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Deep Insight Section

The IKK-related kinases, unsuspected culprits in oncogenesis?

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Abstact

Following pathogen infection, the IκB kinase (IKK) - related kinases TBK1 and IKKi phosphorylate and activate members of the Interferon Regulatory Factor transcription factors that control the trans-cription of a repertoire of genes with anti-viral activity. Studies have revealed that these kinases also target NF-κB and c-Jun transcription factors thereby suggesting that they could also control inflammatory responses. The link between chronic inflammation and cancer is under intensive investigations, the canonical IκB kinases (IKKα and IKKβ) and NF-κB being proposed as major culprits. New findings now suggest that the IKK-related kinases TBK1 and IKKi, in addition to their essential functions in controlling the innate immune response, also participate to signaling pathways that impact on cell transformation and tumor prog-ression. This review will therefore discuss the role of TBK1 and IKKi in innate immunity and summarize their proposed roles in cellular trans-formation and oncogenesis by focusing on their regulation and downstream substrates.

Introduction

Recognition of invading pathogens such as viruses and bacteria by cells is known to trigger the rapid activation of multiple latent transcription factors, namely NF-κB, AP-1 (ATF-2/c-Jun) and the Interferon Regulatory Factors (IRFs). Once activated, these transcription factors activate in turn a set of immunomodulatory genes that produce protein messengers in the form of cytokines. The best-characterized cytokines of the innate host defense to virus is the family of transcriptionally activated interferon (IFN) proteins, which includes type I IFN-α and IFN-β. Once produced, these secreted proteins act in autocrine and paracrine fashion to modulate gene expression through engagement of cell surface type I IFN α/β receptor (IFNAR) and activation of the Jak-STAT signaling pathway. STAT1/2 heterodimers, in conjunction with interferon-stimulated gene factor 3γ (ISGF3γ/IRF-9) bind to interferon-stimulated response elements (ISRE) found in numerous type I IFN-induced genes, such as 2'-5' oligoadenylate synthase, dsRNA activated kinase (PKR), RNA editing enzymes (APOBEC3, ADAR), IFN-Stimu-lated Genes (ISG)-56, -54 and -15, resulting in growth inhibition, apoptosis, and impaired viral gene expression and replication (Grandvaux et al., 2002b). Through their distinct effects on immune cells such as maturation of dendritic cells, clonal expansion of CD8+ T cells and stimulation of NK cells, type I IFNs also link innate and adaptive antiviral immunity (Theofilopoulos et al., 2005).

Pattern Recognition Receptors involved in IFN type I production

Following exposure to pathogen-associated mole-cular patterns (PAMPs), the innate immune response and the inflammation reaction that follows rely on evolutionarily conserved receptors termed pattern-recognition receptors (PRRs). Some of these receptors are located at the plasma membrane and include molecules such as the Toll-like receptors (TLR 1, 2, 4, 5, 6) and scavenger receptors (CD36 and SR-A). Others occur in acidic endosomes (TLR 3, 7, 8, and 9), and still others can be found in the cytoplasm [PKR, the RIG-I-like receptors (RLRs) (RIG-I and MDA5), the DNA-dependent activator of IRFs (DAI), and the nucleotide-binding oligomerization domain (NOD)

receptors] (Lee and Kim, 2007; Tamura et al., 2008). Whereas the vast majority of these PRRs induce intracellular signaling cascades leading to the activation of NF-κB and AP-1 transcription factors, only a subset of them, namely the nucleotide-sensing receptors (TLR3, -7, -8, -9, RIG-I and MDA5) and TLR4, which recognize bacterial lipopolysaccharide (LPS) and viral components such as respiratory syncytial virus F protein, will lead to the activation of IRF3 and -7 and type I IFN synthesis (see (Bowie and Unterholzner, 2008) for a recent review).

Signaling cascades leading to the activation of the NF-κB pathway

The underlying mechanism controlling the activa-tion of NF-κB members began to be elucidated in 1997 when the IKK complex was first discovered (DiDonato et al., 1997). In resting cells, NF-κB members are sequestered in the cytoplasm in inactive forms through their association with one of several inhibitory molecules, namely IκB-α, -β, -ε, p105, and p100. These inhibitors mask the nuclear localization signal (NLS) in the Rel Homology Domain (RHD) of NF-κB members, thereby preventing them from entering the nucleus (see (Perkins, 2007) for a recent review). The rate-limiting step in the activation of NF-κB members is therefore the phosphorylation-dependent degrada-tion of IκB-α, -β, -ε. The best-characterized IκB protein is IκBα. Following PRR engagement or treatment with prototypical activators such as tumor necrosis factor (TNF)- α, IL-1β or phorbol 12-
myristate 13-acetate (PMA), IκBα is phosphoryla-ted at Ser-32 and Ser-36 in the N-terminal signal responsive domain by the canonical IκB kinase (IKK) complex which is composed of two catalytic subunits called IKKα and β and one regulatory subunit called IKKγ or NF-κB essential modulator (NEMO) (Perkins, 2007). Phosphorylated IκBα is subsequently polyubiquitinated by the E3 ubiquitin ligase $SCF^{\beta TrCP}$ and targeted to the 26S proteasome complex for degradation, resulting in the release and nuclear translocation of NF-κB members, which can now stimulate target gene transcription (Perkins, 2007). Alternative signaling pathways activating the NF-κB pathway have also been characterized (Perkins, 2007). They are classified as alternative pathways because they do not rely on the classical IKK complex. One such alternative pathway is the phosphorylation of p100 by IKKα homodimers, which leads to its processing to p52.

