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Commentary

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

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ABSTRACT

The innate immune system forms our first line of defense against invading pathogens and relies for a major part on the activation of two transcription factors, NF-κB and IRF3. Signaling pathways that activate these transcription factors are intertwined at the level of the canonical IκB kinases (IKKα, IKKβ) and non-canonical IKK-related kinases (IKKε, TBK1). Recently, significant progress has been made in understanding the function and mechanism of action of IKKε in immune signaling. In addition, IKKε impacts on cell proliferation and transformation, and is thereby also classified as an oncogene. Studies with IKKε knockout mice have illustrated a key role for IKKε in inflammatory and metabolic diseases. In this review we will highlight the mechanisms by which IKKε impacts on signaling pathways involved in disease development and discuss its potential as a novel therapeutic target.

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-inducible or IKK-i), 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

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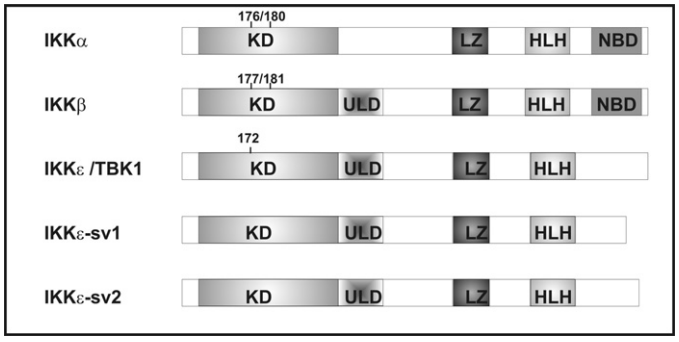

Fig. 1. Domain structure of the IKK family.
The IKK family can be divided in two groups: the classical or canonical IKKs (IKKα and IKKβ) and the non-canonical IKKs (TBK1 and IKKε). In addition, two splice variants of IKKε (IKKε-sv1 and IKKε-sv2), respectively missing 25 and 13 amino acids at their C-terminal 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.

liver [7], whereas IKKε 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 IKKε in various cell types (see also section 2). Moreover, TBK1 and IKKε may have nonredundant 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 IKKε, NIK phosphorylation was highly specific for TBK1. Since TBK1 and IKKε share the same substrate consensus phosphorylation motif [11], substrate specificity is likely driven by recruitment of TBK1 or IKKε to discrete signaling complexes, possibly involving specific scaffolding proteins (see Section 4). Given the ever-growing number of TBK1 and IKKε interaction partners and substrates [12], localized kinase activation and substrate modification could be an effective strategy to confer TBK1 or IKKε specific functions.

2. Expression of IKKε and TBK1

While IKKα, IKKβ and TBK1 are constitutively expressed in most cell types, basal IKKε 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) IKKε 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-i [12,13]. The promoter of the *IKKε* 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 IKKε expression induces kinase activation is currently still unclear. Recently, two IKKε splice variants, missing 25 amino acids (IKKε-sv1) or 13 amino acids (IKKε-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. IKKε 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 IKKε and TBK1 show many overlapping activities that are relevant to innate immunity and inflammation, the more restricted expression of IKKε is of particular interest. In this review we will therefore focus on IKKε and mainly refer to TBK1 for reasons of comparison.

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

The identification of TBK1 and IKKε as IκBα kinases has always been very controversial and the source of many debates. Evidence is based primarily on the observation that overexpression of IKKε or TBK1 in cultured cells leads to phosphorylation of IκBα, be it only at one phosphoacceptor site (Ser36 or Ser32, respectively), driving increased IκBα turnover [13]. However, both IKKε and TBK1 deficient MEF cells display normal IκBα 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 IKKε [7,8]. Therefore it is believed that IKKε influences NF-κB signaling and concomitant gene expression downstream of IκBα [12] (Fig. 2). In this context, several studies support a role for IKKε-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 IKKε-enzymatic activity to κB-containing inflammatory gene promoters in the nucleus, enabling IKKε to phosphorylate adjacent c-Jun and initiate nuclear receptor corepressor clearance [20]. These results suggest that p65-recruitment of IKKε to promoters that exhibit AP1 and κB sites in close proximity (e.g. *inos, cxcl2, cxcl9, cxcl10, ccl4, tnfaip3*) may regulate their activation by initiating corepressor turnover. Besides p65, IKKε/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 IKKε 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-I, 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 IKKε and TBK1 directly phosphorylate IRF3 and IRF7 at their C-termini, 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 IKKε upon viral infection of innate immune cells [22]. IKKε 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 IKKε double deficient MEFs [9]. IKKε overexpression, but not its kinase dead mutant, could restore IRF3 activation in TBK1 deficient cells, suggesting redundancy between both kinases [24]. Although IKKε and TBK1 seem to exert overlapping functions in IRF3 activation, mechanistic differences in the activation of TBK1 and IKKε following virus infection have been observed (e.g. mitochondrial localization of IKKε 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 IKKε but not TBK1-mediated IRF3 phosphorylation [25], indicating that both kinases can also be differentially regulated.

