Two HIFs may be better than one

HIF-2α overexpression directly contributes to renal clear cell tumorigenesis: evidence for HIF as a tumor promoter.

As they expand, solid tumors can rapidly outgrow the carrying capacity of the local vasculature; this phenomenon is typically aggravated by the chaotic vascular structures that form during malignant growth. Thus, tumors are often riddled with areas of lowered oxygen content, or hypoxia, which has many therapeutic ramifications. First, hypoxic regions of tumors are much more resistant to radiation, an important problem in radiotherapy. Second, hypoxia induces release of angiogenic factors such as VEGF (vascular endothelial growth factor), and thus contributes to tumor vascularization. Finally, chronic hypoxia within tumors can select for cells resistant to hypoxia-induced apoptosis; these cells often have mutations in the *p53* gene; therefore, hypoxia can indirectly contribute to the process of malignant progression at the genetic level (Graeber et al., 1996).

When exposed to lowered levels of oxygen, tissues compensate in a variety of ways, ranging from the systemic adjustments caused by increased erythropoietin production to the tissue-specific effects of increased *VEGF* expression and the largely cell-autonomous effects of increased glycolysis (reviewed in Semenza, 1999). All of these adaptations to hypoxia are regulated wholly or in part by the hypoxia-inducible transcription factor (HIF) complex. The oxygen-regulated components of this complex are the HIFα subunits. To date, three members of the *HIFα* family have been cloned: *HIF-1α*, *HIF-2α*, and *HIF-3α*. Of the HIFα subunits, the function of HIF-1α has been the most extensively characterized. Of note as well, thus far, there appears to be little redundancy between *HIF-1α* and *HIF-2α* function based on phenotypes of mice lacking either gene.

During normoxia, the HIFα subunits are rapidly degraded, directed by their interaction with the von Hippel-Lindau (VHL) tumor suppressor protein, in a complex that induces ubiquitination and eventual destruction of the subunit by the proteasome (reviewed in Kondo and Kaelin, 2001). In familial disease, heterozygosity for the von Hippel-Lindau mutations (*VHL*) gives rise to a wide range of solid tumors, including clear cell renal carcinomas, the most common subtype of renal cell carcinomas (RCC). Spontaneous deletion of *VHL* is also a common cause of sporadic renal clear-cell carcinoma; mutations in *VHL* are found in 50%–80% of these tumors. In the United States, almost 30,000 patients are diagnosed with RCC each year; these tumors tend to be highly vascularized; when metastatic, the median survival is less than one year (Karumanchi et al., 2002).

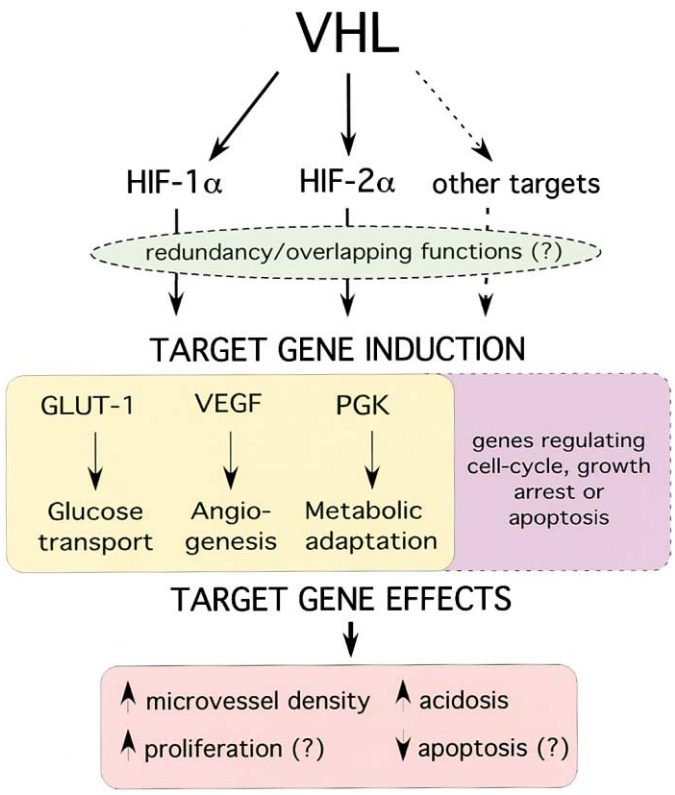

Figure 1. The pathway from VHL to renal cell carcinoma

The steps from loss of VHL to tumorigenesis are becoming more defined. Solid arrows and lines represent the correlations between known genetic pathways and corresponding specific genes that are affected by loss of VHL; dotted arrows and lines represent relationships that have been described, but remain uncertain. In summary, loss of VHL permits constitutive expression of HIF-1α and HIF-2α, and possibly other yet to be identified targets of proteasomal degradation. In this issue of *Cancer Cell*, Kondo et al. (2002) and Maranchie et al. (2002) have described the importance of HIF-2α with respect to growth of renal cell carcinoma; however, it remains unknown whether other HIFα subunits contribute to RCC via redundant or overlapping functions. Overexpression of HIFα subunits results in the induction of several classical HIF-1 target genes, even in the absence of hypoxia, such as Glut-1, VEGF, and PGK. These target genes are in turn responsible for stimulating the angiogenic and metabolic changes that manifest as increased tumor microvessel density and acidosis, hallmarks of a variety of tumor types. The role that hypoxia plays with respect to cell cycle progression/arrest and/or apoptosis is less well-defined. Nevertheless, it is likely that dysregulation at any point in the hierarchy contributes to the development of renal cell carcinoma, as well as other malignancies observed in patients with VHL mutations.

