Kidney Cancer: Now Available in a New Flavor

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The role of the von Hippel-Lindau tumor suppressor protein (pVHL) in kidney cancer has provided a rationale for treating this disease with hypoxia-inducible factor (HIF) antagonists. In this issue, Simon and coworkers show that the molecular signature of *VHL*$^{-/-}$ kidney cancers is profoundly influenced by whether they produce both HIF2α and HIF1α or HIF2α alone.

People who are "lumpers" look for commonalities among seemingly disparate entities, while people who are "splitters" looked for differences among seemingly similar entities. In this issue of *Cancer Cell*, Simon and coworkers (Gordan et al., 2008) make a case for splitting clear cell renal carcinomas into three groups (Figure 1).

In 2008, over 50,000 Americans will be diagnosed with cancer of the kidney and renal pelvis, and over 13,000 will die of this disease, making it one of the ten leading causes of cancer death in the United States. Worldwide, over 250,000 new cases are diagnosed each year.

Kidney cancers account for 95% of cancer of the kidney and renal pelvis. Surgery is the mainstay of treatment for early-stage (organ-confined) kidney cancer and is often curative in this setting. Unfortunately, kidney cancers that cannot be removed surgically, or that recur after initial attempts at surgical resection, are usually fatal. A small subset of patients with advanced kidney cancers experience durable remissions after treatment with high-dose interleukin-2. Unfortunately, this therapy is very toxic, and it is impossible to predict which patients will benefit. Kidney cancer is refractory to conventional forms of chemotherapy and radiotherapy. In fact, until recently, it was ethical to include a placebo arm in randomized clinical trials of new agents for advanced kidney cancer patients who had failed or were not eligible for immunostimulants such as interleukin-2.

Clear cell renal carcinomas (ccRCCs) account for ~75% of all kidney cancers. Individuals with von Hippel-Lindau (*VHL*) tumor suppressor gene germline mutations have an increased risk of developing ccRCC and biallelic *VHL* inactivation, due to somatic mutations or, less frequently, promoter hypermethylation, occurs in 50%–75% of sporadic ccRCCs. Moreover, restoration of *VHL* function in *VHL*$^{-/-}$ renal carcinomas suppresses tumor growth. In short, *VHL* inactivation plays a causal role in hereditary and sporadic ccRCC.

Among its many functions, the *VHL* gene product, pVHL, is the substrate recognition component of a ubiquitin ligase that inhibits the transcription factor hypoxia-inducible factor (HIF), which consists of an unstable α subunit and a stable β subunit (Kaelin, 2008). Specifically, the pVHL complex targets the HIFα subunit for destruction when oxygen is present. Accordingly, *VHL*$^{-/-}$ kidney cancers overproduce HIF and HIF-responsive gene products such as VEGF. Drugs that inhibit VEGF or its receptor KDR have now demonstrated significant activity in the treatment of kidney cancer, as have mTOR inhibitors, which indirectly downregulate HIF (Kaelin, 2008).

Humans have three *HIF*α genes. HIF1α and HIF2α are both capable of activating transcription. Whether HIF3α activates transcription is less clear, and certain HIF3α isoforms are dominant-negative inhibitors with respect to HIF1α and HIF2α. HIF1α is ubiquitously expressed, while HIF2α expression is more restricted. For this and other historical reasons, HIF1α has been the most intensively studied HIF family member. Nonetheless, important quantitative and qualitative differences are beginning to emerge between HIF1α and HIF2α.

One difference is that the genes activated by HIF1α and HIF2α are overlapping but not entirely congruent (Kaelin and Ratcliffe, 2008). For example, HIF1α plays a particularly important role in the activation of genes involved in glucose metabolism, while HIF2α has been linked to the induction of genes such as the stem cell factor gene *Oct4* and the erythropoietic hormone gene erythropoietin (*EPO*). HIF1α can, both directly and indirectly, cooperate with or antagonize c-Myc in a context-dependent manner (Kaelin and Ratcliffe, 2008). Importantly, HIF2α cooperates with c-Myc and promotes cell proliferation under conditions in which HIF1α antagonizes c-Myc (Gordan et al., 2007). The biochemistry underlying these various differences is incompletely understood but might include differential sensitivity to FIH1, an asparaginyl hydroxylase that hydroxylates and thereby silences one of HIFα's two transactivation domains, and/or differential binding of HIF1α and HIF2α to non-HIF transcription factors such as c-Myc and Notch (Kaelin and Ratcliffe, 2008). A second difference is that HIF1α, but not HIF2α, remains susceptible to ubiquitinylation in *VHL*$^{-/-}$ cells, implying the existence of additional HIF1α ubiquitin ligases that do not require pVHL (Kaelin, 2008). Finally, synthesis of HIF1α is more sensitive to rapalogs, which inhibit the mTOR-containing TORC1 complex, than is the synthesis of HIF2α (Toschi et al., 2008). Instead, synthesis of HIF2α appears to be controlled by the mTOR-containing TORC2 complex, which is relatively insensitive to rapalogs.