4. Mechanism and regulation of IKKε/TBK1 activation

Although IKKε/TBK1 can be activated by several inflammatory stimuli, their activation by TLR3, TLR4 and RIG-I receptors has been best documented [26,27]. Receptor stimulation induces the recruitment of specific adaptor proteins to the receptor (TRIF for

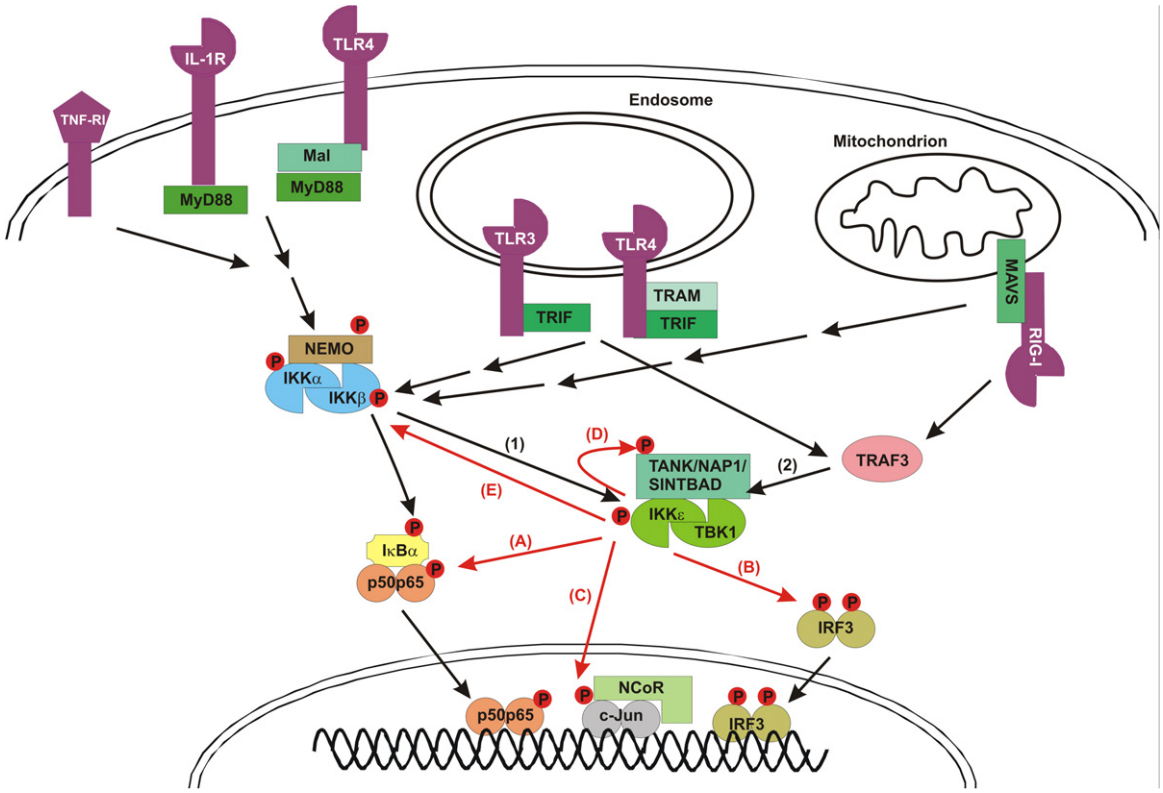

Fig. 2. IKKε mediated signaling to NF-κB and IRF3 in response to specific receptors.
Non-canonical IKKs (IKKε and TBK1) can be activated by two signaling pathways: (1) IKKα/β mediated activation of IKKε/TBK1. Ligand binding to several receptors (TNF-R1, IL-1R, TLR4, RIG-I) initiates the recruitment of 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 IκBα, 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 IKKε/TBK1 autoactivation. TRIF-dependent TLR3 and TLR4 signaling, as well as MAVS-dependent RIG-I signaling, induce IKKε/TBK1 autoactivation via TRAF3. This also requires the binding of IKKε/TBK1 to different scaffold proteins (TANK, NAP1, SINTBAD). IKKε/TBK1 then mediate different activities: (A) IKKε/TBK1 can phosphorylate NF-κB (p65), contributing to NF-κB dependent expression of specific genes. (B) IKKε/TBK1 can phosphorylate IRF3 (and IRF7; not shown), leading to its homodimerization and nuclear translocation. (C) IKKε/TBK1 can phosphorylate c-jun, leading to the release of the nuclear repressor complex (NCoR). (D) IKKε/TBK1 can phosphorylate their own scaffold proteins (TANK, NAP1 or SINTBAD), the function of which is still unclear. (E) IKKε/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 IKKε and TBK1 [28]. At later time points, the presence of IKKε 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, IKKε (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 IKKε/TBK1 at Ser172 [13,31], activation of