IN THE SPOTLIGHT

TBK1 Activation by VHL Loss in Renal Cell Carcinoma: A Novel HIF-Independent Vulnerability

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Summary: The Von Hippel-Lindau gene product is a tumor suppressor whose ubiquitin ligase function is key to oxygen-sensing in cells, whereas Tank-binding kinase (TBK1) is a kinase mostly implicated in innate immune response. The study by Hu and colleagues in this issue reveals that VHL suppresses TBK1 activity under normoxic conditions, and that loss of VHL in kidney cancer cells renders them sensitive to TBK1 inhibition, providing a new potential target for the treatment of clear cell renal cell carcinoma.

See related article by Hu et al., p. 460 (8).

VHL is a tumor suppressor gene whose gene product hydroxylates hypoxia-inducible factor (HIF) α, leading to its degradation by the proteasome. However, in the absence of oxygen, HIF α accumulates and promotes pro- and antitumorigenic innate immune pathways. TBK1 is a kinase that can regulate these pathways; it is activated by hypoxia or when VHL function is lost. This study shows that VHL prevents the phosphorylation of TBK1 on Serine 172, which is necessary for its activation.

The functional loss of both copies of the Von Hippel Lindau (VHL) gene is a necessary, but insufficient, step in the pathogenesis of both hereditary ccRCC and sporadic ccRCC. The protein encoded by the VHL gene forms a complex (along with elongins C and B, CUL2, and RBX1), called VCB–CR, which has E3 ubiquitin ligase activity. Under normal oxygenation conditions, HIF α is hydroxylated by EGLN1 and VHL. However, under hypoxic conditions (when the oxygen cofactor is missing) or when VHL function is lost, HIF α accumulates and dimersizes with constitutively expressed HIFβ to activate an array of hypoxia-inducible genes, including those implicated in erythropoiesis, angiogenesis, and glycolysis. This process forms the basis for the development of ccRCC, and the discovery of this biological sequence partly led to the Nobel Prize in Physiology or Medicine in 2019.

TANK-binding kinase 1 (TBK1), and its homolog IκB kinase epsilon (IKKe), are noncanonical members of the IKK family. Their roles in innate immune signaling and cancer have been well characterized, including promotion of cell survival, autophagy, and mTOR–AKT signaling, and TBK1 activation promotes KRAS-driven tumorigenesis. Furthermore, TBK1 signaling in both cancer and immune cells can promote immunosuppression, and potent/specific TBK1 inhibitors have been shown to potentiate ICI responsiveness in preclinical models. TBK1 also activates type 1 IFN signaling downstream of the cGAS–STING pathway and other viral and pathogen sensors, and thus can regulate both pro- and antitumorigenic innate immune pathways.

In elegant work published in this issue of Cancer Discovery (8), Hu and colleagues show that TBK1 activity is suppressed by VHL under normoxic conditions. Either hypoxia or VHL loss leads to an increase in TBK1 S172 phosphorylation (phospho-TBK1) and consequently increased TBK1 activity. In an interesting parallel to the regulation of HIFα by VHL, VHL was found to bind TBK1 on a proline residue (P48) hydroxylated by EGLN1 (Fig. 1). However, VHL regulation of phospho-TBK1 differed in important ways from that of HIFα: (i) VHL was not found to lower the total amount of the TBK1 protein but rather only the active phosphorylated form; (ii) the mechanism by which VHL decreased
phospho-TBK1 was found to be through recruitment of PPM1B (phosphatase of phospho-TBK1), which dephosphorylates phospho-TBK1, and not through ubiquitination of phospho-TBK1; and (iii) the downstream effects of the accumulated phospho-TBK1 seem to be mediated by the phosphorylation, and ensuing stabilization, of p62 (a kidney cancer oncogene, implicated in autophagy) and not by activation of hypoxia-responsive element (HRE) sequences in gene promoters. Overall, these findings add to the list of the lesser-known HIF-independent non–ubiquitination-mediated functions of VHL and propose a novel non–HRE-dependent mechanism of hypoxia or pseudohypoxia responsiveness.

In addition to these novel mechanistic insights, the results of this study could also form the basis for the development of novel therapeutic agents for ccRCC. Indeed, Hu and colleagues showed that VHL-null ccRCC cell lines, compared with isogenic VHL-restored cell lines, developed very significant proliferative defects either when TBK1 was depleted or when a catalytically inactive form of TBK1 (K38A) was expressed, suggesting that TBK1 loss is synthetically lethal with VHL loss. Importantly, the selective proliferative defects with TBK1 inhibition in VHL-null (but not VHL-restored) ccRCC cell lines could be reproduced with three distinct pharmacologic inhibitors, as well as by using a TBK1 proteolysis targeting chimera (PROTAC), which allows greater specificity in targeting TBK1 compared with pharmacologic inhibitors.

