays in different tumor types might differ, potentially limiting the inhibitors to certain cancers. Likewise, many companies aim to target different inflammatory diseases, with results from clinical trials still outstanding. However, IKK inhibitors seem also to have promise in conditions such as diabetes or muscle atrophy based on genetic mouse models and pharmacological *in vivo* animal studies [69,89,93]. Results from clinical trials will certainly be important for judging whether excitement on current IKKβ inhibitors as potential drugs for such a wide range of diseases is justified.

3. Conclusion

After the identification of a great variety of signaling molecules involved in NF-κB activation via IKKβ, deciphering the exact pathways from cell surface receptors via adapter proteins and kinases to IKKβ as converging point and key enzyme is an ongoing process. Recent data demonstrate that the signaling requires activating ubiquitinations which form K63-linked polyubiquitin chain platforms for the binding of signaling molecules. This may be crucial for scaffolding effector molecules in order to pass on the signal. Based on these findings, chemical substances interfering with activating ubiquitinations might be interesting future drugs.

Competing interests

The authors declare to have no competing interests.

References

[1] Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. Sci STKE 2006;2006:re13.
[2] Perkins ND. Integrating cell-signalling pathways with NF-kappaB and IKK function. Nat Rev Mol Cell Biol 2007;8:49–62.
[3] Scheidereit C. IkappaB kinase complexes: gateways to NF-kappaB activation and transcription. Oncogene 2006;25:6685–705.