the endogenous kinases does not only involve autophosphorylation. Indeed, cells treated with the TBK1 and IKKε inhibitor BX795 still showed phosphorylation of TBK1 and IKKε 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 IKKε/TBK1 is impaired in IKKα deficient cells treated with an IKKε inhibitor [31], indicating IKKα as a potential IKKε kinase. In line with these observations, TNF-induced activation of IKKε/TBK1 seems to be mediated solely by the canonical IKKs [31]. Interestingly, IKKε/TBK1 activation by the canonical IKK 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 IKKε activation involves specific adaptor proteins. So far, three scaffold proteins have been shown to bind IKKε/TBK1 and to promote IKKε/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 IKKε complexes under distinct conditions, providing signaling specificity. Whether or not these scaffold proteins assemble hetero- or homodimers of IKKε and TBK1 is currently still unknown.

In contrast to the positive regulation of NF-κB dependent gene expression by IKKε/TBK1, the latter have also been described to negatively regulate NF-κB activation in response to TNF or IL-1 stimulation. More specifically, IKKε/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 IKKε/TBK1 was impaired in TANK-deficient macrophages [36]. Using these cells it was also shown that TANK is required for IKKε/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 with Tax1 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 TRIP 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 I 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 GSK3α [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 by TLR4 [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 *IKBKE* 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

