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Transcriptional control of kidney cancer:

An *in vitro* screen for novel pVHL substrates identifies the oncoprotein ZHX2 in renal cancer

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Clear cell renal cell carcinoma (ccRCC) is a subtype of kidney cancer characterized by von Hippel-Lindau (*VHL*) gene inactivation in ~90% of patients. *VHL* encodes pVHL, the recognition component of an E3-ubiquitin ligase complex that targets hydroxylated substrates for proteasomal degradation (1). The canonical targets of pVHL are the α -subunits of hypoxia-inducible factors (HIFs), which are constitutively stabilized early in ccRCC development and direct transcriptional programs increasing angiogenesis and rewiring intracellular metabolism (2–4). Identifying pVHL substrates other than HIF- α 's that escape degradation in ccRCC and contribute to tumorigenesis could represent a novel therapeutic approach for this disease. On page XXX of this issue, Zhang, *et al.* (5) demonstrate for the first time that “zinc fingers and homeoboxes” 2 (ZHX2) is a novel pVHL target. Moreover, the ZHX2 transcription factor promotes ccRCC tumorigenesis through regulation of NF- κ B signaling.

The ZHX family of DNA-binding proteins has been reported to play critical roles in cell cycle progression through their function as transcriptional repressors of nuclear factor- κ B, controlling the expression of cyclin-dependent kinases (6). A previous publication on ZHX proteins in ccRCC analyzed RNA-seq data from The Cancer Genome Atlas and found that ZHX1 and ZHX3 are underexpressed, while ZHX2 is overexpressed, in ccRCC relative to healthy kidney tissue (7). Low expression of ZHX1 and ZHX3 within ccRCC tumors was also associated with worse overall patient survival. However, the particular function of ZHX2 in ccRCC and its mode of regulation had not been previously described.

In this issue of *Science*, Zhang and colleagues identify ZHX2 from an *in vitro* screen designed to detect proteins that can be out-competed for binding to pVHL by hydroxylated HIF- α . Using a number of primary patient samples, the authors determined that ZHX2 accumulates in the nucleus of *VHL*-deficient, but not *VHL*-wildtype, ccRCC. Functionally, ZHX2 was shown to maintain proliferation of multiple ccRCC cell lines in 2D- and 3D-cell

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culture experiments as well as *in vivo* through orthotopic and subcutaneous xenograft assays.

To determine the mechanism by which ZHX2 acts as an oncoprotein in ccRCC, the authors employed genome-wide approaches (including ChIP-seq and RNA-seq) and found that ZHX2 regulates the expression of a number of NF- κ B target genes. NF- κ B is an anti-apoptotic signaling pathway that is activated following *VHL* loss in ccRCC (8). ZHX2, in complex with the canonical NF- κ B subunit p65/RelA, binds to NF- κ B consensus motifs at a number of sites marked by H3K4me3 and H3K27ac, associated with active gene transcription (Figure 1). In agreement with this, Zhang, *et al.* demonstrated that genetic or pharmacologic inhibition of NF- κ B signaling attenuates ccRCC cell growth, and that elevated expression of a number of the NF- κ B target genes identified is associated with worse patient survival in ccRCC. These findings may explain why ZHX2 is overexpressed, while ZHX1 and ZHX3 are underexpressed, in renal cancer.

Earlier screens to identify undiscovered targets of pVHL generated hits such as NDRG3, an oxygen-regulated substrate that activates Raf-ERK signaling and promotes tumor growth (9). In the work by Lee, *et al.*, lactate accumulation in hypoxic tumor microenvironments results in lactate modification of NDRG3 to inhibit the prolyl hydroxylase/pVHL machinery, thereby preventing its degradation. The hits identified in the *in vitro* screen performed by Zhang and colleagues likely captures proteins that would be stabilized early in disease development, as biallelic inactivation of *VHL* is amongst the earliest tumorigenic events preceded only by chromosome 3p loss (10). That said, it is possible that lactate buildup in hypoxic ccRCC tumors could contribute to additional factors being stabilized that would not have been found through the *in vitro* screening methodology presented here. Notably, NDRG3 was not reported as a pVHL substrate in the Zhang, *et al.* study. Future efforts could combine the Zhang, *et al.* approach with materials reflecting lactate-mediated and other post-translational modifications of proteins in late-stage ccRCC patient material.

A current challenge in the field of cancer biology relates to stratifying patients to increase frequency of responses to targeted chemotherapeutics. For example, genomic and pre-clinical data suggests small molecule inhibition of HIF-2 α would be efficacious in treating a large majority of ccRCC patients. However, in practice, HIF-2 α inhibition does not work on some tumors expressing HIF-2 α , and resistance develops even in patients that were initially sensitive to the treatment (11, 12). Other efforts have focused on identifying consistent “targetable” metabolic adaptations in ccRCC that might benefit a larger patient population, regardless of the diverse genetic background of primary tumors (13). The identification of novel biomarkers such as ZHX2 could represent a way to stratify patients sensitive to combinatorial therapies and delineate new clinically actionable pathways in ccRCC, such as NF- κ B.

