Assessment of TANK-binding kinase 1 as a therapeutic target in cancer

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Abstract

TANK-binding kinase 1 (TBK1) is central to multiple biological processes that promote tumorigenesis including cell division, autophagy, innate immune response and AKT-pro survival signaling. TBK1 is well studied and most known for its function in innate immunity. However, the serine threonine protein kinase received significant attention as a synthetic lethal partner and effector of the major oncogene, *RAS*. This review summarizes newly identified cancer promoting functions of TBK1 and evaluates the therapeutic potential of targeting TBK1 in cancer.

Keywords Autophagy · Cancer therapeutic target · Pancreatic cancer · RAS · TBK1

Abbreviations

AALe	Immortalized tracheobronchial epithelial cells
AKT	Protein kinase B
BRAFi	BRAF inhibitor
CCL5	Chemokine ligand 5
CEP1	Centrosomal protein 170
DC	Dendritic cell
DKO	Dendritic cell knockout
EMT	Epithelial-to-mesenchymal transition
GEMM	Genetically engineered mouse model
GFP	Green fluorescent protein
IFN- α	Type I interferon
IFN- β	Interferon β
IFNAR1	Interferon alpha/beta receptor 1
IKB	Inhibitor of NF- κ B
IKK	I κ B kinase
IL1	Interleukin 1
IL6	Interleukin 6
IRF3	Interferon regulator factor 3
LATS1	Large tumor suppressor kinase 1
LC3	Microtubule-associated protein-1 light chain 3
MEFs	Mouse embryonic fibroblasts
MEKi	MEK inhibitor
mTOR	Mammalian target of rapamycin
NF- κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
NSCLC	Non-small cell lung cancer

NSG	NOD scid gamma
NUMA	Nuclear mitotic apparatus protein
OPTN	Optineurin
PD-1	Programmed cell death protein 1
PDA	Pancreatic ductal adenocarcinoma
PDK1	3-phosphoinositide-dependent protein kinase-1
PLK1	Polo-like kinase 1
RAL	Ras-related protein
RAS	Rat sarcoma virus
S6K	p70 S6 kinase
SILAC	Stable isotope labeling by amino acids in cell culture
siRNA	Small interfering RNA
STAT3	Signal transducer and activator of transcription 3
STING	Stimulator of interferon genes
TANK	TRAF family member associated NF- κ B activator
TBK1	TANK-binding kinase 1
TBK1i	TBK1 inhibitor
TCGA	The cancer genome atlas
TGFB	Transforming growth factor β
WT	Wild-type
YAP1	Yes associated protein 1

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Introduction

As a member of the inhibitor of nuclear factor- κ B (IKB) kinase (IKK) family, TANK-binding kinase 1 (TBK1) plays a central role in driving inflammation and innate immunity. Upon receptor-mediated pathogen detection, TBK1 can mobilize the interferon response pathway or activate the NF- κ B pathway in host cell defense (Helgason et al. 2013). However, there is now a growing body of evidence implicating aberrant TBK1 activity in numerous cancer types.

Initial studies linking TBK1 to cancer involved the RAS effector, RALB (Chien et al. 2006, Ou et al. 2011). In NSCLC

cells, RALB activated TBK1, leading to restriction of apoptosis, while having little effect on survival of non-tumorigenic epithelial cells. Moreover, expression of an oncogenic *K-Ras* allele in TBK1-deficient murine embryonic fibroblasts (MEFs) induced cell death, suggesting that TBK1 is integral for cells to tolerate transforming levels of oncogenic RAS. The critical contribution of RALB and TBK1 to RAS-induced lung cancer growth was corroborated in an RNAi screen of synthetic lethal partners of oncogenic K-RAS, where *RALB* and *TBK1* were identified as top hits (Barbie et al. 2009). While RAS signaling through RALB and TBK1 is well established, the mechanism by which TBK1 promotes tumorigenesis remains unclear. Dozens of publications related to TBK1 signaling in cancer have resulted since these initial discoveries were made several years ago. In this review, we highlight some of these impactful discoveries, discuss the viability of TBK1 as a therapeutic target and evaluate the efficacy of TBK1 inhibition from recent clinical trials.

