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Commentary

IκB kinase ε (IKKe): A therapeutic target in inflammation and cancer

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1. Introduction to canonical versus non-canonical IκB kinases (IKK)

Our first line of defense against viral and bacterial attacks relies on the innate immune system, which detects non-self products by means of specific surface, endosomal or cytosolic receptors. For example, bacterial lipopolysaccharide (LPS) is sensed by Toll-like receptor (TLR)-4 on the cell surface, whereas viral RNA is sensed by endosomal TLR3 or cytosolic retinoid acid-inducible gene (RIG-I) [1]. A major output from these receptors is the activation of transcription factors belonging to the nuclear factor-kappa B (NF-κB) and interferon (IFN) regulatory factor (IRF) family, which control the expression of multiple immune regulatory genes. In this review we will focus on p50/p65 NF-κB and IRF3, whose activation involves specific members of the inhibitor of κB (IκB) kinase (IKK) family. The canonical IKKs, IKKα and IKKβ, form a complex with the adaptor protein NEMO (also known as IKKγ), which has a regulatory role. This IKK complex is required for proper NF-κB signaling in response to multiple proinflammatory stimuli such as TNF and TLR ligands. IKKα and IKKβ are Ser/Thr kinases that phosphorylate the NF-κB inhibitor protein IκBα, resulting in its Lys48-linked polyubiquitination and subsequent proteasomal degradation. This allows NF-κB to translocate to the nucleus and bind to specific DNA elements [2]. IKKα and IKKβ contain an N-terminal catalytic kinase domain (KD), a more central leucine zipper (LZ) and helix loop helix (HLH) domain, and a C-terminal NEMO-binding domain (NBD) (Fig. 1). The major impact of the IKKs on NF-κB signaling inspired many researchers to search intensively for IKK-related kinases. Based on sequence similarities with IKKα and IKKβ, two IKK-related kinases, TANK binding kinase 1 (TBK1) and IKKε (also known as IKK-δ or IKK-e), were discovered. IKKε and TBK1 are known as the non-canonical IKKs and expand the IKK family to four members [3,4]. The kinase domain of IKKε shares 33% and 31% amino acid identity with the corresponding domains in IKKα and IKKβ, respectively, and 67% with TBK1 [5]. Furthermore, TBK1 and IKKε have a similar domain composition as the canonical IKKs. IKKβ and the non-canonical IKKs also share an ubiquitin-like domain (ULD), which is required for optimal kinase activity [6]. Importantly, IKKε and TBK1 lack a NBD and do not interact with NEMO, but each forms similar complexes with other specific scaffolding proteins (see Section 4).

Although IKKε and TBK1 were originally classified as IKKs based on their ability to phosphorylate IκBα upon overexpression, studies with IKKε and TBK1 deficient cells revealed that these kinases are dispensable for IκBα phosphorylation [7,8]. Instead, both kinases were shown to contribute to LPS- and virus-induced phosphorylation of IRF3 and IRF7, allowing their homodimerization, nuclear import, and activation of type I IFN genes (IFN-α and IFN-β) [9]. IKKε and TBK1 are similar in their ability to activate IRF3 and IRF7 and their ability to phosphorylate the IκBα inhibitor of NF-κB, but they present some differences that may be of importance. For instance, deletion of the TBK1 gene leads to embryonic lethality at day 15 due to TNF-induced apoptosis in the...
The IKK family can be divided into two groups: the classical or canonical IKKs (IKKα and IKKβ) and the non-canonical IKKs (TBK1 and IKKc). In addition, two splice variants of IKKc (IKKc-sv1 and IKKc-sv2), respectively missing 25 and 13 amino acids at their C-terminal end, have been described. The following domains are depicted: kinase domain (KD), leucine zipper (LZ), helix-loop-helix (HLH), NEMO-binding domain (NBD), ubiquitin-like domain (ULD). Numbers indicate kinase-activating phosphorylation sites.

Fig. 1. Domain structure of the IKK family.

Liver [7], whereas IKKc deficient mice are viable [9]. The reason for these different phenotypes is still unclear but may in part reflect the differential expression and use of TBK1 and IKKc in various cell types (see also section 2). Moreover, TBK1 and IKKc may have non-redundant functions in other signaling pathways than those controlling IRF and canonical NF-κB activity. For example, recently published work demonstrated a specific role for TBK1 as a negative regulator of non-canonical NF-κB signaling in B cells stimulated with BAFF or anti-CD40, which was associated with the inducible TBK1 mediated phosphorylation of the kinase NIK, leading to its degradation [10]. Although B cells express both TBK1 and IKKc, NIK phosphorylation was highly specific for TBK1. Since TBK1 and IKKc share the same substrate consensus phosphorylation motif [11], substrate specificity is likely driven by recruitment of TBK1 or IKKc to discrete signaling complexes, possibly involving specific scaffolding proteins (see Section 4). Given the ever-growing number of TBK1 and IKKc interaction partners and substrates [12], localized kinase activation and substrate modification could be an effective strategy to confer TBK1 or IKKc specific functions.