[4] Hayden MS, Ghosh S. Signaling to NF-{kappa}B. Genes Develop 2004;18:2195–224.
[5] Perkins ND, Gilmore TD. Good cop, bad cop: the different faces of NF-kappaB. Cell Death Differ 2006;13:759–72.
[6] Karin M. NF-kappaB and cancer: mechanisms and targets. Mol Carcinog 2006;45:355–61.
[7] Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. Nat Rev Immunol 2005;5: 749–59.
[8] Basseres DS, Baldwin AS. Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. Oncogene 2006;25:6817–30.
[9] Courtois G, Gilmore TD. Mutations in the NF-kappaB signaling pathway: implications for human disease. Oncogene 2006;25:6831–43.
[10] Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. Oncogene 2006;25:6758–80.
[11] Kim HJ, Hawke N, Baldwin AS. NF-kappaB and IKK as therapeutic targets in cancer. Cell Death Differ 2006;13:738–47.
[12] Yamamoto Y, Gaynor RB. Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. J Clin Invest 2001;107:135–42.
[13] Nolan GP, Ghosh S, Liou HC, Tempst P, Baltimore D. DNA binding and I kappa B inhibition of the cloned p65 subunit of NF-kappa B, a rel-related polypeptide. Cell 1991;64:961–9.
[14] Huxford T, Huang DB, Malek S, Ghosh G. The crystal structure of the IkappaBalpha/NF-kappaB complex reveals mechanisms of NF-kappaB inactivation. Cell 1998;95:759–70.
[15] Jacobs MD, Harrison SC. Structure of an IkappaBalpha/NF-kappaB complex. Cell 1998;95:749–58.
[16] Birbach A, Gold P, Binder BR, Hofer E, de Martin R, Schmid JA. Signaling molecules of the NF-kappa B pathway shuttle constitutively between cytoplasm and nucleus. J Biol Chem 2002;277:10842–51.
[17] Huang TT, Kudo N, Yoshida M, Miyamoto S. A nuclear export signal in the N-terminal regulatory domain of IkappaBalpha controls cytoplasmic localization of inactive NF-kappaB/IkappaBalpha complexes. Proc Natl Acad Sci USA 2000;97:1014–9.
[18] Johnson C, Van Antwerp D, Hope TJ. An N-terminal nuclear export signal is required for the nucleocytoplasmic shuttling of IkappaBalpha. Embo J 1999;18:6682–93.
[19] Fuchs SY, Spiegelman VS, Kumar KG. The many faces of beta-TrCP E3 ubiquitin ligases: reflections in the magic mirror of cancer. Oncogene 2004;23:2028–36.
[20] Tan P, Fuchs SY, Chen A, Wu K, Gomez C, Ronai Z, et al. Recruitment of a ROC1-CUL1 ubiquitin ligase by Skp1 and HOS to catalyze the ubiquitination of I kappa B alpha. Mol Cell 1999;3:527–33.
[21] Yaron A, Hatzubai A, Davis M, Lavon I, Amit S, Manning AM, et al. Identification of the receptor component of the IkappaBalpha-ubiquitin ligase. Nature 1998;396:590–4.
[22] DiDonato JA, Hayakawa M, Rothwarf DM, Zandi E, Karin M. A cytokine-responsive IkappaB kinase that activates the transcription factor NF-kappaB. Nature 1997;388:548–54.
[23] Mercurio F, Zhu H, Murray BW, Shevchenko A, Bennett BL, Li J, et al. IKK-1 and IKK-2: cytokine-activated IkappaB kinases essential for NF-kappaB activation. Science 1997;278:860–6.
[24] Woronicz JD, Gao X, Cao Z, Rothe M, Goeddel DV. IkappaB kinase-beta: NF-kappaB activation and complex formation with IkappaB kinase-alpha and NIK. Science 1997;278:866–9.
[25] Zandi E, Rothwarf DM, Delhase M, Hayakawa M, Karin M. The IkappaB kinase complex (IKK) contains two kinase subunits, IKKalpha and IKKbeta, necessary for IkappaB phosphorylation and NF-kappaB activation. Cell 1997;91:243–52.
[26] Rothwarf DM, Zandi E, Natoli G, Karin M. IKK-gamma is an essential regulatory subunit of the IkappaB kinase complex. Nature 1998;395:297–300.
[27] Yamaoka S, Courtois G, Bessia C, Whiteside ST, Weil R, Agou F, et al. Complementation cloning of NEMO, a component of the IkappaB kinase complex essential for NF-kappaB activation. Cell 1998;93: 1231–40.
[28] Solt LA, Madge LA, Orange JS, May MJ. Interleukin-1-induced NF-kappaB activation is NEMO-dependent but does not require IKKbeta. J Biol Chem 2007;282:8724–33.
[29] Sato S, Sanjo H, Takeda K, Ninomiya-Tsuji J, Yamamoto M, Kawai T, et al. Essential function for the kinase TAK1 in innate and adaptive immune responses. Nat Immunol 2005;6:1087–95.
[30] Yang J, Lin Y, Guo Z, Cheng J, Huang J, Deng L, et al. The essential role of MEKK3 in TNF-induced NF-kappaB activation. Nat Immunol 2001;2:620–4.
[31] Inohara N, Koseki T, Lin J, del Peso L, Lucas PC, Chen FF, et al. An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways. J Biol Chem 2000;275:27823–31.
[32] Tegethoff S, Behlke J, Scheidereit C. Tetrameric oligomerization of IkappaB kinase gamma (IKKgamma) is obligatory for IKK complex activity and NF-kappaB activation. Mol Cell Biol 2003;23:2029–41.
[33] Chen ZJ. Ubiquitin signalling in the NF-kappaB pathway. Nat Cell Biol 2005;7:758–65.
[34] Chen ZJ, Parent L, Maniatis T. Site-specific phosphorylation of IkappaBalpha by a novel ubiquitination-dependent protein kinase activity. Cell 1996;84:853–62.
[35] Deng L, Wang C, Spencer E, Yang L, Braun A, You J, et al. Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. Cell 2000;103:351–61.
[36] Wang C, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. Nature 2001;412:346–51.
[37] Chen ZJ, Bhoj V, Seth RB. Ubiquitin, TAK1 and IKK: is there a connection? Cell Death Differ 2006.
[38] May MJ, Larsen SE, Shim JH, Madge LA, Ghosh S. A novel ubiquitin-like domain in IkappaB kinase beta is required for functional activity of the kinase. J Biol Chem 2004;279:45528–39.
[39] Boone DL, Turer EE, Lee EG, Ahmad RC, Wheeler MT, Tsui C, et al. The ubiquitin-modifying enzyme A20 is required for termination of Toll-like receptor responses. Nat Immunol 2004;5:1052–60.
[40] Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, et al. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature 2004;430:694–9.
[41] Brummelkamp TR, Nijman SM, Dirac AM, Bernards R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature 2003;424:797–801.
[42] Kovalenko A, Chable-Bessia C, Cantarella G, Israel A, Wallach D, Courtois G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature 2003;424:801–5.
[43] Karin M. How NF-kappaB is activated: the role of the IkappaB kinase (IKK) complex. Oncogene 1999;18:6867–74.
[44] Prajapati S, Verma U, Yamamoto Y, Kwak YT, Gaynor RB. Protein phosphatase 2Cbeta association with the IkappaB kinase complex is involved in regulating NF-kappaB activity. J Biol Chem 2004;279:1739–46.
[45] Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. Nat Rev Mol Cell Biol 2004;5:392–401.
[46] Yang F, Tang E, Guan K, Wang CY. IKK beta plays an essential role in the phosphorylation of RelA/p65 on serine 536 induced by lipopolysaccharide. J Immunol 2003;170:5630–5.
[47] Beinke S, Robinson MJ, Hugunin M, Ley SC. Lipopolysaccharide activation of the TPL-2/MEK/extracellular signal-regulated kinase mitogen-activated protein kinase cascade is regulated by IkappaB kinase-induced proteolysis of NF-kappaB1 p105. Mol Cell Biol 2004;24:9658–67.
[48] Waterfield M, Jin W, Reiley W, Zhang M, Sun SC. IkappaB kinase is an essential component of the Tpl2 signaling pathway. Mol Cell Biol 2004;24:6040–8.
[49] Wegener E, Oeckinghaus A, Papadopoulou N, Lavitas L, Schmidt-Supprian M, Ferch U, et al. Essential role for IkappaB kinase beta in