VHL$^{-/-}$ renal carcinoma cell lines produce either HIF1α and HIF2α or HIF2α alone. Moreover, examination of preneoplastic lesions in kidneys from VHL patients indicates that HIF activation is an early event, with the appearance of HIF2α correlating with increased evidence of transformation (Mandriota et al., 2002). Importantly, overproduction of HIF2α (but not HIF1α) is sufficient to override pVHL's

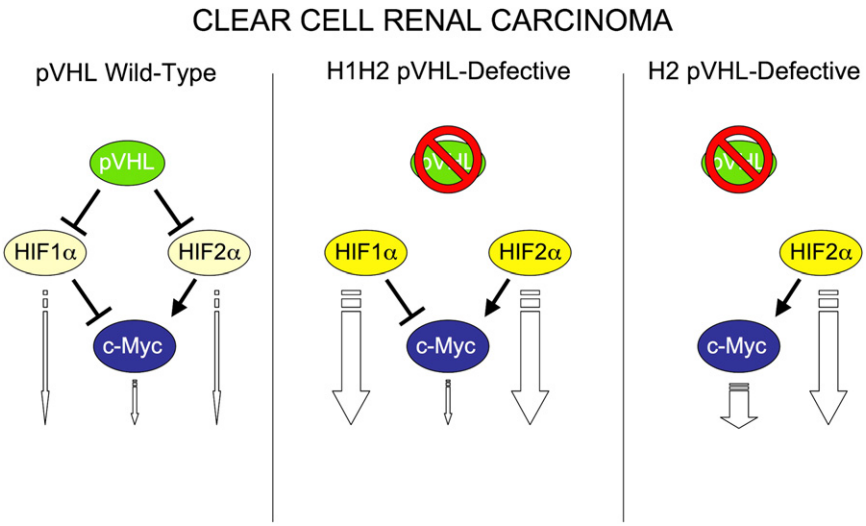

Figure 1. Clear Cell Renal Carcinoma Subtypes
pVHL targets HIFα for proteasomal degradation. Accordingly, tumors with wild-type pVHL have low levels of HIFα. pVHL-defective tumors can be subdivided based on whether they accumulate both HIF1α and HIF2α (H1H2) or HIF2α alone (H2). In the former, HIF1α antagonizes c-Myc. In the latter, this antagonism is lost and c-Myc activity is therefore increased.

ability to suppress *VHL*$^{-/-}$ renal carcinoma tumor growth in nude mouse assays (Kondo et al., 2002; Maranchie et al., 2002). Conversely, silencing HIF2α is sufficient to impair *VHL*$^{-/-}$ renal carcinoma tumor growth in vivo (Kondo et al., 2003). These results suggest that downregulation of HIF2α is both necessary and sufficient for pVHL to suppress renal carcinoma growth. Moreover, deregulation of HIF2α appears to be both necessary and sufficient for the development of pathology in genetically engineered mice lacking pVHL in various organs (Kim et al., 2006; Rankin et al., 2008). Collectively, these results suggest that HIF2α, rather than the canonical HIFα family member HIF1α, drives the development of *VHL*$^{-/-}$ renal carcinomas.