Cellular mechanisms of TBK1-mediated cancer growth

Cell division

TBK1 was originally linked to cell division in a phosphoproteomics screen performed in A549 lung adenocarcinoma cells (Kim et al. 2013). The stable isotope labeling by amino acids in cell culture (SILAC) mass spectrometry technique was used in control shRNA and shTBK1 knock-down A549 cells to define TBK1-regulated signaling networks based on quantitative differences in phosphoproteins. Pathway analyses and subsequent experimental validation revealed that TBK1 is induced at mitosis and directly phosphorylates the mitotic kinase, Polo-like kinase 1 (PLK1). In 2015, Pillai and colleagues (Pillai et al. 2015) confirmed TBK1 induction during mitosis in non-small cell lung cancer (NSCLC) cell lines. However, PLK1 overexpression did not rescue mitotic progression in cells treated with siRNA targeting TBK. This led to a search for and ultimately identification of novel mitotic TBK1 substrates including CEP170 and NUMA, proteins that promote microtubule stability and mitosis.

Given the relatively high frequency of *KRAS* mutations in lung cancer, one consideration is whether TBK1 facilitates mitotic spindle formation independent of mutant *KRAS*. The discovery of CEP170 and NUMA as mitotic TBK1 substrates was made in both mutant and wild-type (WT) *KRAS* NSCLC lines, suggesting that mutant *KRAS* may not be initiating this function of TBK1. Thus the identity of factors upstream that direct TBK1 to the centrosome during mitosis and induce TBK1 expression is of great interest. Evidence from recent studies indicates that TBK1 activation is dependent upon its subcellular localization as well as local TBK1 concentration

(Ma et al. 2012; Helgason et al. 2013). Numerous adaptor proteins have been shown to escort TBK1 to various signaling complexes for distinctive cellular responses. Additionally, TBK1 can autophosphorylate itself through inter-dimer interactions between locally concentrated TBK1 molecules. Moving forward, it will be important to test these findings in vivo to understand the clinical significance of targeting TBK1 in NSCLC. Mitotic defects resulting from TBK1 inhibition in vitro indicate that therapeutically targeting TBK1 would likely have a cytotoxic effect by preventing tumor cell division. Inhibiting cancer cell proliferation by blocking TBK1 activity could be especially beneficial in combination with drugs that function independently of the cell cycle.

Autophagy

TBK1 has been shown to promote the intracellular degradation pathway, autophagy that is often deregulated in human cancers (Newman et al. 2012; Yang et al. 2016). Autophagy is a fundamental biological process of self-digestion, whereby a cell degrades various intracellular components, including damaged or excessive proteins and organelles, as a reactive survival mechanism or as a strategy to maintain cellular energy production. Autophagy is induced by various physiological stressors including hypoxia, nutrient deprivation, high temperatures and innate immune signals (Levine and Klionsky 2004). Deregulation of autophagy is implicated in various disease states, including cancer. However, the function of autophagy in cancer cells is complex with reports indicating it has oncogenic and tumor-suppressive roles. Depending on the tumor source and/or stage, autophagy can function as a tumor suppressor pathway that prevents tumor formation. For example, in the initial stages of pancreatic cancer, autophagy can limit inflammation and cell injury, processes that are critical for tumor development and progression (Gukovsky et al. 2013). Autophagy also functions as a pro-survival pathway in pancreas cancer, allowing tumor cells to tolerate metabolic stress and resist cell death induced by chemotherapy. In fact, several studies have reported elevated basal autophagy levels in human pancreatic ductal adenocarcinoma (PDA) cell lines and primary tumor tissues. Inhibition of autophagy in culture and in PDA mouse models caused marked growth suppression, indicating a large subset of PDA cells depend on autophagy (Yang et al. 2011; Rosenfeldt et al. 2013).