To date, the only proteins that are clearly substrates of the VHL complex are HIF-1α and HIF-2α. In response to hypoxia, HIF-α proteins are stabilized; therefore, loss of *VHL* expression represents a potent mechanism to achieve overexpression of the HIFα subunits. In fact, previous studies in cell lines and in vivo have suggested that most, if not all, phenotypic effects of the loss of *VHL* may be due to the normoxic stabilization of HIF and increased expression of HIF dependent genes such as *VEGF* (Wiesener et al., 2001).

A key question with regards to the biology of the hypoxic response has been whether HIF is a positive or negative regulator of tumor growth. In contrast to most normal tissues, which lack detectable HIFα staining, expression of HIF-1α has been reported in a variety of human tumors, including prostate, breast, and colon cancers (Zhong et al., 1999). The staining was noted especially in

areas of necrosis or on the borders of necrotic cells, presumably the cells exposed to the most severe hypoxia. In contrast, in RCC, HIF-1α staining is detected throughout the tumor, presumably due to constitutive overexpression (Karumanchi et al., 2002).

In mouse xenograft models, striking differences in growth rates of tumors have been reported using genetically modified cell lines that lack HIF-1 activity. In three studies, *HIF-1α* has been identified as a positive regulator of tumor growth (Maxwell et al., 1997; Ryan et al., 1998, 2000). A variety of HIF-1 null cell types were used in these studies, including embryonic stem (ES) cells lacking *HIF-1α*, transformed fibroblasts lacking *HIF-1α*, and hepatoma cells lacking *HIF-1β*. In contrast to these results, other investigators have reported that tumors derived from ES cells lacking *HIF-1α* can grow faster than wild-type tumors as a result of decreased rates of apoptosis (Carmeliet et al., 1998). The reasons for these discrepancies remain unclear, although clonal effects are likely to be the source of some of the differences seen.

Another key question is whether HIFα subunits are necessary/sufficient to induce increased microvessel density (MVD) observed in a majority of human tumors. Again, there is controversy regarding the role of HIF-1α in tumor angiogenesis, since depending on the cell type utilized to derive HIF-1α tumors, the tumors either exhibit decreased or equivalent MVD. For example, a recent study of 60 human ovarian tumors did not find a significant relationship between MVD and HIF-1α expression, nor a correlation between MVD and HIF-1α expression in survival (Nakayama et al., 2002).

The recent work by Maranchie et al. (2002) and Kondo et al. (2002), presented in this issue of *Cancer Cell*, is the first attempt to directly test and compare the individual contribution of HIFα subunits to tumorigenesis. Their results support the growing consensus that the HIFα proteins function to promote tumor growth of a variety of tumor types.

Both Maranchie and Kondo have utilized an RCC line derived from a sporadic renal clear cell carcinoma (786-0), in which one allele of VHL has been lost, and the other inactivated by a truncation at amino acid 104. This cell line has been previously reported to overexpress HIF-2α, as well as HIF target genes such as *VEGF* and glucose-transporter-1 (*Glut-1*). These cells lack detectable expression of HIF-1α protein. Both groups utilized RCC lines previously created by Illiopoulos et al. (1995), in which either VHL or empty vector were stably transfected into parental 786-0 cells in order to create VHL wild-type or null RCC lines. Although both groups use mouse xenograft models of their modified RCC lines to arrive at similar conclusions, they approach the question of the role of the HIFα subunits in renal cell tumorigenesis in differing ways, by independently investigating the effects of overexpression of either HIF-1α and HIF-2α (the latter indirectly) (Maranchie et al., 2002) or HIF-2α (Kondo et al., 2002) independently of VHL status.

In light of recent observations that proline hydroxylase activity is required for interaction with VHL and subsequent degradation of the HIFα subunits, both groups mutated key proline residues of either HIF-1α or HIF-2α to allow constitutive expression. Kondo et al. (2002) compare the growth of tumors in VHL wild-type cells to cells that express both wild-type VHL as well as a point mutant of HIF-2α (P531A). Similarly, Maranchie et al. (2002) have mutated P564 of HIF-1α and compared tumor growth to VHL wild-type or null cells. In addition, Maranchie et al. (2002) have created cells that express a blocking peptide derived from the VHL binding domain of HIF-1α (the oxygen-dependent domain, ODD) that functions to prevent association of either HIFα with pVHL, allowing constitutive overexpression of HIF-2α (since these cells lack HIF-1α expression).