The observations outlined above, however, left open the question of whether the differences between HIF1α and HIF2α observed in cell lines and mouse models would play out in clear cell carcinomas arising in patients. In this issue of *Cancer Cell*, Gordan and coworkers studied over 160 sporadic ccRCCs. As expected, they found that the majority of these tumors were pVHL defective. Interestingly, these tumors could be subdivided into two distinct subtypes based on immunohistochemical assays and mRNA profiling (Figure 1). One subtype was characterized by overproduction of both HIF1α and HIF2α ("H1H2" tumors), and the other by exclusive production of HIF2α ("H2") (Figure 1). The H2 tumors were associated with enhanced c-Myc activity and enhanced cellular proliferation relative to H1H2 tumors, irrespective of stage, in keeping with the idea that HIF1α antagonizes c-Myc in vivo. H2 tumors also displayed increased expression of genes involved in DNA repair, which was associated with decreased levels of endogenous DNA damage and fewer genomic copy number changes. Consistent with cell culture studies, H1H2 tumors were associated with increased expression of glycolytic genes relative to H2 tumors. In addition, proliferation of H1H2 tumors, in contrast to H2 tumors, appeared to be driven by increased activity of TORC1, as determined by phospho-S6 staining, and MAPK activity, as determined by phospho-ERK staining.

These findings are important for several reasons. First, they solidify the importance of HIF2α in human renal carcinogenesis. Interestingly, increased expression of HIF2α has been noted in a variety of nonrenal tumors as well, despite its more restricted expression in normal tissues. In addition, replacement of HIF1α with HIF2α in murine embryonic stem cells promotes their ability to form teratomas in vivo (Covello et al., 2005). Therefore, the role of HIF2α as an oncoprotein might extend beyond *VHL*$^{-/-}$ renal carcinomas. Second, they suggest that there are two biologically distinct types of *VHL*$^{-/-}$ renal carcinomas: those that produce HIF1α and those that do not. It will be of interest to determine whether the presence of HIF1α has predictive value with respect to response to therapy, such as therapy with VEGF inhibitors and rapalogs, and prognostic value with respect to natural history. It will also be of interest to determine whether the absence of HIF1α in H2 tumors reflects a cell-of-origin issue or perhaps genetic (or epigenetic) changes that occurred during tumor progression. Indeed, the current study by Gordan et al. raises the somewhat heretical possibility that HIF1α acts as a tumor suppressor in the context of *VHL*$^{-/-}$ ccRCC. In any event, future kidney cancer clinical trials, especially those that directly or indirectly take aim at HIF, would be well advised to consider the presence or absence of HIF1α as a potential confounder.

REFERENCES

Covello, K.L., Simon, M.C., and Keith, B. (2005). Cancer Res. *65*, 2277–2286.

Gordan, J.D., Bertout, J.A., Hu, C.J., Diehl, J.A., and Simon, M.C. (2007). Cancer Cell *11*, 335–347.

Gordan, J.D., Lal, P., Dondeti, V.R., Letrero, R., Parekh, K.N., Oquendo, C.E., Greenberg, R.A., Flaherty, K.T., Rathmell, W.K., Keith, B., et al. (2008). Cancer Cell *14*, this issue, 435–446.

Kaelin, W.G., Jr. (2008). Nat. Rev. Cancer *8*, 865–873.

Kaelin, W.G., Jr., and Ratcliffe, P.J. (2008). Mol. Cell *30*, 393–402.

Kim, W.Y., Safran, M., Buckley, M.R., Ebert, B.L., Glickman, J., Bosenberg, M., Regan, M., and Kaelin, W.G., Jr. (2006). EMBO J. *25*, 4650–4662.

Kondo, K., Klco, J., Nakamura, E., Lechpammer, M., and Kaelin, W.G. (2002). Cancer Cell *1*, 237–246.

Kondo, K., Kim, W.Y., Lechpammer, M., and Kaelin, W.G., Jr. (2003). PLoS Biol. *1*, E83.

Mandriota, S.J., Turner, K.J., Davies, D.R., Murray, P.G., Morgan, N.V., Sowter, H.M., Wykoff, C.C., Maher, E.R., Harris, A.L., Ratcliffe, P.J., and Maxwell, P.H. (2002). Cancer Cell *1*, 459–468.

Maranchie, J.K., Vasselli, J.R., Riss, J., Bonifacino, J.S., Linehan, W.M., and Klausner, R.D. (2002). Cancer Cell *1*, 247–255.

Rankin, E.B., Rha, J., Unger, T.L., Wu, C.H., Shutt, H.P., Johnson, R.S., Simon, M.C., Keith, B., and Haase, V.H. (2008). Oncogene *27*, 5354–5358.

Toschi, A., Lee, E., Gadir, N., Ohh, M., and Foster, D.A. (2008). J. Biol. Chem. Published online October 22, 2008. 10.1074/jbc.C800170200.