Originally, autophagy was thought to be a nonselective bulk degradation pathway in response to cellular stress. Recent studies have revealed that through the use of autophagic receptors and adaptors, the antibacterial form of autophagy, referred to as xenophagy, can selectively degrade intracellular pathogens (Reggiori et al. 2012; Fimia et al. 2013). In xenophagy, TBK1 activates the adaptor proteins OPTN and p62 that bind to and escort invading pathogens for rapid autophagic clearance (Wild et al. 2011; Pilli et al. 2012; Jo et al.

2013). We, along with another group (Pilli et al. 2012), observed an increase in the autophagic marker, LC3, in *Tbk1*-deficient mouse embryonic fibroblasts (MEFs) without any pathogenic stimulus (Fig. 1a). Additionally we detected an increase in autophagic markers, LC3 and p62, in tumor tissues collected from *Tbk1* mutant mice (*Tbk1*^{Δ/Δ}) that were crossed into a genetically engineered mouse model of PDA (*Kras*^{LSL-G12D}; *Cdkn2a*^{lox/lox}; *Ptf1a*^{Cre/+}) compared to *Tbk1*^{+/+}: PDA tumors (Fig. 1b). *Tbk1*^{Δ/Δ} mice have a truncated form of TBK1 that lacks the catalytic domain and is expressed at very low levels globally (Marchlik et al. 2010). These findings demonstrate that autophagy is altered in the absence of *Tbk1* and suggest that TBK1 may regulate autophagy induced by stimuli other than bacteria. Furthermore, it is plausible that TBK1 contributes to the pro-survival effect of autophagy in tumors, including PDA.

Similarly, a recent study by Yang et al. (2016) demonstrated a link between autophagy inhibition and TBK1 signaling in pancreas cancer. They claim that a negative feedback mechanism exists between TBK1 and autophagy, whereby active TBK1 promotes basal autophagy in PDA cells and is then degraded in the autophagic process to limit over-activation

of autophagy and TBK1-induced cytokine production, both of which fuel neoplasia. Accordingly, utilization of the multi-target JAK/TBK1/IKKε inhibitor CYT387 (mometinib) proved advantageous in blocking autophagy and cytokine production in a PDA cell line with high basal autophagy and in a PDA mouse model, resulting in more intact pancreatic acinar tissue and limited pre-neoplastic lesions relative to vehicle control. While the authors mention that autophagy restricts TBK1 and cytokine activity, it isn't clear how cell lines with low basal levels of autophagy would respond to the multi-target inhibitor CYT387. It is intriguing to consider but currently unclear whether baseline autophagy levels could serve as a predictor of CYT387 sensitivity. Or whether inflammation could function as a biomarker for this TBK1 inhibitor. It is also unclear if mutant active *KRAS* is necessary for the negative feedback between TBK1 and autophagy in PDA cells. This is especially relevant considering that oncogenic *KRAS* can induce autophagy and is commonly mutated in PDA (Guo et al. 2011). It is worth pointing out that CYT387 has undergone testing in multiple clinical trials including in combination with chemotherapy in PDA and in combination with the MEK inhibitor, Trametinib, in NCSLC. Results from

Fig. 1 Autophagy is dysregulated in the absence of *Tbk1*. **a** MEFs isolated at E13.5 days were cultured in DMEM-10% serum. **b** GFP-LC3⁺ puncta were quantified in a minimum of four microscopic fields per cell line (3 cell lines/genotype). Results are presented as mean ±

SEM. ***p*<0.01, student's *t*-test. **c** Tumor tissues from *Tbk1*^{+/+}: PDA and *Tbk1*^{Δ/Δ}: PDA mice were stained for autophagy markers LC3 and p62 to analyze autophagic flux. Images are representative of 4 images/tumor, 5 tumors/genotype

these trials will be very informative from multiple interest points, including that of *KRAS*-driven cancers.