2. Expression of IKKc and TBK1

While IKKα, IKKβ and TBK1 are constitutively expressed in most cell types, basal IKKc expression is only observed in specific tissues (pancreas, thymus and spleen) and cell types (T-cells and peripheral blood leukocytes). However, in other cell types (e.g. fibroblasts) IKKc is rapidly upregulated by cytokines (e.g. TNF, IL-1, IL-6, IFN-γ), microbial products (e.g. LPS, viral RNA), and phorbol esters (PMA), and has therefore also been called inducible IKK or IKK-1 [12,13]. The promoter of the IKKc gene contains two putative NF-κB binding sites, of which only one was shown to be functionally active [14], as well as seven STAT3-binding sites, of which two are active [15]. Whether the increase in IKKc expression induces kinase activation is currently still unclear. Recently, two IKKc splice variants, missing 25 amino acids (IKKc-sv1) or 13 amino acids (IKKc-sv2) at their C-terminal end, were detected in human peripheral blood mononuclear cells [16]. While these splice variants are ubiquitously expressed at the mRNA level, both protein isoforms are selectively upregulated by TNF stimulation or virus infection. IKKc is frequently overexpressed in a number of human cancers, in particular breast, ovarian and pancreatic cancer and has been implicated in tumorigenesis (see Section 6). Although IKKc and TBK1 show many overlapping activities that are relevant to innate immunity and inflammation, the more restricted expression of IKKc is of particular interest. In this review we will therefore focus on IKKc and mainly refer to TBK1 for reasons of comparison.

3. Role of IKKc/TBK1 in NF-κB and IRF signaling

The identification of TBK1 and IKKc as IkBα kinases has always been very controversial and the source of many debates. Evidence is based primarily on the observation that overexpression of IKKc or TBK1 in cultured cells leads to phosphorylation of IkBα, but it only at one phosphoacceptor site (Ser36 or Ser32, respectively), driving increased IkBα turnover [13]. However, both IKKc and TBK1 deficient MEF cells display normal IkBα degradation in response to TNF, IL-1 or LPS, contradicting the overexpression data [7,8]. Nevertheless, TNF-, IL-1- or LPS-induced NF-κB dependent gene expression is abrogated in the absence of IKKc [7,8]. Therefore it is believed that IKKc influences NF-κB signaling and concomitant gene expression downstream of IkBα [12] (Fig. 2). In this context, several studies support a role for IKKc-mediated Ser468 and Ser536 phosphorylation of the p65 NF-κB subunit in the expression of a specific subset of NF-κB target genes in response to pro-inflammatory signals and viral infection [12,17–19]. It has also been shown that activated p65 (phosphorylated on Ser468 or Ser536) serves as a docking site to bring IKKc-enzymatic activity to κB-containing inflammatory gene promoters in the nucleus, enabling IKKc to phosphorylate adjacent c-Jun and initiate nuclear receptor corepressor clearance [20]. These results suggest that p65-activation of IKKc to promoters that exhibit AP1 and κB sites in close proximity (e.g. inos, cxc12, cxc9, cxc10, cc4, tnfaip3) may regulate their activation by initiating corepressor turnover. Besides p65, IKKc/TBK1 also targets c-Rel NF-κB, leading to its nuclear accumulation and activation of NF-κB dependent gene expression [21].

Next to their role in NF-κB signaling, TBK1 and IKKc are also implicated in IRF3 and IRF7 signaling in response to viral infection that is sensed by a diversity of receptors, such as TLR3 and RIG-1, leading to the production of type I IFNs [12] (Fig. 2). While IRF3 is constitutively expressed in the cytoplasm of many cells, IRF7 needs to be transcriptionally upregulated. Both IKKc and TBK1 directly phosphorylate IRF3 and IRF7 at their C-term, resulting in their homo- and heterodimerization and translocation to the nucleus. Mass spectrometry pinpointed Ser386, Ser396 and Ser402 in IRF3 as redundant phosphorylation sites for IKKc: upon viral infection of innate immune cells [22]. IKKc deficient MEF cells showed no change in IRF3 activation [9], whereas TBK1 deficient MEF cells showed reduced IRF3 activation upon TLR3 and TLR4 triggering [9,23,24]. However, IRF3 activation was completely abolished in TBK1 and IKKc double deficient MEFs [9]. IKKc overexpression, but not its kinase dead mutant, could restore IRF3 activation in TBK1 deficient cells, suggesting redundancy between both kinases [24]. Although IKKc and TBK1 seem to exert overlapping functions in IRF3 activation, mechanistic differences in the activation of TBK1 and IKKc: following virus infection have been observed (e.g. mitochondrial localization of IKKc versus cytoplasmic localization of TBK1) [25]. Moreover, partially different substrates have been reported for both kinases. It is interesting to note that the TLR adaptor protein MyD88 was recently shown to abrogate TLR3-induced IFN production by preventing IKKc: but not TBK1-mediated IRF3 phosphorylation [25], indicating that both kinases can also be differentially regulated.

4. Mechanism and regulation of IKKc/TBK1 activation

Although IKKc/TBK1 can be activated by several inflammatory stimuli, their activation by TLR3, TLR4 and RIG-1 receptors has been best documented [26,27]. Receptor stimulation induces the recruitment of specific adaptor proteins to the receptor (TRIF for
Fig. 2. IKKc mediated signaling to NF-κB and IRF3 in response to specific receptors.

Non-canonical IKKs (IKKc and TBK1) can be activated by two signaling pathways: (1) IKKc/β mediated activation of IKKc/TBK1. Ligand binding to several receptors (TNF-R1, IL-1R, TLR3, TLR4, RIG-I) initiates the recruitment of specific adaptor proteins (e.g. Mal, MyD88, TRAM, TRIF, MAVS), E3 ubiquitin ligases and kinases (not shown) to the receptor, eventually resulting in the activation of the canonical IKK (IKKα/IKKβ/NEMO) complex. This leads to the IKKβ-mediated phosphorylation and subsequent Lys48-linked polyubiquitination of IkBα, resulting in its proteasomal degradation and release of the p50-p65 NF-κB heterodimer, which then translocates to the nucleus. (2) IKKα/IKKβ-independent and TRAF3-dependent IKKc/TBK1 autoactivation. TRIF-dependent TLR3 and TLR4 signaling, as well as MAVS-dependent RIG-I signaling, induce IKKc/TBK1 autoactivation via TRAF3. This also requires the binding of IKKc/TBK1 to different scaffold proteins (TANK, NAP1, SINTBAD). IKKc/TBK1 then mediate different activities: (A) IKKc/TBK1 can phosphorylate NF-κB (p65), contributing to NF-κB dependent expression of specific genes. (B) IKKc/TBK1 can phosphorylate IRF3 (and IRF7; not shown), leading to its homodimerization and nuclear translocation. (C) IKKc/TBK1 can phosphorylate c-jun, leading to the release of the nuclear repressor complex (NCoR). (D) IKKc/TBK1 can phosphorylate their own scaffold proteins (TANK, NAP1 or SINTBAD), the function of which is still unclear. (E) IKKc/TBK1 is also able to phosphorylate the canonical IKKs, leading to their inactivation.