Discovery of the IKK-related kinases

The importance of the IKK complex in the regulation of NF-κB has fueled intensive research efforts by several groups leading to the discovery of the IKK-related kinases (for detailed reviews, see (Hacker and Karin, 2006) and (Peters and Maniatis, 2001)). Using degenerate primers containing sequences common to IKKα and IKKβ, a portion of NF-κB-activating kinase (NAK) was amplified by PCR (Tojima et al., 2000). This NAK, also called TANK Binding Kinase 1 (TBK1) or TRAF2-associated kinase (T2K), was shown to act as an IKK-activating kinase responsible for NF-κB activation in response to growth factors that stimulate PKCε. TBK1 was originally identified in a yeast two-hybrid screen using TANK as a bait (Pomerantz and Baltimore, 1999).
T2K was cloned as part of a TRAF2-binding

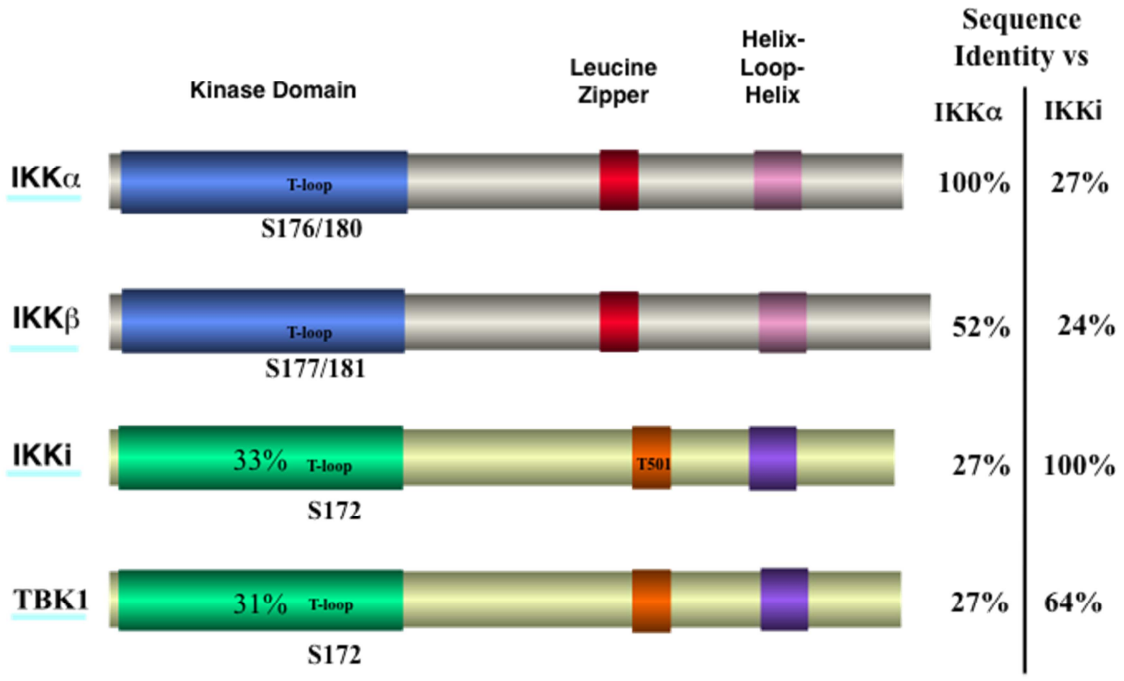

Figure 1: Schematic representation of the kinase subunit of the IKK complex (IKKα and IKKβ) and the IKK-related kinases (IKKi and TBK1).

complex that exhibits kinase activity towards IκBα in vitro (Bonnard et al., 2000). At almost exactly the same time, another IKK-related kinase was also characterized and named IKKε/IKKi.

Peters and colleagues found IKKε in a database search for proteins similar to IKKα and IKKβ, whereas Akira's group isolated IKKi (the "i" standing for inducible IKK, see below) in a subtractive hybridization screen of a cDNA library from LPS-stimulated mouse macrophages (Peters et al., 2000; Shimada et al., 1999). TBK1 and IKKi display 64% overall sequence identity (Fig. 1). In contrast to the kinase subunits of the canonical IKK complex (IKKα and IKKβ) and TBK1, which are constitutively expressed in virtually all cell types, IKKi is predominantly expressed in specific tissues such as the pancreas, thymus, spleen, and peripheral blood leukocytes (Shimada et al., 1999). It is also expressed at very low levels in specific cell lines (Bibeau-Poirier et al., 2006; Gravel and Servant, 2005; Honda et al., 2005). Importantly, IKKi mRNA can be induced in response to exposure to LPS, viral infection, or a number of NF-κB-inducing cytokines (Bibeau-Poirier et al., 2006; Kravchenko et al., 2003; Shimada et al., 1999; Wang et al., 2005).

Like IKKα and IKKβ, the IKK-related kinases contain an N-terminal kinase domain and a C-terminal Leucine Zipper (LZ) thought to be involved in homo and heterodimerization of the protein kinases (Fig. 1). A potential Helix-Loop-Helix (HLH) motif is also present and, as demonstrated for IKKβ (Delhase et al., 1999), may be involved in the regulation of phosphotransferase activity. The kinase domain of IKKi shares 33% and 31% sequence identity with IKKα and IKKβ respectively. As such, overexpression of both kinases induces NF-κB activation (Peters et al., 2000; Pomerantz and Baltimore, 1999; Shimada et al., 1999). However, the IKK-related kinases are not part of the classical IKKγ-containing IKK complex (Fujita et al., 2003; Peters et al., 2000; Shimada et al., 1999) and their role in controling the NF-κB pathway in response to proinflammatory stimuli or PRR engagement remains uncertain. The only evidence in favor of a role of TBK1 in regulating NF-κB activation are indirect and come from mouse models that revealed functional similarities between IKKβ and the IKK-related kinases. Notably, TBK1-deficient mice are phenol-typically similar to IKKγ, IKKβ- and p65-deficient mice, which die at E14.5 due to liver degeneration and apoptosis (Bonnard et al., 2000). However, mouse embryonic fibroblasts (MEFs) deficient for IKKi or TBK1 show normal IκBα degradation and NF-κB DNA-binding activity in response to classical NF-κB inducers including TNFα, IL-1β and LPS (Bonnard et al., 2000; Hemmi et al., 2004; Kravchenko et al., 2003). Intriguingly, the induction of well-characterized NF-κB target genes such as IL6, MCP1, COX2 and ICAM1 was abrogated in MEFs lacking either IKKi or TBK1. Therefore, phosphorylation of NF-κB subunits by TBK1 was proposed to explain the observed defects in the transactivation of specific NF-κB target genes (Bonnard et al., 2000). It is worth mentioning that phosphorylation of p65/RelA at serine 536 by IKKβ and IKKα was shown to be an essential modification required in the second phase of NF-κB activation. This event plays a key role in determining both the strength and duration of the NF-κB-mediated transcriptional response (Chen et al., 2005; Lawrence et al., 2005). Interestingly, both IKKi and TBK1 also target p65/RelA at serine 536 (see Table I), a process clearly involved in the induction of NF-κB-regulated genes (Buss et al., 2004; Fujita et al., 2003). However, neither a defect in p65 phosphorylation or in NF-κB dependent gene transcription was confirmed in more recent studies using MEFs isolated from TBK1-IKKi double knockout mice (Adli and Baldwin, 2006; Hemmi et al., 2004). Thus, the physiological role of the IKK-related kinases in NF-κB activation in response to proinflammatory cytokines or PRR engagement is still not fully understood.