Immune response

Innate immune sensing is a critical step in promoting T-cell priming and infiltration in tumors. Better understanding of the cross talk between innate and adaptive immune systems is needed to pharmacologically facilitate enduring anti-tumor immunity. TBK1 is best known as an innate immune kinase that is downstream of the transmembrane protein stimulator of interferon genes (STING) in the type I interferon (IFN-I) response pathway. STING senses the presence of nucleic acids from intracellular pathogen infection and in turn initiates a downstream signaling cascade that includes TBK1-mediated activation of IRF3 and Stat6, resulting in IFN-I and cytokine production (Ishikawa and Barber 2008; Chen et al. 2011). Multiple STING agonists have been shown to generate anti-tumor immunity through interferon β (IFN- β) production in the tumor microenvironment (Woo et al. 2014; Corrales et al. 2015). Intratumoral injection of synthetic derived cyclic dinucleotides to activate STING resulted in primary tumor regression in three different murine tumor models, including those of B16 melanoma, as well as 4T1 mammary and CT26 colon carcinomas. In each of these models, a systemic immune response involving IFN- β and cytokine production, priming of CD8 $^{+}$ T cells and immunologic memory was elicited that prevented primary tumor growth and the development of distal lesions. This work supports the development of innate immune modulatory strategies for anti-tumor efficacy and implicates TBK1 as a potential anti-tumor protein.

Contrary to these findings, a recent study of dendritic cell (DC) conditional *Tbk1* knockout mice (*Tbk1*-DKO) found that *Tbk1*-DKO mice injected subcutaneously with tumor cells lived longer and had smaller tumors compared to *WT* control mice (Xiao et al. 2017). Bone marrow and spleen from *Tbk1*-DKO and *WT* control mice showed similar peripheral immune profiles, implying that TBK1 is not critical to myeloid cell development. Yet the assessment of anti-tumor immunity in *Tbk1*-DKO animals containing B16 melanoma tumors revealed greater T-effector cell infiltration into tumors and lymph nodes as well as synergy with anti-PD-1 treatment. These results were corroborated with two additional tumor cell lines (EG7-OVA and EL4 lymphoma cells). Gene expression profiling of DCs isolated from *Tbk1*-DKO spleens and tumors showed enhanced interferon-responsive gene expression compared to *WT* control DCs. To see if the *Tbk1*-DKO tumor suppressive phenotype was dependent upon interferon signaling, DCs harvested from *Tbk1*-DKO mice crossed into *interferon alpha/beta receptor 1* (*Ifnar1*) KO mice were utilized in DC-based tumor immunotherapy in *WT* mice with established B16 melanoma tumors. Treatment with double *TBK1*^{DKO} *IFNAR*^{KO} DCs allowed for tumor development

unlike in the *TBK1*^{DKO} *IFNAR*^{WT} DC therapy group where tumor growth was restrained. Collectively, these observations support a pro-tumor function for TBK1 in DCs that suppresses *IFNAR1* signaling to mediate immune tolerance and enable tumor growth.

While the interpretation of the function of TBK1 in tumor immunity from these two publications (Corrales et al. 2015; Xiao et al. 2017) are conflicting, we can glean that the function of TBK1 is likely variable between different stromal cell types within a tumor and even within cancer types. Further work is needed to understand whether there are distinct tumor types that would respond better to modulation of TBK1 activity. In the previous publication (Xiao et al. 2017) for example, synergy was observed in *Tbk1*-DKO animals containing B16 melanoma tumors treated with anti-PD-1. Thus, it is plausible that PD-L1 expressing tumors may show a stronger response to TBK1 inhibition. Xiao and colleagues (Xiao et al. 2017) also showed greater T-effector cell tumor infiltration in tumor bearing *Tbk1*-DKO animals compared to tumor bearing *WT* control animals. Therefore, it may also be therapeutically beneficial to pretreat immune therapy non-responder patients with a TBK1 inhibitor in an effort to augment T-cell infiltration into tumors. These papers highlight the importance in understanding the unique function of TBK1 in each relevant cell type within a tumor as well as different tumor types and mouse models. This is especially pertinent when considering the therapeutic benefit of pharmacological modulation of TBK1 in cancer patients. If TBK1 does function differently between immune cell types, then going forward it would be important to evaluate whether one cell type is more abundant in a particular tumor or has more of a dominant effect on tumor growth.