TLR3 and TLR4, MAVS for RIG-I). Subsequently, these adaptors interact with the E3 ubiquitin ligase TRAF3 to activate IRF3, or with the E3 ubiquitin ligase TRAF6 and RIP1 kinase to activate NF-κB. Autoubiquitination of TRAF3 is needed for the recruitment and activation of IKKc and TBK1 [28]. At later time points, the presence of IKKc in the MAVS complex eventually leads to the release of TRAF3, with the shutdown of IFN signaling as a consequence [29].

At the protein level, IKKc (and TBK1) activity is regulated by phosphorylation at a single site (Ser172) in its activation loop [30], in contrast to IKKα and IKKβ, which require phosphorylation at two sites (IKKα: Ser176 and Ser180; IKKβ: Ser177 and Ser181) for their function. Although overexpression studies indicated a role for autophosphorylation of IKKc/TBK1 at Ser172 [13,31], activation of the endogenous kinases does not only involve autophosphorylation. Indeed, cells treated with the TBK1 and IKKc inhibitor BX795 still showed phosphorylation of TBK1 and IKKc at Ser172 in response to poly(I:C), LPS, TNF and IL-1 [32], suggesting the involvement of other kinases. Clark and co-workers could show that Ser172 phosphorylation of IKKc/TBK1 is impaired in IKKα deficient cells treated with an IKKc inhibitor [31], indicating IKKc as a potential IKKc kinase. In line with these observations, TNF-induced activation of IKKc/TBK1 seems to be mediated solely by the canonical IKKs [31]. Interestingly, IKKc/TBK1 activation by the canonical IK pathway only results in NF-κB activation and not IRF3 activation.

Similar to the canonical kinases IKKα and IKKβ, which require the formation of a complex with the adaptor protein NEMO, also TBK1 and IKKc activation involves specific adaptor proteins. So far, three scaffold proteins have been shown to bind IKKc/TBK1 and to promote IKKc/TBK1-mediated phosphorylation of IRF3 and IRF7: NAK associated protein 1 (NAP1), TNF receptor-associated factor (TRAF) family member-associated NF-κB activator (TANK) and similar to NAP and TBK1 adaptor (SINTBAD) [30,33–35]. Therefore, it is an attractive idea that different scaffold proteins might assemble distinct TBK1 and IKKc complexes under distinct conditions, providing signaling specificity. Whether or not these scaffold proteins assemble hetero- or homodimers of IKKc and TBK1 is currently still unknown.

In contrast to the positive regulation of NF-κB dependent gene expression by IKKc/TBK1, the latter have also been described to negatively regulate NF-κB activation in response to TNF or IL-1 stimulation. More specifically, IKKc/TBK1 was shown to directly phosphorylate the canonical IKKs outside their activation loop, restricting their activity [31] (Fig. 2). Interestingly, this negative regulation of the canonical IKKs by IKKc/TBK1 was impaired in TANK-deficient macrophages [36]. Using these cells it was also shown that TANK is required for IKc/TBK1 and NF-κB activation in response to LPS via both the MyD88- and TRIF-dependent pathways, and mediates the interaction of the non-canonical IKKs with the canonical IKKs. The latter most likely involves the binding of TANK to the canonical IKK adaptor protein NEMO [37]. Together,
these findings demonstrate a key role for the IKKε/TBK1 adaptor protein TANK in enabling the canonical and non-canonical IKKs to regulate each other.

In analogy to NEMO, one can expect that the mechanisms driving IKKε/TBK1 activation most likely require different post-translational modifications of their scaffold proteins. Indeed, IKKε/TBK1 dependent phosphorylation and Lys63-linked polyubiquitination of TANK have already been described in response to LPS stimulation [33], but their significance is still unclear.

Multiple negative regulatory mechanisms are in place to avoid excessive IKKε/TBK1 activity and IFN production. For example, the deubiquitinase DUBA reverses TRAF3 ubiquitination, disconnecting TRAF3 from its substrates TBK1 and IKKε [38]. Also the deubiquitinase CYLD negatively regulates RIG-I induced interferon production by deubiquitinating RIG-I and TBK1/IKKε [39,40]. Conversely, IKKε directly phosphorylates CYLD at Ser418, hereby decreasing its deubiquitinating potential but increasing the IKKε-induced cell transformation [41] (Fig. 3) (see Section 6). Others have shown that the ubiquitin-editing enzyme A20, in conjunction withTax1 binding protein 1 (TAX1BP1), antagonizes Lys63-polyubiquitination of TBK1 and IKKε. Surprisingly, A20-mediated inhibition of TBK1/IKKε-Lys63-polyubiquitination was independent of its DUB function [42]. Recently, the E3 ubiquitin ligases TRIP13 and DTX4 have been shown to negatively regulate IRF3 activation by inducing TBK1 Lys48-polyubiquitination and proteosomal degradation [43,44]. DTX4 showed weak or no activity against IKKε, while the activity of TRIP against IKKε was not studied. Interestingly, also many viruses themselves interfere with the IRF pathway and antiviral IFN production at the level of IKKε and/or TBK1. For example, specific viral components disturb the interaction of IKKε/TBK1 with other signaling proteins (e.g. Ebola virus VP35 protein [45], M protein of Corona virus [46], Hepatitis B or C virus [47,48]). Alternatively, viral proteins may act as alternative substrates for IKKε and TBK1, thereby targeting these kinases for degradation (e.g. Borna disease virus P protein, paramyxovirus V proteins [49,50]). These virus adaptations further emphasize the importance of the non-canonical kinases in antiviral signaling.