It is possible that under conditions of overexpres-sion (as observed in certain cancers (see below)) and/or hyperactivation, the NF-κB pathway could become a significant target of IKK-related kinases. Indeed, when overexpressed, TBK1 and IKKi activate the NF-κB pathway and are able to induce the phosphorylation of IκBα (Peters et al., 2000; Pomerantz and Baltimore, 1999; Shimada et al., 1999). Interestingly, in contrast to IKKβ, the enzymes TBK1 and IKKi phosphorylate only one (serine 36) of the two phosphoacceptor sites present in IκBα (Peters et al., 2000; Shimada et al., 1999; Tojima et al., 2000). Why only one phosphorylation event occurs in the IκBα phosphodegron motif –DSGXXS- is unclear. Overexpression of IKKi is known to effectively drive IκBα degradation (Boehm et al., 2007; Eddy et al., 2005), indicating that phosphorylation of serine 36 may facilitate the phosphorylation of the protein on serine 32 by another kinase, leading to its degradation. Phosphorylation of the adaptor protein TANK by IKKi could also induce the release of TRAF2 followed by the subsequent activation of the IKK complex (Nomura et al., 2000) (see Table I). Furthermore, overexpression of these kinases was shown to phosphorylate NF-κB members such as p65 and c-Rel (Adli and Baldwin, 2006; Harris et al., 2006).

The IKK-related kinases, key players in the induction of type I interferon

Whereas the role of the IKK-related kinases in regulating the canonical NF-κB pathway is still uncertain, their roles in type I IFN production through the phosphorylation and activation of IRF-3 and IRF-7 transcription factors is well established (Fitzgerald et al., 2003; Sharma et al., 2003). In most cells, including fibroblasts, macrophages and conventional dendritic cells, IRF-3 and IRF-7 are important players in the induction of type I IFN following RLRs and TLR3/4 engagement (Honda et al., 2005; Sato et al., 2000;

Tamura et al., 2008) and both kinases phosphorylate IRF-3 and IRF-7 at key C-terminal residues (Caillaud et al., 2005; Clement et al., 2008a; Mori et al., 2004; Panne et al., 2007; tenOever et al., 2004). Whereas IRF-7 can be induced at the transcriptional level, IRF-3 is found latent in the cytoplasm. Upon phosphorylation of the C-terminal region by TBK1 or IKKi, IRF-3 homodimerizes or heterodimerizes with IRF-7 followed by its nuclear accumulation and binding to ISRE found in numerous genes such as type I IFN (see (Tamura et al., 2008) for a recent review). Another IRF family member, IRF-5, is also involved in IFN type I induction (Yanai et al., 2007) and can also be phosphorylated by TBK1 or IKKi (Cheng et al., 2006; Lin et al., 2005; Schoenemeyer et al., 2005). However, how IRF-5 participates in the transcriptional regulation of type I IFNs genes is not completely understood.

Since the discovery of TBK1 and IKKi, extensive characterization of the signaling pathways leading to the activation of IRF-3 and IRF-7 in response to pathogen infection has led to the identification of several positive scaffolding proteins regulating the phosphotransferase activities of the two kinases. These regulators include NEMO/IKKγ (Zhao et al., 2007), FADD (Balachandran et al., 2004), TRADD (Michallet et al., 2008), TRAF3 (Hacker et al., 2006; Oganesyan et al., 2006), TANK (Gatot et al., 2007; Guo and Cheng, 2007; Nomura et al., 2000; Rothe et al., 1996), NAP1 (Fujita et al., 2003; Sasai et al., 2005), HSP90 (Yang et al., 2006), SINTBAD (Ryzhakov and Randow, 2007), DDX3 (Schroder et al., 2008; Soulat et al., 2008) and STING/MITA (Ishikawa and Barber, 2008; Zhong et al., 2008). In addition, intracellular RNA sensors such as RIG-1 (Yoneyama et al., 2004) and MDA-5 (Andrejeva et al., 2004), which signal through the mitochondrial antiviral signaling adaptor MAVS (also known as Cardif, VISA, and IPS-1) (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005), have been found to activate the IKK-related kinases in response to infection by RNA viruses. The detection of intracellular DNA (particulary B-DNA) by DNA sensors, which rely on IRF-3 for type I IFN production, was also recently proposed to impact on the activity of IKK-related kinases. DAI (also know as DLM-1/ZBP1) is a DNA sensor that assembles with TBK1 and IRF-3 upon dsDNA treatment and was suggested to be involved in type I IFN response (Takaoka et al., 2007). However, recent studies in DAI-deficient cells failed to identify a phenotype suggesting the implication of other DNA sensors (Charrel-Dennis et al., 2008; Wang et al., 2008). Importantly, whereas TBK1 and IKKi play redundant roles in macrophages following RIG-I engagement (Perry et al., 2004), a TBK1-dependent pathway is dominant for type I IFN response upon intracellular DNA recognition (Charrel-Dennis et al., 2008; Miyahira et al., 2009; Nociari et al., 2009). This possibly suggests an important role of the scaffolding regulators described above in ordering the formation and/or recruitment of specific oligomeric complexes upon exposition to different stimuli. At the level of transcriptional regulation, GRIP1 was recently identified as a new cofactor of IRF-3 dimers (Reily et al., 2006). Negative regulators including the SHP-2 phosphatase (An et al., 2006), SIKE (Huang et al., 2005), A20 (Lin et al., 2006), LGP-2 (Rothenfusser et al., 2005), NLXR1 (Moore et al., 2008) and CYLD (Zhang et al., 2008) add to the complex puzzle. Some of these important findings have been extensively reviewed elsewhere (Bowie and Unterholzner, 2008; Hacker and Karin, 2006; Honda and Taniguchi, 2006; Kassel and Herrlich, 2007; Seth et al., 2006; Tamura et al., 2008) and will not be discussed further here. More recently, the IKK-related kinases have been implicated in oncogenesis (Boehm et al., 2007; Chien et al., 2006; Clement et al., 2008b; Eddy et al., 2005; Korherr et al., 2006; Lee and Hung, 2008). The following discussion will focus on the potential oncogenic roles of the IKK-related kinases and their different substrates (Table 1).