AKT pro-survival signaling

One of the original reports linking TBK1 with RAS signaling identified AKT as a direct substrate of TBK1 in NSCLC (Ou et al. 2011). Follow up from these results has led to the characterization of TBK1 as a molecular vulnerability in subtypes of melanoma, in addition to NSCLC, in two recent studies (Cooper et al. 2017; Eskiocak et al. 2017). In the melanoma study (Eskiocak et al. 2017), a chemical compound library was employed in screening a panel of melanoma cell lines annotated with drug resistance status to identify molecular liabilities unique to BRAF inhibitor (BRAFi) resistant tumors. Bx795, a TBK1/PDK1 (3-phosphoinositide-dependent protein kinase-1) dual inhibitor was the top hit from the screen with selective toxicity in BRAFi resistant cell lines. Since Bx795 is a dual TBK1/PDK1 inhibitor, the mechanism of action causing toxicity in the drug resistant melanoma cell lines was unclear. Additional scaffolds targeting TBK1 were tested, including compound II, MRT6737 and momelotinib, and these showed similar toxicity profiles across the cell

panel, incriminating TBK1 inhibition as the cause for cell death. Of note, TBK1 inhibitor (TBK1i) sensitivity positively correlated with MEK inhibitor resistance. TBK1 inhibitor compound II was selected for further testing in vivo in NSG (*Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ*) mice subcutaneously implanted with three melanoma cell lines that were already proven to be sensitive to compound II in vitro. As expected, tumor growth in these immunodeficient animals was reduced by compound II. Though compound II does show therapeutic benefit in this model, the growth curves of the treated tumors have a positive slope indicating that tumor growth is slowed but not inhibited. In these TBK1-dependent cell lines, compound II treatment reduced AKT activity, yet chemical inhibition of AKT was not sufficient to recapitulate the level of toxicity associated with TBK1 inhibition. Interestingly, two members of the hippo tumor suppressor pathway, LATS1 and YAP1, co-immunoprecipitated with TBK1. Inhibition of TBK1 activated the hippo tumor suppressor pathway while combined siRNA-mediated knock down of YAP1 with AKT inhibition resulted in apoptosis at a level comparable to compound II-mediated apoptosis. Thus, the authors concluded that the basis of TBK1 addiction in drug resistant melanoma cells is through the combined activation of AKT survival signaling and suppression of hippo tumor suppressor pathway activity.

In the NSCLC study (Cooper et al. 2017), sensitivity to TBK1 inhibitors Bx795 and compound II along with gene expression data for 100 NSCLC cell lines were pooled to distinguish biological features of TBK1-dependent cell lines. This data included a screen of chemical compounds in pursuit of scaffolds with similar activity profiles. As in the melanoma study (Eskiocak et al. 2017), the TBK1i sensitivity profiles correlated well with one another. They also were highly correlative with the profiles of multiple AKT/mTOR pathway inhibitors, particularly in the mutant *KRAS* NSCLC lines, suggesting a mechanistic relationship between TBK1 and the mTOR pathway. Subsequent experiments in fibroblasts with genetic modulation or pharmacological inhibition of TBK1 uncovered a physical interaction between TBK1 and multiple components of the mTOR pathway. TBK1 promoted mTOR activation through direct phosphorylation of upstream activator AKT and downstream substrate S6K predominantly in the transition from the amino acid-starved to fed state. Further analysis of the TBK1i sensitive NSCLC lines revealed mutations in *RAS* family members and greater mesenchymal gene expression compared to the resistant lines that had a more epithelial gene expression profile. Intriguingly, TGF β treatment in a TBK1i resistant cell line induced epithelial-to-mesenchymal transition (EMT) and sensitized the cell line to TBK1i in a reversible manner. All in all, this report characterized a TBK1-dependent subset of NSCLC lines that contain mesenchymal gene expression, *RAS* class member mutations and are liable to mTOR pathway inhibitors. A limitation of this study is that the mechanism of TBK1 dependency in the

subset of NSCLC lines is attributed to TBK1-mediated activation of the mTOR/AKT pro-survival pathway that was observed primarily in fibroblasts. This investigation sheds light on the effect of TBK1 inhibition in a relevant stromal cell type which is informative considering small molecule inhibitors do not target cancer cells exclusively. However, additional studies to validate these interactions in lung cancer cell lines would substantiate the proposed mechanism in the NSCLC context. Additionally, it would be interesting to examine the non-tumor cell autonomous consequences of inhibiting TBK1-mediated mTOR activation in cancer associated fibroblasts or other stromal cells on overall tumor growth.