remodeling Carma1–Bcl10–Malt1 complexes upon T cell activation. Mol Cell 2006;23:13–23.
[50] Wu ZH, Shi Y, Tibbetts RS, Miyamoto S. Molecular linkage between the kinase ATM and NF-kappaB signaling in response to genotoxic stimuli. Science 2006;311:1141–6.
[51] Hu MC, Lee DF, Xia W, Golfman LS, Ou-Yang F, Yang JY, et al. IkappaB kinase promotes tumorigenesis through inhibition of forkhead FOXO3a. Cell 2004;117:225–37.
[52] Gringhuis SI, Garcia-Vallejo JJ, van Het Hof B, van Dijk W. Convergent actions of I kappa B kinase beta and protein kinase C delta modulate mRNA stability through phosphorylation of 14-3-3 beta complexed with tristetraprolin. Mol Cell Biol 2005;25:6454–63.
[53] Gao Z, Hwang D, Bataille F, Lefevre M, York D, Quon MJ, et al. Serine phosphorylation of insulin receptor substrate 1 by inhibitor kappa B kinase complex. J Biol Chem 2002;277:48115–21.
[54] Lee S, Andrieu C, Saltel F, Destaing O, Auclair J, Pouchkine V, et al. IkappaB kinase beta phosphorylates Dok1 serines in response to TNF, IL-1, or gamma radiation. Proc Natl Acad Sci USA 2004;101:17416–21.
[55] Gerondakis S, Grumont R, Gugasyan R, Wong L, Isomura I, Ho W, et al. Unravelling the complexities of the NF-kappaB signalling pathway using mouse knockout and transgenic models. Oncogene 2006;25:6781–99.
[56] Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM. Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. Science 1999;284:321–5.
[57] Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. Nature 1995;376:167–70.
[58] Park JM, Greten FR, Wong A, Westrick RJ, Arthur JS, Otsu K, et al. Signaling pathways and genes that inhibit pathogen-induced macrophage apoptosis—CREB and NF-kappaB as key regulators. Immunity 2005;23:319–29.
[59] Ruocco MG, Maeda S, Park JM, Lawrence T, Hsu LC, Cao Y, et al. I{kappa}B kinase (IKK){beta}, but not IKK{alpha}, is a critical mediator of osteoclast survival and is required for inflammation-induced bone loss. J Exp Med 2005;201:1677–87.
[60] Chen LW, Egan L, Li ZW, Greten FR, Kagnoff MF, Karin M. The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. Nat Med 2003;9:575–81.
[61] Herrmann O, Baumann B, de Lorenzi R, Muhammad S, Zhang W, Kleesiek J, et al. IKK mediates ischemia-induced neuronal death. Nat Med 2005;11:1322–9.
[62] Pasparakis M, Courtois G, Hafner M, Schmidt-Supprian M, Nenci A, Toksoy A, et al. TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. Nature 2002;417:861–6.
[63] Maeda S, Chang L, Li ZW, Luo JL, Leffert H, Karin M. IKKbeta is required for prevention of apoptosis mediated by cell-bound but not by circulating TNFalpha. Immunity 2003;19:725–37.
[64] Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–90.
[65] Lawrence T, Bebien M, Liu GY, Nizet V, Karin M. IKKalpha limits macrophage NF-kappaB activation and contributes to the resolution of inflammation. Nature 2005;434:1138–43.
[66] Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. Nat Med 2005;11:191–8.
[67] Pasparakis M, Schmidt-Supprian M, Rajewsky K. IkappaB kinase signaling is essential for maintenance of mature B cells. J Exp Med 2002;196:743–52.
[68] Schmidt-Supprian M, Tian J, Grant EP, Pasparakis M, Maehr R, Ovaa H, et al. Differential dependence of CD4+CD25+ regulatory and natural killer-like T cells on signals leading to NF-kappaB activation. Proc Natl Acad Sci USA 2004;101:4566–71.
[69] Cai D, Frantz JD, Tawa Jr NE, Melendez PA, Oh BC, Lidov HG, et al. IKKbeta/NF-kappaB activation causes severe muscle wasting in mice. Cell 2004;119:285–98.