The IKK-related kinases in oncogenesis

The role of the NF-κB pathway in the molecular etiology of cancer has been well established using mouse models with tissue-specific ablation of the IKKβ gene in epithelial cells, bone-marrow-derived macrophages and hepatocytes (Greten et al., 2004; Maeda et al., 2005) and see (Coussens and Werb, 2002; Karin, 2008; Karin and Greten, 2005; Karin et al., 2006) for reviews. Moreover, study of $IKK\alpha^{AA}$ knock-in mice, which express a catalytically inactive kinase mutant, revealed an important role for IKKα in metastatogenesis (Luo et al., 2007). The fact that ectopic expression of the IKK-related kinases leads to activation of the NF-κB pathway (see above) might suggest that these kinases could also be involved in cancer develop-ment. Notably, recent findings point towards a role for TBK1 and IKKi in Ras-induced oncogenic transformation (Fig. 2). TBK1 was identified as a RalB effector in the Ral-guanine nucleotide exchange factor (GEF) pathway that is required for Ras-induced transformation of certain cell types. On the other hand, a functional screen identified IKKi as a kinase that acts downstream of the phosphatidylinositol 3-kinase (PI3K)-Akt pathway and cooperate with activated MEK1/2 to promote cellular transformation.

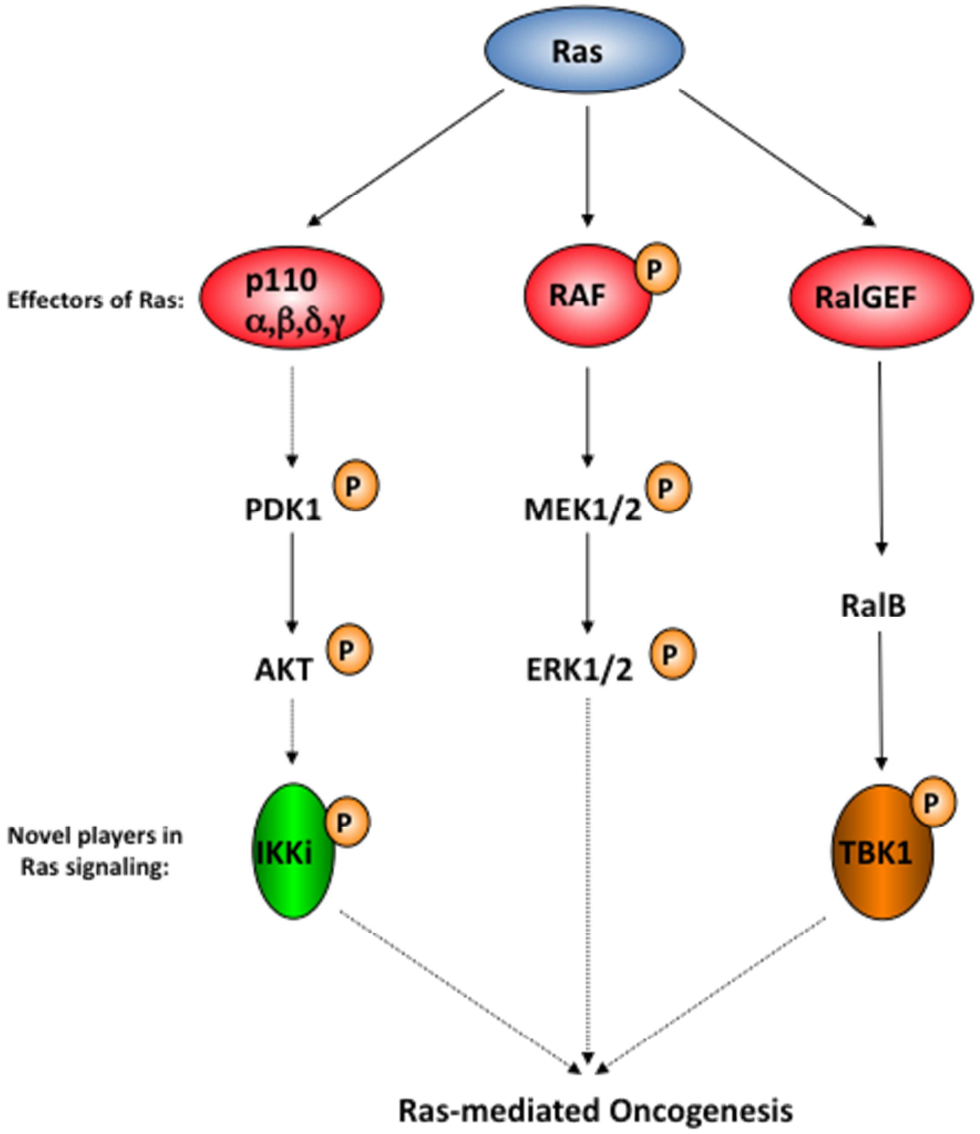

Figure 2: Pivotal contribution of the IKK-related kinases to oncogenic Ras-induced tumorigenesis. Recent findings highlight a role of TBK1 and IKKi in Ras-induced oncogenic transformation. In the uncontrolled proliferation of transformed cells, IKKi plays a role downstream of the PI3KAkt pathway, whereas TBK1 most likely acts as a RalB effector in the RalGEF pathway.