These two studies (Cooper et al. 2017; Eskiocak et al. 2017) highlight the potential of TBK1 as a therapeutic target in BRAF/MEKi resistant melanoma together with mutant *RAS* NSCLC. Moving forward, it will be essential to evaluate the therapeutic benefit of TBK1 inhibition in preclinical cancer models, particularly in immune-competent animals. The tumor xenograft models from the melanoma study were performed in immunodeficient animals so there is a gap in understanding how well TBK1 inhibition will affect the immune-competent tumor microenvironment. Also, the TBK1-dependent melanoma cell lines were BRAF/MEKi resistant and displayed an “innate immune” expression profile. This specific combination of drug resistance and gene expression is associated with a decreased response to immune therapy (Hugo et al. 2015). Therefore, TBK1 inhibition may be a viable alternative to the subset of melanoma patients who don’t respond to immune therapy. It is also possible that pretreatment of these patients with TBK1i could enhance immune therapy response. An unexplored question stemming from the melanoma study is whether *NRAS* mutation status, which can be found in up to 30% of melanoma patients, had any correlation with TBK1i sensitivity. One group observed that TBK1 is active in mutant *NRAS* melanoma and promoted migration and invasion of these cells (Vu and Aplin 2014). The group also demonstrated cooperation between TBK1 and MEK inhibition to promote apoptosis in mutant *NRAS* MEKi resistant melanomas, suggesting clinical utility for combined TBK1/MEK inhibition. While mutant *NRAS* is common in melanoma, *KRAS* activating mutations dominate PDA and to a lesser extent colon and lung cancers, respectively. Given the high sensitivity to TBK1i seen in NSCLCs with *RAS* class mutations it would also be appropriate to investigate TBK1 dependency in these other *RAS*-driven cancers. In our own analysis, data from a cohort of PDA patients within The Cancer Genome Atlas (TCGA) revealed a correlation between high *TBK1* RNA expression and poorer overall survival (Fig. 2a). Expression of TBK1 homolog, *IKBKE*, on the other hand showed no correlation with overall survival (Fig. 2b). Though not causative, this data strengthens the relevance of TBK1 in *RAS*-driven PDA.

Fig. 2 High *TBK1* expression is associated with poor prognosis. Kaplan Meier curves of a cohort of PDA patients from TCGA showing the overall survival of **a** the *TBK1*-high (upper 25 %) and *TBK1*-low (lower 75 %) expression subgroups and **b** the *IKBKE*-high (upper 25 %) and *IKBKE*-low (lower 75 %) expression subgroups. Cox regression was used to calculate hazard ratios and Kaplan Meier survival analyses

TBK1 inhibitors

Over the past decade, research and interest in TBK1 have expanded along with the identification and development of small molecules targeting TBK1. There are at least six distinct small molecules that are known to inhibit TBK1 including Bx795, compound II, CYT387, MRT67307, GSK2292978A and amlexanox. Though most of these compounds are quite potent towards TBK1 and homolog IKK ϵ , not all of them are highly selective. Bx795 inhibits the serine/threonine kinase, PDK1, in addition to TBK1 and IKK ϵ . CYT387 or momelotinib was originally described as a selective JAK inhibitor but was recently discovered to inhibit TBK1 with low nanomolar potency (Zhu et al. 2014). Three years ago, momelotinib began testing in combination with either chemotherapy or Trametinib (MEKi) in multiple clinical trials for metastatic *KRAS* mutant NSCLC and PDA (NCT02258607, NCT02244489, NCT02101021). Unfortunately all three trials were terminated early by the sponsor, Gilead Sciences without the release of further information.