5. Role of IKKε in inflammatory and metabolic diseases

IKKε activity has been linked to the pathology of inflammatory diseases such as rheumatoid arthritis (RA) [51–53]. For instance, IKKε is constitutively expressed and phosphorylated in synovial intimal lining of RA patients, resulting in uncontrolled IRF3-driven production of proinflammatory mediators such as IFN-β, matrix metalloproteinases and chemokines [52]. Further supporting a major contribution of IKKε to the pathogenesis of RA is the finding that mice deficient in IKKε show less synovial inflammation in a passive K/BxN arthritis model due to lower expression of inflammatory mediators [51]. Furthermore, IKKε: single nucleotide polymorphisms have been associated with the early stages of RA [54], and genome wide associated studies revealed IKKε as a susceptibility locus for systemic lupus erythematosus (SLE), in which type 1 IFNs play a crucial role [55]. Computational analysis of predicted protein–protein interactions also pinpointed IKKε as a potential therapeutic target in psoriasis [56].

IKKε has also been implicated in pulmonary inflammation. In this context, IKKε deficient mice showed fewer infiltrating neutrophils and diminished expression of proinflammatory cytokines and chemokines after intranasal administration of IL-17. Surprisingly, in this model IKKε deficiency did not affect NF-κB activation, but did reduce MAP kinase signaling [57]. More specifically, IKKε was shown to be responsible for phosphorylation of the IL-17 receptor adaptor Act-1, thereby activating the TRAF2/ TRAF5 pathway leading to increased MAP kinase activation and leaving the TRAF6/NF-κB axis emanating from Act-1 undisturbed. Increased MAP kinase activation led to increased chemokine mRNA stability, contributing to increased chemokine production and neutrophil infiltration into the lungs. A role for IKKε in the regulation of IL-17 responses is also illustrated by the recent finding that IKKε can promote the AKT-mTOR signaling pathway, which mediates IL-1 induced Th17 maintenance, by phosphorylating and inactivating GSK3β [58]. Taken together, many studies indicate an important role of IKKε in the pathophysiology of inflammatory diseases, mainly by regulating NF-κB, IFN and IL-17 responses.

IKKε might also be a potential target to treat inflammatory pain. In inflammatory pain models (hind paw inflammation evoked by injection of zymosan or formalin), IKKε-deficient mice exhibited a significantly reduced nociceptive behavior in comparison with wild type mice, indicating that IKKε contributes to the development of inflammatory hyperalgesia [59]. At the same time, the provoked NF-κB activation in nociceptive neurons (neurons involved in perception of pain) was reduced, as reflected by lower inflammatory gene expression. Of interest, this process is independent of type I IFN responses, suggesting that IKKε is promoting inflammatory hyperalgesia merely by activating the NF-κB pathway.

Finally, IKKε has been reported as an important link between inflammation and obesity. Obese mice have major risks to develop inflammatory or metabolic diseases such as type 2 diabetes [60]. In fact, obesity mimics a permanent low-grade inflammatory condition due to dietary fatty acid recognition byTLR4 [61] or hypoxia [62]. Elevated NF-κB activity in obese mice drives increased IKKε expression in the liver, adipocytes and adipose tissue macrophages [63,64]. Furthermore, mice deficient in IKKε were found to be protected from high fat diet-induced obesity and showed less chronic liver inflammation, hepatic steatosis and insulin resistance. These events are regulated by changes in the expression of regulatory proteins and enzymes that are involved in glucose and lipid metabolism, and by a decrease in the production of proinflammatory cytokines and proteins involved in insulin resistance. Transfection of cultured adipocytes and hepatoma cells with IKKε induced similar changes, suggesting a direct role for IKKε in the regulation of hepatic inflammation. However, contradictory results were reported by another group, showing no difference between IKKε knockout and wild type mice in a model of high fat diet-induced obesity and insulin resistance [65]. Therefore, more work is needed to define the precise role of IKKε in the development of metabolic diseases.

6. Role of IKKε in cancer

IKKε has been associated with the initiation and progression of multiple cancers and might function as an oncogene for malignant transformation. For instance, several breast cancer cell lines and ~30% of primary human breast tumors express high levels of IKKε [66]. Increased IKKε expression is due to an up till now unknown mutation regulating IKKε transcript levels or to an amplification of the 1q32 region comprising the IKKBE locus [67,68]. Ectopic expression of IKKε in immortalized mammary epithelial cells at levels found in human cancer cells renders them tumorigenic, confirming that the allele amplified in breast cancer specimens is transforming [67]. High IKKε expression is often associated with the accumulation of c-Rel and p65 NF-κB subunits in the nucleus (e.g. in primary breast tumors), and IKKε silencing in several breast cancer cell lines was shown to reduce NF-κB activation and cell proliferation [69]. Similarly, introducing a kinase inactive IKKε mutant (IKKε K38A) in breast cancer cells, which exerts a dominant negative effect on endogenous IKKε, reduced NF-κB dependent
gene expression (e.g. cyclin D1 and RelB) [70], demonstrating the necessity of IKK\(\alpha\) catalytic activity. Recently, this was further confirmed by demonstrating the requirement of IKK\(\alpha\)-mediated phosphorylation at Ser418 of the tumor suppressor CYLD, which prevents its deubiquitinating activity on NF-\(\kappa\)B signaling proteins such as TRAF2 and NEMO [41,71] (Fig. 3). Moreover, IKK\(\alpha\) also activates the E3 ubiquitin ligase TRAF2 by direct phosphorylation at Ser11, resulting in increased TRAF2 ubiquitination [71]. Together these events lead to enhanced NF-\(\kappa\)B activation, which promotes survival, transformation and proliferation of mammary epithelial cells (Fig. 3). Furthermore, IKK\(\alpha\) (as well as TBK1) may contribute to enhanced NF-\(\kappa\)B activity and tumorigenesis by directly phosphorylating NF-\(\kappa\)B p65 (as described above) or by phosphorylating Akt, which then phosphorylates and activates p65 [72,73]. In addition, elevated IKK\(\alpha\) levels are also associated with STAT1 activation in different primary tumors and cell lines derived from a diversity of cancers, like lung and breast carcinoma [15,74,75], which may also contribute to the oncogenic activities of IKK\(\alpha\). As IRF3 suppression did not affect IKK\(\alpha\)-induced cell transformation, the oncogenic potential of IKK\(\alpha\) seems to be independent of its IRF3 signaling function [67].