TBK1 and RalB signaling

The Ras-like (Ral) GTPases RalA and RalB are two close relatives of the founding member of the Ras GTPase superfamily. The Ral proteins are involved in a variety of regulatory systems known to participate in oncogenic signaling cascades. Indeed, modulation of Ral GTPase activity was shown to influence the state of activation of Src, Jnk, and p38 kinases, phospholipase D, and NF-κB, Stat3 and transcription factors of the Forkhead family (Camonis and White, 2005). Importantly, RalGEF-induced Ral activation was found to be necessary and sufficient for Ras-dependent transformation of a variety of human cell types (Hamad et al., 2002; Lim et al., 2006; Rangarajan et al., 2004). Ral GTPases also control secretory events through the exocyst. The exocyst is a multisubunit complex whose core elements include Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70 and Exo84. It is involved in the intricate secretory vesicle sorting and delivery events required establishing function-ally and architecturally discrete plasma membrane domains (Camonis and White, 2005). Sec5 and Exo84 are both direct effectors of Ral. While studying the contribution of Ral GTPase effector proteins to Ral function in normal and tumorigenic epithelial cells, Chien and colleagues observed that RNAi-mediated depletion of Sec5 induces apoptosis of a variety of tumor cell lines (Chien et al., 2006). To understand the molecular basis of this effect, they immunoisolated the exocyst complex from epithelial cells and identified TBK1 as a co-precipitating protein. They further showed that RalB activation promotes TBK1/Sec5 assembly and that TBK1 can phosphorylate Sec5 in vitro (see Fig. 3A and Table I). Silencing of TBK1 expression by RNAi was found to

mimic depletion of RalB or Sec5 and induce tumor cell apoptosis. Interestingly, non-tumorigenic epithelial cells do not depend on TBK1 for survival (Chien et al., 2006). The selective dependence of transformed cells on TBK1 was also reflected in the enzyme's phosphotransferase activity, which was higher in transformed cells than in non-tumorigenic epithelial cells. Furthermore, studies using $TBK1^{-/-}$ fibroblasts revealed that TBK1 expression was required for cells to tolerate transforming levels of oncogenic $K\text{-}Ras^{G12V}$. This study also established that RalB and Sec5 were required for activation of the host defense pathway. Whereas Ras activity was suppressed in non-transformed epithelial cells in response to TLR3 signaling stimulated by poly(I:C) treatment, Ral GTPases were activated and this activation correlated with the phosphorylation of IRF-3. Moreover, siRNA-mediated depletion of Sec5 severely impaired not only the nuclear accumulation of IRF-3, but also the induction of ISG56 and IFNβ following Sendai virus (SeV) infection or poly(I:C) treatment. In summary, the RalB/TBK1 pathway is required for the survival of Ras-transformed cells and to activate the innate immune response in non-tumorigenic cells (Fig. 4).

Another recent study provides evidence for a role of TBK1 in the tumorigenesis process. Using a genome-wide phenotypic screen based on the overexpression of 250,000 cDNAs in HEK 293 cells combined with the transfer of culture supernatants onto HUVEC cells, Korrher and colleagues identified TBK1 and TRIF as new effectors of vascularization (Korherr et al., 2006). Overexpression of TRIF and TBK1 in HEK 293 cells resulted in the secretion of a complex mixture containing endothelial growth factors such as IL-8 and RANTES, as well as the anti-proliferative cytokine IFNβ. The secreted mixture displayed significant proangiogenic properties on endothelial cells. In addition to HEK 293 cells, the authors analyzed the proliferative response of endothelial cells to supernatants isolated from MCF-7, PC-3, and KB-3-1 cancer cells transiently transfected with the gene encoding TBK1. They confirmed that the autocrine effect of TBK1 is observed in various cancer cell lines. Interestingly, these cancer cell lines already show increased activity of TBK1 (see above). Their study also showed some unexpected findings: TBK1 was up-regulated under hypoxic conditions, as well as in solid breast and colon tumors. This is the first report demonstrating modulation of TBK1 at the protein level. Overall, these two studies propose a role of TBK1 in tumor cell survival and angiogenesis. However, the molecular mechanisms explaining the possible effects of TBK1 in oncogenesis remain to be characterized.

Breast cancer and the NF-κB pathway. Is IKKi the missing link?

Inflammatory breast cancer is the most aggressive form of locally advanced breast cancer with a high metastatic potential. At the molecular level, microarray and qPCR analyses have revealed an 'NF-κB signature' in this form of breast cancer (Lerebours et al., 2008; Van Laere et al., 2006). Interestingly, the first evidence implicating IKKi in tumor development was in breast cancer. IKKi was found to be constitutively expressed in two-thirds of the human breast cancer samples analyzed, as well as in several human breast cancer cell lines (Eddy et al., 2005). Casein kinase 2 (CK2) was proposed to be involved in the increased expression level of IKKi. Most importantly, the NF-κB pathway is thought to contribute to the uncontrolled cellular proliferation of IKKi-expressing cells, since overexpression of an inactive mutant of IKKi reduces the expression of the cyclin D1 gene targeted by NF-κB (Fig. 3B). It also reduces the ability of breast cancer cells to grow in soft agar and to form invasive colonies in Matrigel.

Mutation of Ras proteins or hyperactivation of downstream effector pathways, principally the mitogen-activated protein kinase (MAPK) and PI3K pathways, occurs in several epithelial cancers including breast cancers ((Downward, 2003) and Fig. 2). An elegant functional genomic approach was used to identify protein kinases that have the potential to replace activated Akt and cooperate with a constitutively active MAPK pathway to drive tumorigenesis of immortalized human embryonic kidney epithelial cells (Boehm et al., 2007). In this study, IKKi was identified among four kinases with transforming potential (Fig. 3B). Interestingly, the authors found copy-number amplification of the 1q32 region, which includes the IKBKE locus, in 16.3% of breast cancer cell lines and in several primary human breast cancer specimens. No somatic mutation was found in the IKBKE gene, and no association was observed between the IKBKE copy-number gain and the estrogen receptor or HER2/neu status. IKBKE amplification correlated with overexpression of IKKi in several breast cancer cell lines such as MCF-7 and ZR-75-1. Silencing experiments with shRNAs confirmed that IKKi is required for the proliferation and survival of ZR-75-1 cells. Interestingly, knocking down the expression levels of both IKKi and TBK1 was required to diminish the viability of MCF-7 cells. This observation also supports a role for TBK1 in oncogenesis as discussed above. Amplification of chromosome 1q is also observed in other cancers such as multiple myeloma (Kuehl and Bergsagel, 2002) and head and neck cancer (Bauer et al., 2008). Thus, it would be interesting to verify whether IKKi is also up-regulated in these cancers.

At the molecular level, the overexpression of IKKi observed in breast cancer cell lines resulted in increased expression of two NF-κB-regulated genes, MMP9 and BCL2, both of which were down-regulated when IKBKE expression was suppressed. Moreover, immunohistochemical studies in patient-derived breast cancer tissue samples revealed nuclear staining for c-Rel, a recently described substrate of IKKi (Table I).