The only other TBK1i known to enter clinical trial testing in human patients is amlexanox in a phase 2 study for the

treatment of type 2 diabetes, nonalcoholic fatty liver disease or obesity (NCT01975935, NCT01842282). Amlexanox was identified in a compound library screen for inhibitors of TBK1/IKK ϵ , which had previously been shown to promote metabolic syndrome in an obese rodent model (Chiang et al. 2009; Reilly et al. 2013). Takeda developed amlexanox in the 1980s for the treatment of asthma and conjunctivitis in Japan. Though the mechanism of action was unknown until recently, amlexanox is still sold there today for treating asthma. Despite the fact that TBK1 inhibition in patients with metabolic syndrome is a different biology than cancer, the side effects from systemic TBK1 inhibition in this trial are applicable to the evaluation of TBK1 as a viable therapeutic target in cancer patients. In the open label trial to ensure the safe administration of amlexanox, there were no serious adverse events reported (Oral et al. 2017). However, mild to moderate events included two rash incidents that resolved in the presence of continued amlexanox treatment. The rashes, confirmed as perivascular inflammation by biopsy, are reminiscent of the reported phenotype seen in the previously mentioned (Marchlik et al. 2010) kinase dead *Tbk1* $\Delta\Delta$ mice. These mice exhibited mild levels of immune cell infiltrates in multiple

Table 1 Summary of TBK1-mediated malignant growth signaling

Upstream/Predictor	Downstream/Substrate	Context	Reference
-	PLK1	NSCLC	Kim et al. 2013
-	CEP170, NUMA	NSCLC*	Pillai et al. 2015
KRAS, IL1	CCL5, IL6	PDA	Zhu et al. 2014; Yang et al. 2016
STING	IRF3	Melanoma	Corrales et al. 2015
-	IFNAR1, STAT3	Melanoma, Lymphoma	Xiao et al. 2017
BRAF ⁱ /MEKi resistance	AKT, YAP1, LATS1	Melanoma	Eskicak et al. 2017
RAS class	S6K, AKT	NSCLC, Fibroblasts	Cooper et al. 2017

- Undefined/unmentioned by authors

*Experiments were carried out primarily in NSCLC cell lines but some experiments were validated in cell lines of other origins including myeloid leukemia, Daudi Burkitt lymphoma, HeLa, AALE cells

organs and tissues including skin. It is likely that modulation of the innate immune kinase TBK1 will result in some form of an altered immune reactivity. The question then becomes in what way and how will that affect a tumor? Aside from that, the mild side effects observed from the amlexanox trial are promising in terms of tolerability of systemic TBK1 inhibition, at least in the context of amlexanox.

Conclusion

The multifunctional kinase, TBK1, mediates numerous signaling pathways that drive malignant growth (Table 1). TBK1 facilitates cell division in lung cancer cells through its interaction with mitotic substrates PLK1, CEP170 and NUMA to promote microtubule stability and mitosis. TBK1 can also promote autophagy in pancreatic cancer cells to silence proinflammatory signals that elicit an immune response. Furthermore, the DC-specific function of TBK1 suppresses IFNAR1 signaling to enable tumor growth. In a subset of melanoma and NSCLC cancers, TBK1 has been implicated in the activation of AKT/mTOR signaling to promote cancer cell survival. To date, the majority of TBK1-related cancer research has conferred a pro-tumorigenic role for TBK1. Further investigations targeting TBK1 in immune-competent animals are needed to better predict response in human cancer patients and to analyze the effect on tumor metastases. It is estimated that nearly 90% of cancer mortalities are due to metastases, yet studies to date have evaluated the effect of TBK1 inhibition on primary tumor burden. Gene expression data from the melanoma and NSCLC studies (Cooper et al. 2017; Eskiocak et al. 2017) suggest TBK1 is involved in EMT, which is a process commonly associated with metastatic potential. Both subsets of TBK1i sensitive melanoma and NSCLC cell lines comprised a mesenchymal gene signature. Looking ahead, it will be critical to test the effect of targeting TBK1 on tumor cell migration, invasion and overall metastatic burden. These studies will aid in our overall understanding of the clinical benefit of targeted TBK1 inhibition.

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