More recently, human ovarian cancer cell lines and primary tumors were also shown to have elevated levels and activity of IKK\(\alpha\), which was associated with a lower overall survival rate [76,77]. Furthermore, alterations of IKK\(\beta\) were associated with late-stage and high-grade tumors, suggesting a role of IKK\(\beta\) in ovarian tumor progression rather than in tumor initiation. Finally, IKK\(\beta\) was also described as an oncogene in prostate and oesophageal squamous cell carcinoma with increased levels of different NF-\(\kappa\)B family members [78–81], and in clear cell renal cell carcinoma [82].
High levels of IKKε coincide with resistance to well-established chemotherapeutics [83]. This is also the case for elevated NF-κB activation, which is known to contribute to cancer cell survival and to reduce sensitivity to chemotherapeutic agents and ionizing radiation [84]. Unfortunately, breast cancer cells in which IKKε was inactivated retained their resistance to chemotherapeutics despite a lowered NF-κB activation, suggesting that additional mechanisms must be involved in the regulation of cell death [69,77,85]. Finally, IKKε also accumulates in subnuclear promyelocytic leukemia (PML) bodies upon genotoxic stress, where it undergoes SUMOylation, leading to its activation and ultimately resulting in NF-κB-mediated anti-apoptotic responses [86]. In this respect, it is worthwhile to mention that several glioma cell lines and human primary glioma tissues exhibit elevated levels of IKKε and are less sensitive to DNA damage-induced apoptosis [87]. Suppression of IKKε, however, renders the cells more sensitive, confirming its pro-survival function.

7. Development and characterization of IKKε inhibitors

The role of IKKε in the development of inflammatory and metabolic diseases, as well as cancer, indicate the potential of IKKε as a therapeutic target. So far, only few small molecule inhibitors of IKKε have been described. BX-795 was the first IKKε inhibitor on the market and suppresses TBK1 and IKKε activity at nanomolar concentrations in vitro [32]. The specificity of BX-795 is however an issue since it was originally developed as an inhibitor of 3-phosphoinositide-dependent protein kinase 1 (PKD1) and only later found to also inhibit IKKε [88]. Moreover, BX-795 shows off-target effects towards several other kinases, including JNK and p38 MAP kinases [31,32]. A modified version of BX-795, MRT67307, no longer inhibits JNK or p38 MAP kinases, but still interferes with the activity of TBK1 and PDK1 [31,32]. More recently, a series of azabenzimidole derivatives and 2,4-diamino-5-cyclopropyl pyrimidines with improved kinase selectivity and drug-like properties were described [88,89]. However, these compounds still inhibit both IKKε and TBK1, with a slightly higher potency against TBK1. In mice, the pyrimidines significantly inhibited LPS-induced release of IFN-β, although toxicity was observed at higher doses [88,89]. It is worth mentioning that also a number of naturally occurring compounds (polyphenoles such as (--)-epigallocatechin-3-gallate, lutolin, quercetin) with anti-inflammatory properties have been shown to target TBK1 [90,91]. Activities against IKKε have not been described, but in general the specificity of these polyphenoles is low and most of them have multiple targets.

The development of more specific and better IKKε inhibitors may be enabled by the recent elucidation of the substrate specificity of IKKε and TBK1 [11]. These studies demonstrated that the consensus phosphorylation motif of IKKε differs from that of IKKβ, but is identical to that of TBK1, suggesting that the development of IKKε inhibitors that do not target TBK1 may be very difficult. Nevertheless, high-throughput screening using a specific IKKε/TBK1 substrate peptide resulted in several lead molecules that showed selectivity for either IKKε or TBK1 [11]. However, as IKKε and TBK1 show many overlapping functions in oncogenic and inflammatory signaling pathways, it is likely that the therapeutic effectiveness of IKKε specific inhibitors may be hampered by the redundant activity of TBK1. Therefore, at least in some cases inhibition of the activity of both kinases may be a better therapeutic approach. Finally, as overexpression of IKKε is linked with tumorigenesis, therapeutic approaches that would reduce IKKε expression to normal levels may be a valid alternative. Therefore, more fundamental research on the molecular mechanisms that regulate IKKε expression may further boost the development of IKKε targeting drugs.