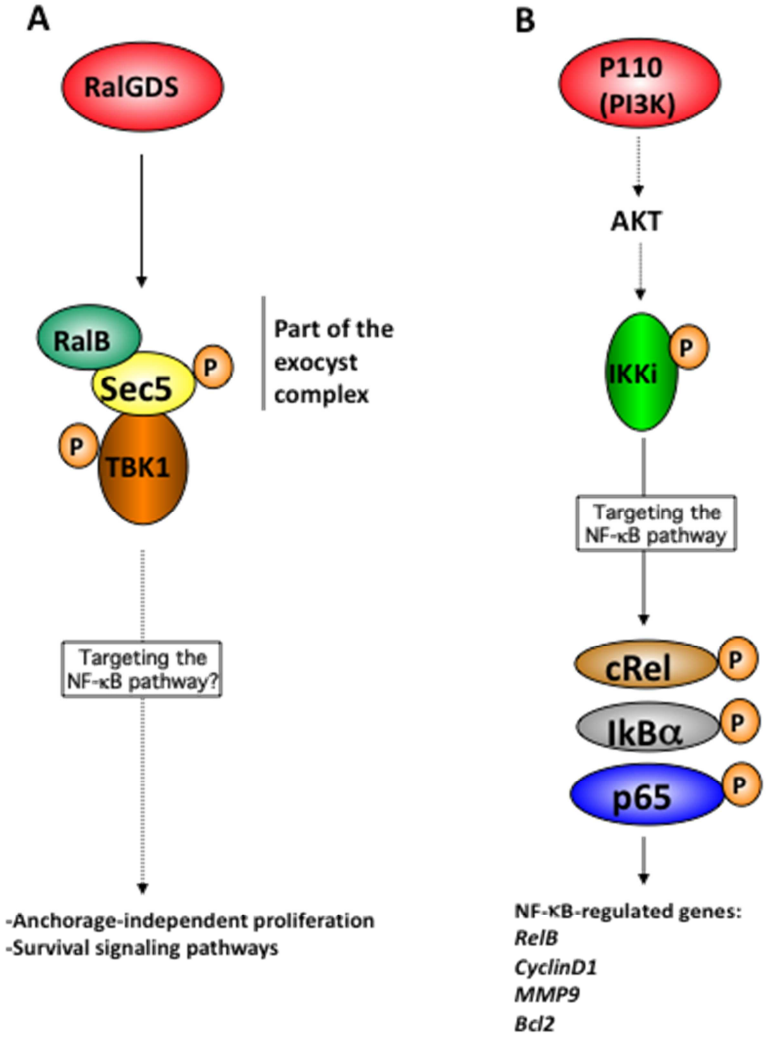

Figure 3: (A) TBK1 interacts with Sec5 in the exocyst complex. The contribution of Sec5 to TBK1 activation occurs independently of exocyst integrity. Rather, the proposed model suggests that RalB-Sec5 complex formation exposes a cryptic TBK1 interaction domain that recruits TBK1 into a kinase activation complex. The molecular mecha-nism responsible for TBK1-dependent tumor cell survival remains to be characterized but could involve the NF-κB pathway. **(B) IKKi is an essential effector of Akt cellular transformation.** The NF-κB pathway contributes to the oncogenic potential of IKKi. How the PI3-kinase pathway activates IKKi is presently unknown.

This protein had previously been reported to accumulate in the nuclei of breast cancer cells (Sovak et al., 1997) and to contribute to tumorigenesis of the mammary gland in an MMTV-LTR-c-Rel transgenic mouse model (Romieu-Mourez et al., 2003). IKKi modulates c-Rel activity through direct phosphorylation of the transactivation domain (Harris et al., 2006).

However, even though nuclear accumulation of c-Rel was observed in HEK 293T cells, IKKi-induced phosphorylation was insuffi-cient to significantly induce its transactivation potential. Thus, additional signaling cascades are likely to be required for full activation of c-Rel.

In addition to c-Rel and RelB (Eddy et al., 2005), RelA/p65 was observed to accumulate in the nuclei of primary human breast tumor specimens (Sovak et al., 1997). Furthermore, in many cancer cell lines, expression levels of IKKi correlate with p65 phosphorylation at serine 536 (Adli and Baldwin, 2006). It is worth mentioning that IKKi was also shown to enhance the activity of p65 following its phosphorylation at serine 468 (Mattioli et al., 2006). Finally, IKKi also interacts with NF-κB2/p100 and p52 following TNFα stimulation (Wietek et al., 2006). Gel filtration experiments suggest that IKKi and p52 are components of a 600-kDa complex. This interaction promotes transcription of p52-dependent genes in a ternary complex with p65/RelA. Although the kinase activity of IKKi is essential for this process, p52 does not seem to be a direct target, again suggesting that p65/RelA may be the preferred substrate of the kinase.

Are other substrates of IKK-related kinases involved in tumorigenesis?

It is intriguing that while the activity of IKK-related kinases is linked to cancer cell survival (see above),

	Targeted region	Phosphorylation site	Suggested Function
IKK-related common substrates			
RelA/p65	TAD	Ser 536	Transactivation
IkBα	Phosphodegron	Ser 36	?
TANK	aa 192-247	?	Proper assembly of signaling complexes TRAF2 dissociation
c-Rel	TAD	?	Induces nuclear translocation
IRF-3	C-Terminal	Ser 396, Ser 402 Thr 404, Ser 405 Ser 386	Stability/Transactivation/ Rearrangement of the autoinhibitory structure Activation
IRF-7	C-Terminal	Ser 477/479	Activation
IRF-5	C-Terminal	Ser 477/480	Activation
xIAP	?	?	Protein turnover
TBK1 specific substrates			
Sec5	Ral Binding Domain	unknown	?
IKKβ	Activation loop	Ser 177/181	Kinase activation
IKKi specific substrates			
STAT1	C-terminus	Ser 708	ISGF3 stability
RelA/p65	TAD	Ser 468	Regulation of gene expression
p100/p52	N/A	Not phosphorylated	Transactivation via p65
DmIKKe substrate			
DIAP1	?	?	Protein turnover

Table I. Substrates of the IKK-related kinases.

these kinases act on transcription factors with tumor suppressor properties, namely IRF-3, -5, and -7 (Table I and Fig. 4). Upon activation by TBK1 or IKKi following viral or bacterial infections, these transcription factors ultimately lead to pathogen clearance. This occurs through the induction of type I IFN and of several IFN-stimulated genes (ISGs) involved in host defense cellular processes.