Recently, HIF2α inhibitors have been developed and are currently under investigation in clinical trials for the treatment of multiple cancers, including ccRCC. These agents hold great promise; patients heavily pretreated by multiple lines of therapy (including VEGFR-TKIs and ICIs) showed clinical responses (overall response rate of 14% including one durable complete response) in a recent phase I trial of the first-generation inhibitor, PT2385 (9), while the next generation of anti-HIF2α inhibitors is currently being evaluated in clinical trials (NCT03634540, NCT02974738, and NCT04195750). These agents seem especially effective in VHL-null tumors, in which HIF2α is overexpressed, and therefore elucidating the role of the synthetic lethality of TBK1 with VHL within this context may be crucial for future therapeutic development. It is therefore interesting that Hu and colleagues show that a ccRCC cell line previously shown to be resistant to PT2399 (a close structural analogue of PT2385), UMRC2, was found to be sensitive to CMPD1, a pharmacologic inhibitor of TBK1. However, PT2399 and CMPD1 were not found to have a cooperative effect on the proliferation of UMRC2. Although the results of testing in UMRC2 suggest that TBK1 inhibitors may be effective for the subset of VHL-null tumors that are intrinsically resistant to HIF2α inhibitors, the potential cooperative effects of these two classes of agents should be further investigated across a range of cell lines with different sensitivities to either single-agent inhibitor alone.

**Figure 1.** The parallels of regulation of HIFα and TBK1 by VHL under normoxic and hypoxic (or pseudohypoxic, due to VHL loss) conditions and mechanisms of action of TBK1 and HIF2α inhibitors. EGLN, Egl nine homolog; O2, oxygen; OH, hydroxyl group; P, phosphoryl group; PPM1B, protein phosphatase, Mg2+/Mn2+ dependent, 1B; Ub, ubiquitin.
To evaluate the role of TBK1 depletion in a ccRCC tumor context, Hu and colleagues first introduced doxycycline-inducible TBK1 short hairpin RNA (shRNA) into UMRC2 ccRCC cell lines that were then injected into mice. They found that the tumor-forming capacity of TBK1 shRNA-expressing cells was impaired in vivo compared with controls. Second, the authors evaluated the relationship between VHL loss (by DNA sequencing and IHC) and the ratio of phospho-TBK1 to TBK1 by IHC in patient tumor–normal pairs of samples and tissue microarrays. Phospho-TBK1 expression was found to be higher in tumors with VHL loss compared with tumors that had normal or increased VHL expression by IHC. Third, the authors found that p62 expression was higher in tumor compared with normal tissue, but they found no correlation between the phospho-TBK1/TBK1 ratio and p62 expression. It is also notable that multiple tumors with VHL loss had low phospho-TBK1 expression and others with normal or high VHL expression had relatively high phospho-TBK1 expression. The authors largely discounted these differences in the patient tumor results versus those observed in preclinical models as due to technical factors related to the clinical samples and the tissue microarrays. However, the intricacies of the relationship between these molecular features in ccRCC tumors merit further investigation. For example, loss of VHL on chromosome 3p co-occurs variably with deletions or mutations in the epigenetic modifiers PBRM1, SETD2, and BAP1, which could influence derepression of specific endogenous retroviruses that can also activate TBK1 (10).

In summary, the study by Hu and colleagues unveils novel mechanistic insights into hypoxia response as well as the functions of VHL and TBK1, identifying TBK1 as a novel synthetic lethal target in tumors with VHL loss. Future studies will need to better elucidate the downstream effects of increased TBK1 activity in ccRCC, including the potential effects of increased p62 expression on autophagy and tumorigenesis. The interplay of VHL, phospho-TBK1, and p62 should also be further assessed in patient ccRCC tumors, as the relationship seems more complex than that found in cell line models, as suggested by the ambiguous results from the patient tissue microarrays presented in the study. Most importantly, the clinical role of TBK1 inhibitors in the treatment of patients with ccRCC will need to be further refined as highly selective TBK1/IKKε inhibitors continue to be developed. Special attention will need to be given to combination therapies with TBK1 inhibition, including with VEGFR-TKIs and HIF2α inhibitors, considering their complementary mechanisms of action, and with ICIs, given the potential to enhance antitumor immune responses.

Disclosure of Potential Conflicts of Interest

D.A. Barbie is a consultant for N of One/Qiagen, is a scientific advisory board member for Tango Biosciences, reports receiving commercial research grants from Novartis, BMS, and Lilly, and has ownership interest (including patents) in Xpshera Biosciences. No potential conflicts of interest were disclosed by the other author.

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