8. Future perspectives

The potential value of IKKε as therapeutic target for anti-inflammatory or anti-cancer therapies requires further investigation into the mechanisms and pathways involved. The pharmaceutical industry has been very active in the development of IKKα/IKKβ inhibitors as novel anti-inflammatory agents, but so far with limited success. Since NF-κB mediates a number of physiological functions, non-selective and complete inhibition of the NF-κB pathway may lead to serious side-effects. This is also illustrated by the fact that p65-, IKKε-, IKKβ-, or NEMO-deficient mice die during embryonic development or perinatally. Compounds that more selectively repress the activation of NF-κB in response to specific receptors or the expression of only a specific subset of NF-κB dependent genes would be associated with fewer side effects. Therefore, IKKε could well be such a target with great clinical value. This is supported by the fact that IKKε knockout mice, in contrast to IKKα or IKKβ knockout mice, are viable and fertile. Since TBK1 knockout mice also die embryonically, the challenge will be to develop IKKε specific inhibitors that do not target TBK1.

In this context, it will also be important to further determine the relative contribution of IKKε and TBK1 in different pathways (e.g. NF-κB versus IRF) and pathophysiological processes. Modulation of IKKε or TBK1 expression and activity in distinct cell types or tissues by means of conditional knockout mice will therefore be of high value. In addition, knowledge of the different IKKε substrates and the physiological relevance of their phosphorylation may help to predict efficiency or side effects of IKKε inhibitors.

The relationship between the canonical and non-canonical IKKεs, and other signaling pathways, is also an open line of investigation. In particular the negative regulatory effect of IKKε/TBK1 on the canonical IKKεs could suggest that inhibition of IKKε/TBK1 may actually have a pro-inflammatory effect. In this context, a more detailed understanding of the molecular mechanisms involved in the counter-regulation in the IKK family could be helpful. Many of the players that are involved (e.g. TANK, NEMO) in the connection between IKK and IKK-related kinases are phosphorylated or modified by different types of ubiquitin chains, but the consequence of these modifications is not fully clear. IKKε is also believed to form distinct complexes with different adaptor proteins (TANK, NAP1, SINTBAD), which could perform different functions. Future studies on the existence and function of IKKε complexes consisting of distinct IKKε adaptor proteins are therefore of high interest as it may allow more selective IKKε targeting. Such studies may also help to explain why some stimuli (e.g. TNF, IL-1, IL-17) activate IKKε/TBK1 without inducing the phosphorylation of IRF3, whereas other stimuli (e.g. LPS, viral RNA) do activate this pathway via IKKα/TBK1. The strong inducibility of IKKε expression and its overexpression in multiple tumors is also worthwhile to examine. What are the upstream signals that can regulate IKKε expression? What determines IKKε stability? Is IKKε overexpression sufficient to be oncogenic? In this context, generating (e.g. mammary) tissue specific IKKε transgenic mice may be very informative.

In summary, the generation and exploitation of IKKε specific inhibitors and conditional knockout/knockin/transgenic mice of IKKε and its regulators or substrates is likely to give answers to many of the above mentioned questions. Eventually, this could lead to the use of IKKε specific inhibitors for the successful treatment of autoimmunity, obesity, diabetes and certain cancers.

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[28] Verstrepen L, Verhelst K, Carpentier I, Beyaert RTAIXBP1, a ubiquitin-binding adaptor protein in innate immunity and beyond. Trends Biochem Sci 2013;38:347–54.

[29] Paz S, Vilasco M, Werden SJ, Arguello M, Joseph-Pillai D, Zhao T, et al. A functional C-terminal TRAF3-binding site in MAVS participates in positive and negative regulation of the IFN antiviral response. Cell Res 2011.

[30] Cloutier TJ, Goria R, Gato JS, Patrascu F, Carpentier I, Chapelle JP, et al. Are the IKKs and IKK-related kinases TBK1 and IKK epsilon similarly activated. Trends Biochem Sci 2008;33:171–80.

[31] Clark K, Peggie M, Plater L, Sorcek RJ, Young ER, Madwed JB, et al. Novel cross-talk within the IKK family controls innate immunity. Biochem J 2011;434:93–104.

[32] Clark K, Plater L, Peggie C, Cohen P. The TRAF-associated protein TANK facilitates cross-talk within the IKK family during Toll-like receptor signaling. Proc Natl Acad Sci USA 2011;108:17093–98.

[33] Chariot A, Leonardi A, Muller J, Bonif M, Brown K, Siebenlist U. Association of the receptor TNF with TRAF3 and the kinase IKK regulator NEMO connects IKK complexes with IKK and TBK1 kinases. J Biol Chem 2002;277:37092–36.

[34] Koyama K, Goto T, Saito Y, Hasegawa D, Ono T, et al. A ubiquitin ligase that regulates type I interferon production. Science 2007;318:1628–32.

[35] Ryzhakov G, Rondon PSINTBD. A novel component of innate antiviral immunity, shares a TBK1-binding domain with NAP1 and TANK. EMBO J 2007;26:3180–90.

[36] Goto K, Takeuchi O, Akira S, Cohen P. The TRAF-associated protein TANK inhibits IRAK-1 function by ubiquitinating IRAK-1. EMBO Rep 2008;9:810–6.

[37] Zhang M, Wu X, Liu C, Zhang M, Wright A, et al. Regulation of IkappaB kinase-related kinases and antiviral responses by tumor suppressor CYLD. J Biol Chem 2008;283:18621–30.

[38] Goto T, Kato K, Kato O, Sato H, Nishimune K, et al. IKK epsilon-induced STING promotes cell transformation. Mol Cell 2009;34:461–72.

[39] Parvatiyar K, Barber GN, Harhay EW. TAXIP1 and A20 inhibit antiviral signaling by targeting TBK1-IKKg kinases. J Biol Chem 2010;285:14999–5009.

[40] Cui J, Li Y, Zhu L, Liu D, Songyang Z, Wang HY, et al. NLRP3 negatively regulates type I interferon signaling by targeting the kinase TBK1 for degradation via the ubiquitin ligase DTX4. Nat Immunol 2012;13:387–95.