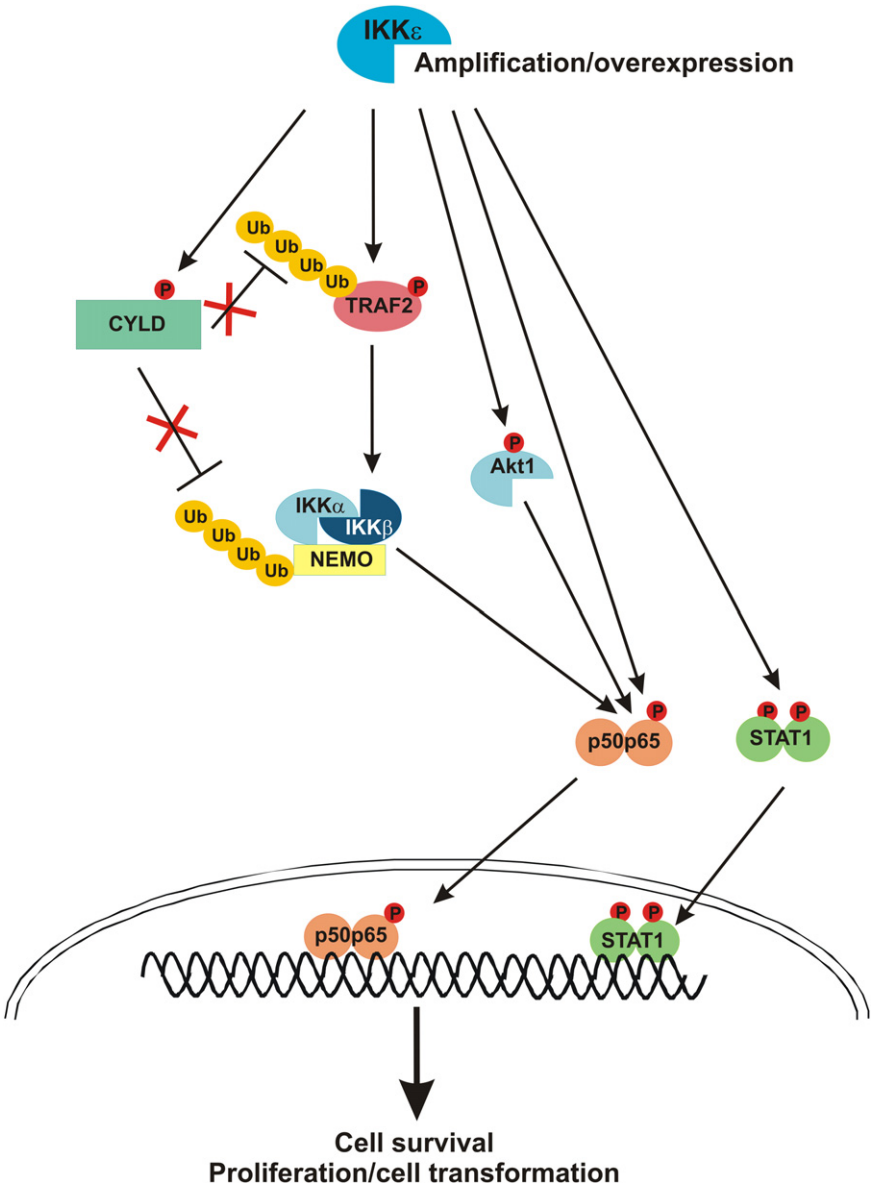

Fig. 3. Mechanisms contributing to the oncogenic potential of IKKε.
Overexpression of IKKε in tumor cells induces cell survival, cell transformation and proliferation by different mechanisms involving IKKε mediated phosphorylation of specific substrates. IKKε can either directly or indirectly (via Akt phosphorylation and activation) phosphorylate NF-κB (p65), leading to increased NF-κB dependent gene expression. IKKε also phosphorylates and inactivates the tumor suppressor CYLD, preventing CYLD from deubiquitinating specific substrates in the NF-κB signaling pathway. In addition, phosphorylation of TRAF2 activates its E3 ubiquitin ligase activity. Both CYLD and TRAF2 phosphorylation thus increase ubiquitin-dependent NF-κB signaling. IKKε also directly phosphorylates STAT1, increasing its gene activating potential.

gene expression (e.g. cyclin D1 and RelB) [70], demonstrating the necessity of IKKε catalytic activity. Recently, this was further confirmed by demonstrating the requirement of IKKε-mediated phosphorylation at Ser418 of the tumor suppressor CYLD, which prevents its deubiquitinating activity on NF-κB signaling proteins such as TRAF2 and NEMO [41,71] (Fig. 3). Moreover, IKKε 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-κB activation, which promotes survival, transformation and proliferation of mammary epithelial cells (Fig. 3). Furthermore, IKKε (as well as TBK1) may contribute to enhanced NF-κB activity and tumorigenesis by directly phosphorylating NF-κB p65 (as described above) or by phosphorylating Akt, which then phosphorylates and activates p65 [72,73]. In addition, elevated IKKε 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 IKKε. As IRF3 suppression did not affect IKKε-induced cell transformation, the oncogenic potential of IKKε 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 IKKε, which was associated with a lower overall survival rate [76,77]. Furthermore, alterations of IKKε were associated with late-stage and high-grade tumors, suggesting a role of IKKε in ovarian tumor progression rather than in tumor initiation. Finally, IKKε was also described as an oncogene in prostate and oesophageal squamous cell carcinoma with increased levels of different NF-κB 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 prosurvival 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 (PDK1) 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-, IKKα-, 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 IKKs, and other signaling pathways, is also an open line of investigation. In particular the negative regulatory effect of IKKε/TBK1 on the canonical IKKs 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 IKKε/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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