[70] Voll RE, Jimi E, Phillips RJ, Barber DF, Rincon M, Hayday AC, et al. NF-kappa B activation by the pre-T cell receptor serves as a selective survival signal in T lymphocyte development. Immunity 2000;13:677–89.
[71] Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature 1998;396:77–80.
[72] Yamamoto Y, Yin MJ, Lin KM, Gaynor RB. Sulindac inhibits activation of the NF-kappaB pathway. J Biol Chem 1999;274:27307–14.
[73] Keifer JA, Guttridge DC, Ashburner BP, Baldwin Jr AS. Inhibition of NF-kappa B activity by thalidomide through suppression of IkappaB kinase activity. J Biol Chem 2001;276:22382–7.
[74] Rossi A, Kapahi P, Natoli G, Takahashi T, Chen Y, Karin M, et al. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of IkappaB kinase. Nature 2000;403:103–8.
[75] Waxman S, Anderson KC. History of the development of arsenic derivatives in cancer therapy. Oncologist 2001;6(Suppl. 2):3–10.
[76] Kapahi P, Takahashi T, Natoli G, Adams SR, Chen Y, Tsien RY, et al. Inhibition of NF-kappa B activation by arsenite through reaction with a critical cysteine in the activation loop of Ikappa B kinase. J Biol Chem 2000;275:36062–6.
[77] Reynaert NL, van der Vliet A, Guala AS, McGovern T, Hristova M, Pantano C, et al. Dynamic redox control of NF-kappaB through glutaredoxin-regulated *S*-glutathionylation of inhibitory kappaB kinase beta. Proc Natl Acad Sci USA 2006;103:13086–91.
[78] Hammaker D, Sweeney S, Firestein GS. Signal transduction networks in rheumatoid arthritis. Ann Rheum Dis 2003;62(Suppl. 2):ii86–9.
[79] Karin M, Yamamoto Y, Wang QM. The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 2004;3:17–26.
[80] Castro AC, Dang LC, Soucy F, Grenier L, Mazdiyasni H, Hottelet M, et al. Novel IKK inhibitors: beta-carbolines. Bioorg Med Chem Lett 2003;13:2419–22.
[81] Kishore N, Sommers C, Mathialagan S, Guzova J, Yao M, Hauser S, et al. A selective IKK-2 inhibitor blocks NF-kappa B-dependent gene expression in interleukin-1 beta-stimulated synovial fibroblasts. J Biol Chem 2003;278:32861–7.
[82] Nagashima K, Sasseville VG, Wen D, Bielecki A, Yang H, Simpson C, et al. Rapid TNFR1-dependent lymphocyte depletion in vivo with a selective chemical inhibitor of IKKbeta. Blood 2006;107:4266–73.
[83] Schopf L, Savinainen A, Anderson K, Kujawa J, DuPont M, Silva M, et al. IKKbeta inhibition protects against bone and cartilage destruction in a rat model of rheumatoid arthritis. Arthritis Rheum 2006;54:3163–73.
[84] Burke JR, Pattoli MA, Gregor KR, Brassil PJ, MacMaster JF, McIntyre KW, et al. BMS-345541 is a highly selective inhibitor of I kappa B kinase that binds at an allosteric site of the enzyme and blocks NF-kappa B-dependent transcription in mice. J Biol Chem 2003;278:1450–6.
[85] McIntyre KW, Shuster DJ, Gillooly KM, Dambach DM, Pattoli MA, Lu P, et al. A highly selective inhibitor of I kappa B kinase, BMS-345541, blocks both joint inflammation and destruction in collagen-induced arthritis in mice. Arthritis Rheum 2003;48:2652–9.
[86] MacMaster JF, Dambach DM, Lee DB, Berry KK, Qiu Y, Zusi FC, et al. An inhibitor of IkappaB kinase, BMS-345541, blocks endothelial cell adhesion molecule expression and reduces the severity of dextran sulfate sodium-induced colitis in mice. Inflamm Res 2003;52:508–11.
[87] Ziegelbauer K, Gantner F, Lukacs NW, Berlin A, Fuchikami K, Niki T, et al. A selective novel low-molecular-weight inhibitor of IkappaB kinase-beta (IKK-beta) prevents pulmonary inflammation and shows broad anti-inflammatory activity. Br J Pharmacol 2005;145:178–92.
[88] Olsen LS, Hjarnaa PJ, Latini S, Holm PK, Larsson R, Bramm E, et al. Anticancer agent CHS 828 suppresses nuclear factor-kappa B activity