[41] Zhang M, Wang L, Zhao X, Zhao K, Meng H, Zhao W, et al. TRAF-interacting protein (TRIP) negatively regulates IFN-beta production and antiviral response by promoting proteasomal degradation of TANK-binding kinase 1. J Exp Med 2009;202:1703–11.

[42] BrinsKC, Cardenas WB, Basler CF. Ebola virus protein VP35 impairs the function of interferon regulatory factor-activating kinases IKBepsilon and TBK-1. J Virol 2008;82:3069–77.

[43] Li JK, Koh KH, Ng MH, Poon VK, Yuen KY, Zheng B, et al. Severe acute respiratory syndrome coronavirus M protein inhibits type I interferon production by impeding the formation of TRAF3.TANK.TBK1-IKKepsilon complex. J Biol Chem 2009;284:16202–09.

[44] Kaukinen P, Sillanpaa M, Nousiainen L, Melen K, Hepatitis Julkuen I. C virus NS2 protease inhibits host cell antiviral response by inhibiting IKKepsilon and TBK1 functions. J Med Virol 2013;85:71–82.

[45] Yu S, Chen J, Wu M, Chen H, Kato N, Hepatitis Y, Z. B virus polymerase induces the IKK-1 and -2-like receptor 3-mediated beta interferon induction in human hepatocytes through interference with interferon regulatory factor 3 activation and dampening of the IFN-beta induction. J Virol 2010;84:3069–77.

[46] Sun J, Sun Y, Zhang Z, Huang X, Li L, et al. Select paramyxovirus V proteins inhibit IFN3 activation by acting as alternative substrates for inhibitor of kappa B kinase epsilon (IKKE)/TBK1. J Biol Chem 2008;283:14269–76.

[47] Untersturb G, Ludwig S, Sonn A, Plamau Z, Dauber B, Krappmann D, et al. Viral interference with the interferon-(beta)-inducing TRAF family member-associated NF-(kappa)B activator (TANK)-binding kinase-1. Proc Natl Acad Sci USA 2005;102:13640–6.

[48] Morr I, Boyle DL, Ronacher L, Flores N, Firestein GS. Synergistic benefit in inflammatory arthritis by targeting I kappa B kinase epsilon and interferon beta. Ann Rheum Dis 2009;68:257–63.

[49] Sweeney SE, Mo L, Firestein GS. Antiviral gene expression in rheumatoid arthritis: role of IKKepsilon and interferon regulatory factor 3. Arthritis Rheum 2007;54:742–51.

[50] Vervoordeldonk MJ, Aalbers CJ, Tak PP. Interferon beta for rheumatoid arthritis: news for clothes for an old kid on the block. Ann Rheum Dis 2009;68:157–8.

[51] Fabbrizio D, Gonzalez-Ramirez P, Calaza M, Perez-Pampin E, Castas J, Torres MA, et al. Genetic variation in the nuclear factor kappaB pathway in relation to susceptibility to rheumatoid arthritis. Ann Rheum Dis 2009;68:579–83.
[55] Sandling JK, Garnier S, Sigurdsson S, Wang C, Nordmark G, Gunnarsson I, et al. A candidate gene study of the type I interferon pathway implicates IKKε and IL12 as risk loci for SLE. Eur J Hum Genet 2011;19:479–84.

[56] Park D, Jeong HO, Kim BC, Ha YM, Young Chung H. Computational approach to identify enzymes that are potential therapeutic candidates for psoriasis. Enzyme Res 2011;2011:826784.

[57] Bulek K, Liu C, Swaidani S, Wang L, Page RC. Gulen MF, et al. The inducible kinase IKKi is required for IL-17-dependent signaling associated with neutrophilia and pulmonary inflammation. Nat Immunol 2011;12:844–52.

[58] Gulen MF, Bulek K, Xiao H, Yu M, Gao J, Sun L, et al. Inactivation of the Enzyme GSKalpha by the Kinase IKKi Promotes AKT-mTOR Signaling Pathway that Mediates Interleukin-1-Induced Th1 Cell Maturation. Immunity 2012;37: 800–12.

[59] Moser CV, Kynast K, Baatz K, Russe OQ, Ferreiros N, Costiuk H, et al. The protein kinase IKKepsilon is a potential target for the treatment of inflammatory hyperglycaemia. J Immunol 2011;187:2617–25.

[60] Shoelson SE, Herrero L, Naza A, Obesity, inflammation, and insulin resistance. Gastroenterology 2007;133:2169–80.

[61] Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prado PO, Hirabara SM, Schenka AA, et al. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 2007;56:1986–98.

[62] Ye J, Gao Z, Yio J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am J Physiol Endocrinol Metab 2007;293:E1118–28.

[63] Chiang SH, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, et al. The protein kinase IKKepsilon regulates energy balance in obese mice. Cell 2009;138:961–75.

[64] Olefsky JM. IKKepsilon: a bridge between obesity and inflammation. Cell 2009;138:834–6.

[65] Scheja L, Heese B, Seedorf K. Beneficial effects of IKKepsilon-deficiency on body weight and insulin sensitivity are lost in high fat diet-induced obesity in mice. Biochem Biophys Res Commun 2011;407:288–94.

[66] Shen RR, Hahn WC. Emerging roles for the non-canonical IKKs in cancer. Oncogene 2011;30:631–41.

[67] Boehm JS, Zhao JJ, Yao J, Kim SY, Firestein R, Dunn IF, et al. Integrative genomic approaches identify IKKbeta as a breast cancer oncogene. Cell 2007;129:1065–79.

[68] Krishnamurthy S, Basu A. Regulation of IKKepsilon Expression by Akt2 iso- form. Genes Cancer 2011;2:1044–50.

[69] Qin B, Cheng K. Silencing of the IKKepsilon gene by siRNA inhibits invasiveness and growth of breast cancer cells. Breast Cancer Res 2010;12:R74.