Additional questions remaining include: what other factors regulate ZHX2 protein stability? It is likely that ZHX2 is dynamically regulated in part through a non-pVHL dependent mechanism, as ZHX2 protein levels did not always correlate with HIF- α abundance nor were they completely eliminated following pVHL add-back. Furthermore, as ZHX2 is expressed to some degree in the renal epithelium of patients with *VHL* mutations, is there a

function for this transcription factor in healthy kidney tissue prior to oncogenic transformation?

Interestingly, in hepatocellular carcinoma and Hodgkin's lymphoma, ZHX2 was reported to be a tumor suppressor and transcriptionally repress expression of cyclins A and E, among other targets (14, 15). This contrasts with its newly elucidated role in ccRCC, where ZHX2 promotes tumorigenesis through its role in NF- κ B gene regulation. Elucidating cell-type-specific patterns of genomic occupancy and functions of ZHX2 across cancer subtypes represents an appealing future direction to this research, particularly in non-pVHL driven disease. These studies highlight the importance of lineage specificity in determining whether a given factor will play an oncogenic or tumor suppressive role.

Collectively, the findings of Zhang, *et al.* describe a novel pVHL substrate that is stabilized in ccRCC and responsible for promoting oncogenic NF- κ B signaling. Targets such as ZHX2 that are present in a large majority of tumors as a consequence of *VHL* inactivation may represent a promising therapeutic strategy, particularly for patients with metastatic disease in which limited treatments are currently effective.

References

1. Kaelin WG Jr, The VhL Tumor Suppressor Gene: Insights Into Oxygen Sensing and Cancer. Transactions of the American Clinical and Climatological Association 128, 298 (2017). [PubMed: 28790514]
2. Ivan M et al., HIF α targeted for VHL-mediated destruction by proline hydroxylation: implications for O₂ sensing. Science 292, 464–468 (2001). [PubMed: 11292862]
3. Jaakkola P et al., Targeting of HIF- α to the von Hippel-Lindau ubiquitylation complex by O₂-regulated prolyl hydroxylation. Science 292, 468–472 (2001). [PubMed: 11292861]
4. Mandriota SJ et al., HIF activation identifies early lesions in VHL kidneys: evidence for site-specific tumor suppressor function in the nephron. Cancer cell 1, 459–468 (2002). [PubMed: 12124175]
5. Zhang J et al., VHL Substrate Transcription Factor ZHX2 As An Oncogenic Driver In Clear Cell Renal Cell Carcinoma. Science, (2018).
6. Kawata H et al., Zinc-fingers and homeoboxes (ZHX) 2, a novel member of the ZHX family, functions as a transcriptional repressor. Biochemical Journal 373, 747–757 (2003). [PubMed: 12741956]
7. Kwon R-J et al., Expression and prognostic significance of zinc fingers and homeoboxes family members in renal cell carcinoma. PloS one 12, e0171036 (2017). [PubMed: 28152006]
8. An J, Rettig MB, Mechanism of von Hippel-Lindau protein-mediated suppression of nuclear factor kappa B activity. Molecular and cellular biology 25, 7546–7556 (2005). [PubMed: 16107702]
9. Lee DC et al., A lactate-induced response to hypoxia. Cell 161, 595–609 (2015). [PubMed: 25892225]
10. Mitchell TJ et al., Timing the landmark events in the evolution of clear cell renal cell cancer: TRACERx renal. Cell 173, 611–623. e617 (2018). [PubMed: 29656891]
11. Cho H et al., On-target efficacy of a HIF-2 α antagonist in preclinical kidney cancer models. Nature 539, 107 (2016). [PubMed: 27595393]
12. Chen W et al., Targeting renal cell carcinoma with a HIF-2 antagonist. Nature 539, 112 (2016). [PubMed: 27595394]
13. Ochocki JD et al., Arginase 2 Suppresses Renal Carcinoma Progression via Biosynthetic Cofactor Pyridoxal Phosphate Depletion and Increased Polyamine Toxicity. Cell metabolism 27, 1263–1280. e1266 (2018). [PubMed: 29754953]
14. Nagel S et al., Transcriptional deregulation of homeobox gene ZHX2 in Hodgkin lymphoma. Leukemia research 36, 646–655 (2012). [PubMed: 22078940]

15. Yue X et al., Zinc fingers and homeoboxes 2 inhibits hepatocellular carcinoma cell proliferation and represses expression of Cyclins A and E. *Gastroenterology* 142, 1559–1570. e1552 (2012). [PubMed: 22406477]

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Figure 1. The novel pVHL substrate ZHX2 promotes NF- κ B gene expression and tumorigenesis in ccRCC

ZHX2 escapes proteasomal degradation in *VHL*-deficient ccRCC and accumulates in the nucleus, where it binds to NF- κ B consensus motifs on DNA along with p65/RelA. Genetic depletion of ZHX2 reduces nuclear accumulation of p65, NF- κ B target gene expression, and tumor growth.