in cancer cells through downregulation of IKK activity. Int J Cancer 2004;111:198–205.
[89] Kamon J, Yamauchi T, Muto S, Takekawa S, Ito Y, Hada Y, et al. A novel IKKbeta inhibitor stimulates adiponectin levels and ameliorates obesity-linked insulin resistance. Biochem Biophys Res Commun 2004;323:242–8.
[90] Gilmore TD, Herscovitch M. Inhibitors of NF-kappaB signaling: 785 and counting. Oncogene 2006;25:6887–99.
[91] Chu L, Gu J, He Z, Xiao T, Liu X. Adenoviral vector expressing CYLD augments antitumor activity of TRAIL by suppression of NF-kappaB survival signaling in hepatocellular carcinoma. Cancer Biol Ther 2006;5:615–22.
[92] Swinney DC, Xu YZ, Scarafia LE, Lee I, Mak AY, Gan QF, et al. A small molecule ubiquitination inhibitor blocks NF-kappa B-dependent cytokine expression in cells and rats. J Biol Chem 2002;277:23573–81.
[93] Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001;293:1673–7.

Johannes A. Schmid received his MSc in 1990 from the Institute of Applied Genetics und Cell Biology at the University of Natural Resources and Applied Life Sciences, Vienna, Austria. He did his PhD thesis at the Institute of Pathophysiology of the Medical University Vienna and graduated in 1994.
After a two-year postdoctoral fellowship at the Novartis Research Institute, Vienna, working on differentiation of keratinocytes, he joined the department of vascular biology and thrombosis research of the medical university, Vienna focusing on different aspects of inflammation and the NF-κB signaling pathway. There he started to investigate IKKβ, one of the key molecules of the canonical NF-κB activation pathway with an emphasis on identifying unknown interaction partners of this kinase and characterizing signaling crosstalk, as well as intracellular dynamics of effector molecules. After receiving the university lecture qualification in 2002, he went on a sabbatical leave to the immunology department of Yale University Medical School in 2003. Back at the Medical University, Vienna, he joined an initiative for the foundation of a new institute, the Ludwig Boltzmann Institute for Cancer Research, which was launched in Sept. 2005 employing about 20 scientists. As deputy director of this institute he is heading a group working on the role of inflammation in cancer development with a focus on prostate cancer.

Andreas Birbach, studied biochemistry at the University of Vienna, Austria, and received his masters degree in 2000 after working on signal transduction pathways in NF-κB activation. He went on to do his PhD thesis at the Friedrich Miescher Institute, Basel, Switzerland, studying actin cytoskeleton regulation in hippocampal neurons and received his PhD from the University of Basel in 2006. Since then he is a postdoctoral researcher at the Ludwig Boltzmann Institute for Cancer Research in Vienna working on molecular aspects of prostate tumorigenesis.