These processes include protein translation shutdown, cell cycle withdrawal, apoptosis, and hematopoietic cell regulation such as dendritic cell activation. Notably, type I IFN signaling induces p53 accumulation (Takaoka et al., 2003). Thus, at first glance, one can argue that the IKK-related kinases may also counteract tumor development through their effect on type I IFN synthesis. However, several cancers exhibit specific mutations in components of the IFN signaling pathway (Stojdl et al., 2000) and are therefore resistant to the growth-inhibitory effects of type I IFN. Since these cytokines are known to act by paracrine and autocrine mechanisms, transformed cells that rely on IKK-related kinases for survival and proliferation are likely to be unresponsive to type I IFN, thus shifting the equilibrium toward tumor progression (Fig. 4). On the other hand, IRF-3 is known to possess tumor suppressor activities that are unrelated to the protein's effect on type I IFN synthesis, but are directly linked to its effect on apoptosis. Studies have revealed that IRF-3 can transactivate a large number of genes, including ISG56, TRAIL, and NOXA, which can abrogate protein synthesis or induce apoptosis in a p53-independent manner (Grandvaux et al., 2002a; Guo et al., 2000; Kirshner et al., 2005; Lallemand et al., 2007; Weaver et al., 2001). IRF-3 also activates transcription of the gene encoding promyelocytic leukemia protein (PML), leading to p53-dependent growth inhibition of cancer cell lines (Kim et al., 2006; Kim et al., 2007).

The role of IRF-7 in oncogenesis is unclear. According to Zhang and colleagues, IRF-7 itself has oncogenic potential, as evidenced by its ability to induce tumor

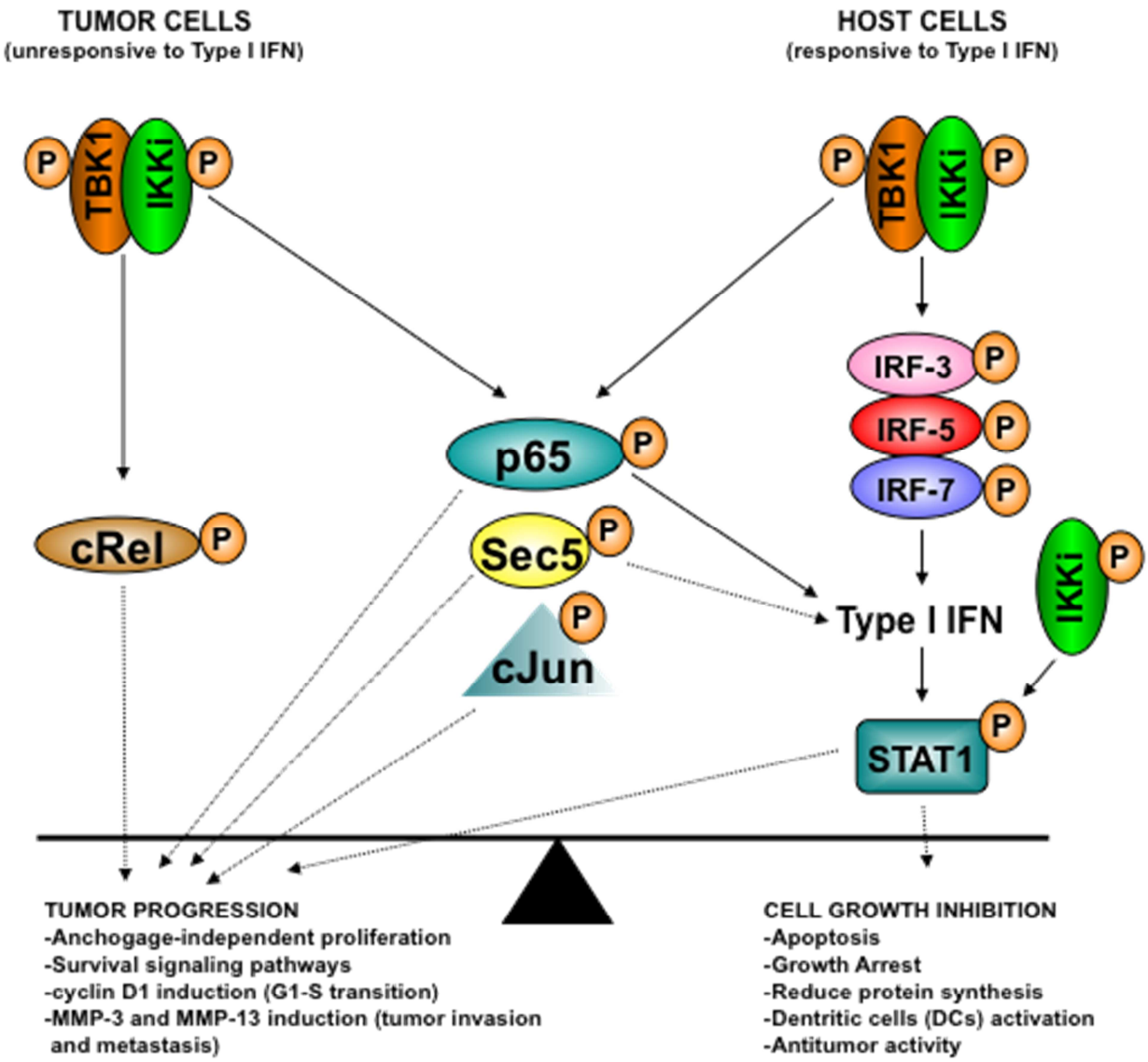

Figure 4: Transformed cells rely on the IKK-related kinases for cell proliferation and viability. Both the expression and the activity of IKKi and TBK1 can be increased in transformed tissues or cancer-derived cell lines. The IKK-related kinases depend on effectors of the NF-κB pathway, such as c-Rel and p65/RelA in order to antagonize apoptotic signals that are normally activated when cells loose contact with the extracellular matrix or following genotoxic insults. Cyclin D1 induction reduces the dependency of growth signals and promotes G1-S transition. The role of phosphoryla-ted Sec5 in TBK1-induced cancer cell survival remains to be elucidated. On the other hand, following exposition to PAMPs, targeted cells use IKK-related kinases to induce a set of anti-viral genes like type I IFN. IFNs are strong inducers of cell cycle arrest. However, several cancers exhibit specific mutations of gene products in the IFN pathway and are therefore resistant to the growth inhibitory effect of type I IFN.