[70] Eddy SF, Guo S, Demico EC, Romieu-Mourez R, Landesman-Bolag E, Seldin DC, et al. Inducible IkappaB kinase/IkkappaB kinase epsilon expression is induced by CK2 and promotes aberrant nuclear factor-kappaB activation in breast cancer cells. Cancer Res 2005;65:11375–83.

[71] Shen RR, Zhou AY, Kim E, Lim E, Habelhah H, Hahn WC, et al. IκB kinase ε: phosphorylates TRAF2 to promote mammary epithelial cell transformation. Mol Cell Biol 2012;32:4756–68.

[72] Guo JP, Coppola D, Cheng JQ. IKKbeta protein activates Akt independent of phosphatidylinositol 3-kinase/PDK1/mTORC2 and the pleckstrin homology domain to sustain malignant transformation. J Biol Chem 2011;286:37389–98.

[73] Xie X, Zhang D, Zhao B, Lu MK, You M, Condorelli G, et al. IkappaB kinase epsilon and TANK-binding kinase 1 activate Akt by direct phosphorylation. Proc Natl Acad Sci USA 2011;108:6474–9.

[74] Teneoever BR, Ng SL, Chua MA, McWhitter SM, Garcia-Sastre A, Maniatis T. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. Science 2007;315:1274–8.

[75] Watson CJ, Neoh K. The Stat family of transcription factors have diverse roles in mammalian gland development. Semin Cell Dev Biol 2008;19:401–6.

[76] Hsu S, Kim M, Hernandez L, Grajales V, Noonan A, Anver M, et al. IKK-ε coordinates invasion and metastasis of ovarian cancer. Cancer Res 2012;72: 5494–504.

[77] Guo JP, Shu SK, He L, Lee YC, Kruk PA, Greneman S, et al. Deregelation of IKKbeta is associated with tumor progression, poor prognosis, and cisplatin resistance in ovarian cancer. Am J Pathol 2009;175:324–33.

[78] Kang MR, Kim MS, Kim SS, Ahn CH, Yoo NJ, Lee SH. NF-kappaB signalling proteins p50/p105, p52/p100, RelA, and IKK epsilon are over-expressed in oesophageal squamous cell carcinomas. Pathology 2009;41:622–5.

[79] Peant B, Diallo JS, Dufour F, Le Page C, Delvoye N, Saad F, et al. Over-expression of IkappaB-kinase-epsilon (IKKepsilon/IKKi) induces secretion of inflammatory cytokines in prostate cancer cell lines. Prostate 2009;69:706–18.

[80] Peant B, Forest V, Trudeau V, Latour M, Mes-Masson AM, Saad F. IkappaB- Kinase-epsilon (IKKepsilon/IKKi)/IKKbeta expression and localization in prostate cancer tissues. Prostate 2011;71:1131–8.

[81] Seo SI, Song SY, Kang MK, Kim MS, Oh JE, Kim YR, et al. Immunohistochemical analysis of NF-kappaB signaling proteins IKK epsilon, p50/p105, p52/p100 and RelA in prostate cancers. APMIS 2009;117:623–8.

[82] Hildebrandt MA, Tan W, Tamboli P, Huang M, Ye Y, Lin J, et al. Kinome expression profiling identifies IKKbeta as a predictor of overall survival in clear cell renal cell carcinoma patients. Carcinogenesis 2012;33:799–803.

[83] Guo JP, Shu SK, Esposito NN, Coppola D, Koomen JM, Cheng JQ. IKKepsilon phosphorylation of ERalpha-Ser167 and contribution to tamoxifen resistance in breast cancer. J Biol Chem 2010;285:3676–84.

[84] Arlt A, Schafer H, NFkappaB-dependent chemoresistance in solid tumors. Int J Clin Pharmacol Ther 2002;40:336–47.

[85] Tapia MA, Gonzalez-Navarrete I, Dalmases A, Bosch M, Rodriguez-Fanjul V, Rolle M, et al. Inhibition of the canonical IKK/NF kappa B pathway sensitizes human cancer cells to doxorubicin. Cell Cycle 2007;6:2284–92.

[86] Renner F, Moreno R, Schmitz ML. SUMOylation-Dependent Localization of IKK epsilon in PML Nuclear Bodies Is Essential for Protection against DNA-Damage-Triggered Cell Death. Mol Cell 2010;37:503–15.

[87] Guan H, Zhang H, Cai J, Wu J, Yuan J, Li J, et al. IKKepsilon is over-expressed in glioma and contributes to resistance of glioma cells to apoptosis via activating NF-kappaB. J Pathol 2011;223:436–45.

[88] Wang T, Block MA, Cowen S, Davies AM, Devereaux E, Gingipalli L, et al. Discovery of azabenzimidazole derivatives as potent, selective inhibitors of TRβ1/IKKepsilon kinases. Bioorg Med Chem Lett 2012;22:2063–9.

[89] McVerr EG, Bryans J, Bircahl K, Chugh J, Drake T, Lewis SJ, et al. Synthesis and structure-activity relationships of a novel series of pyrimidines as potent inhibitors of TRβ1/IKKepsilon kinases. Bioorg Med Chem Lett 2012;22: 7169–73.

[90] Lee JK, Kim SY, Kim YS, Lee WH, Hwang DH, Lee YJ. Suppression of the TRF- dependent signaling pathway of Toll-like receptors by luteolin. Biochem Pharmacol 2009;77:1391–400.

[91] Youn HS, Lee JY, Saitoh SI, Miyake K, Kang KW, Choi YJ, et al. Suppression of MDY88- and TRF-dependent signaling pathways of Toll-like receptor by (-)-epigallocatechin-3-gallate, a polyphenol component of green tea. Biochem Pharmacol 2006;72:850–9.