formation in nude mice and to cause anchorage-independent growth in NIH 3T3 cells (Zhang et al., 2004). These authors suggested that IRF-7 might cooperate with the viral onco-protein LMP-1 in EBV-immortalized B cells or CNS lymphoma cells in order to achieve cellular transformation. Conversely, a recent study pointed to the antitumor effector functions of IRF-7. Adenoviral transduction of an active form of IRF-7 into macrophages was found to up-regulate genes such as TRAIL, IL15, IL12p35, ISG56, and CD80 and also down-regulate transcription of proangio-genic/metastatic genes such as VEGF and MMP2. Thus, IRF-7 increased the antitumor properties of primary macrophages while reducing their pro-tumorigenic effects (Romieu-Mourez et al., 2006).

IRF-5 is also a candidate tumor suppressor gene that mediates cell cycle arrest and apoptosis (reviewed in (Tamura et al., 2008)).

In primary hematological malignancies such as acute lympho-blastic leukemia, chronic lymphocytic leukemia and acute myeloid leukemia, IRF-5 expression is frequently down-regulated or absent (Barnes et al., 2003).

Overexpression of IRF-5 reduces colony formation of A549 lung cancer cells and HCT116 colorectal cancer cells (Mori et al., 2002), and it inhibits growth of BJAB tumor cells in vivo (Barnes et al., 2003).

STAT1 and c-Jun, novel substrates of IKKi

As key players in the interferon pathway, proteins known as signal transducers and activators of transcription (STAT) proteins contribute to the induction of numerous genes known to establish a formidable barrier against viral infection. Recently, Tenoever and colleagues established a molecular link between IKKi and the formation of ISGF3, composed of the STAT1-STAT2 heterodimer and IRF-9 (Tenoever et al., 2007). They showed that IKKi phosphorylates STAT1 following treatment with IFNβ. In vitro kinase assays combined with mass spectrometry identified serine 708 as the phosphorylated residue. This post-translational modifycation allows formation of a more stable STAT1-STAT2 heterodimer, which is likely to be essential for binding to the ISRE sequence located in a subset of genes involved in antiviral immunity. Thus, IKKi may be involved in defining ISGF3 binding specificity.

The STAT proteins have also been extensively studied for their role in oncogenesis. For instance, constitutive activation of STAT1, STAT3, and STAT5 has been observed in different human primary tumors and cell lines derived from breast cancer, multiple myeloma, head and neck cancer, leukemia, lymphoma, and lung cancer (Bowman et al., 2000; Watson, 2001). However, unlike the clear involvement of STAT3 and STAT5 in oncogenesis, the role of STAT1 remains obscure and contro-versial. While STAT3 and STAT5 are clearly recognized as oncogenic transcription factors, the STAT1 pathway has been predominantly associated with the inhibition of cell proliferation and tumor suppression (Desrivieres et al., 2006; Watson, 2001). On one hand, STAT1 can promote tumor cell death through transcriptional regulation of genes encoding proteins such as death receptors and ligands, caspases, iNOS, Bcl-xL, and $p21^{Cip1}$ (for reviews see (Bowman et al., 2000; Bromberg and Darnell, 2000; Kim and Lee, 2007)). On the other hand, according to the recent findings of Yarilina and colleagues, STAT1 in myeloid cells could enhance tumor progression by accelerating inflame-mation during the early stages of inflammation-associated tumorigenesis (Yarilina et al., 2008). Indeed, TNFα, a well-known protumorigenic cyto-kine (Lin and Karin, 2007), can stimulate macrophages to produce low levels of IFNβ, which can in turn activate Jak-STAT signaling. Once activated, the Jak-STAT pathway act in synergy with other TNF-induced signals to maintain the expression of inflammatory cytokines. It also leads to increased expression of IKKi, IRF-7, and STAT1. Thus, STAT1 activation could play opposing roles in cancer development (Fig. 4). As mentioned above, IKKi expression is also increased in breast tumors and it may therefore play a role in the constitutive activation of STAT1 seen in breast tumor-derived cell lines (Bowman et al., 2000; Watson, 2001).

The transcriptional activator c-Jun (cellular Jun) has inherent oncogenic potential (Vogt and Bader, 2005). It has been suggested that IKKi is involved in TNFα and LPS-induced MMP-3 and MMP-13 gene expressions via phosphorylation and activa-tion of c-Jun (Sweeney et al., 2005). The phospho-acceptor site(s) on c-Jun was however not identified and more work is necessary to ascertain whether c-Jun transcriptional activity is regulated by IKKi.

Conclusion

There is now compelling evidence that the transcription factor NF-κB plays a key role in cancer development and progression. Thus, the recent suggestion that the IKK-related kinases TBK1 and IKKi also regulate the proliferation and survival of cancer cells was not totally unexpected. These new findings should increase our under-standing of the complex relationship between innate immune effectors and the signaling events that drive tumor formation. Some of the future questions that should be high on the priority list of investigators analyzing the role of these kinases in tumor development are the following. How the kinase activity of TBK1 is increased in cancer-derived cell lines in the absence of any apparent increased in expression level? Is this related to distinct scaffold proteins that facilitate assembly of TBK1 and IKKi subcomplexes (Chau et al., 2008) or is it related to the a decrease in the expression of the tumor supressor CYLD (Zhang et al., 2008)? Is the effect of TBK1 on tumor cell survival and angiogenesis dependent on the NF-κB pathway? What is the molecular basis of IKKi overexpression in breast tumors in the absence of amplification of the IKBKE locus, and is this related to increase NF-κB signaling? Are IRF-3 and IRF-7 activated in transformed cells that rely on TBK1 or IKKi for their proliferation and survival? If yes, then what is the role of the proapoptotic functions of these transcription factors that are unrelated to their effect on type I IFN synthesis? Are there other substrates for these kinases that could explain their effect on tumor cell proliferation and survival? TBK1 and IKKi are new effectors implicated in tumor progression and might therefore represent new opportunities for drug development. Notably, the availability of compounds like SU6668, which targets TBK1 and other kinases, may help dissect out the role of IKK-related kinases in cancer (Godl et al., 2005).

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