In this issue of *Cancer Cell*, Kondo et al. (2002) demonstrate that introduction of the HIF2αP531A mutant into 786-0 cells wild-type for VHL accelerated tumor growth rate, and increased tumor net weight approximately 50%. Results obtained by Maranchie et al. (2002) indicate that tumor size is approximately equivalent between RCC cells lacking VHL and RCC cells that are wild-type for VHL, but express the ODD blocking peptide; this confirms that overexpression of HIFα subunits promotes tumorigenesis in these cells. Interestingly, overexpression of HIF-1α in a VHL-independent fashion impaired tumor growth; this, together with the blocking peptide data and the data from Kondo et al. (2002), would argue that the tumor-promoting HIFα is in fact HIF-2α in these cell lines.

Although both groups demonstrate that dysregulation of HIF-2α promotes tumorigenesis in RCC, it is interesting to note that there are some histological differences seen in the two papers. Of particular note, the clear cell phenotype is observed in tumors overexpressing HIF-2α (Kondo et al., 2002), but not in the blocking peptide expressing mutants (described in Maranchie et al., 2002), which are also characterized by high levels of HIF-2α expression. Indeed, such discrepancies are common in cell culture models following sequential manipulation and passaging, and will need to be resolved to determine the exact role of HIF-2α with respect to the clear cell morphology.

In summary, these novel investigations of HIFα function have suggested that overexpression of HIF-2α (and, in the case of Maranchie et al. [2002], not HIF-1α) promotes tumor cell growth in an RCC-derived cell line. These observations, in turn, have exciting implications for the specification of the role(s) of HIF-1α and HIF-2α in promoting tumor growth. A model that summarizes the genetic alterations proposed to result in RCC is presented in Figure 1. In addition, there are intriguing suggestions that HIF-1α and HIF-2α differ substantially in their functions during tumorigenesis. Follow-up studies that characterize the specific genetic pathways controlled by these two different hypoxia-responsive transcription factors should provide exciting insights into how hypoxia shapes and controls the growth of solid tumors.

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RUNX: A trilogy of cancer genes

The RUNX family of transcription factors plays pivotal roles during normal development and in neoplasias. Recent data involve RUNX3 as an important tumor suppressor in gastric cancers and pose interesting questions about how perturbed levels and interspecific competition among RUNX family members may contribute to tumorigenesis.

On a worldwide basis, gastric cancer is a major cancer-related killer. In Japan and certain other Asian countries gastric cancer tops the list of cancer-induced deaths. While clear links have been discovered between environmental factors, such as *Helicobacter pylori* infection, dietary components, and gastric cancer frequencies, the genetic basis for gastric cancer development is still largely unclear. In the April 5 issue of *Cell*, Li and coworkers (2002) describe the uncovering of RUNX3/AML2/CBFA3/PEBP2αC as a candidate tumor suppressor in gastric cancer development. RUNX3 belongs to the Runt domain family of transcription factors, which consists of 3 DNA binding α subunits, RUNX1, RUNX2, and RUNX3 (see Table 1 for alternative names), each of which is capable of forming heterodimers with the common β subunit CBFβ. RUNX heterodimers are relatively weakly acting transcriptional regulators, the potency of which can be induced by associations with transcriptional (co)activators, such as MYB, ETS, and p300/CBP, or corepressors such as TLE1 and mSin3A (Perry et al., 2002). The main family feature, the 128 amino acid runt domain named for its high homology to the *Drosophila* pair-rule protein runt, facilitates dimerization and DNA binding. Like their counterparts in *D. melanogaster* and *C. elegans,* mammalian RUNX family transcription factors play important roles in cell fate determination during development.

The work of Li and coworkers (2002) completes the mouse knockout analyses of the *Runx* family and underscores the role of Runt family members as cancer-related genes. As previously found for *Runx1* (Okuda et al., 1996), *Runx2* (Otto et al., 1997), and *Cfb*β (Wang et al., 1996), genetic ablation of *Runx3* has profound effects. While *Runx3* knockout mice are born in Mendelian ratios, they die soon after birth probably due to starvation. *Runx3* is strongly expressed in gastrointestinal organs in the developing embryo and throughout adult life of the mouse and the gastric epithelium in *Runx3* knockouts displays hyperplasia and a reduced apoptotic rate. Interestingly, when analyzed in primary cultures *Runx3*$^{-/-}$ gastric epithelial cells are less sensitive to TGF-β-mediated growth inhibition, based on a marked failure to enter apoptosis when treated with TGF-β. To investigate the potential connection between RUNX3 and gastric cancers in humans, Li et al. (2002) analyze a series of gastric cancer cell lines and primary human gastric tumors. Out of 46 primary human tumors, 30% displayed hemizygosity of *RUNX3* with a significant correlation between *RUNX3* loss and gastric cancer progression stage. Furthermore, RUNX3 expression analysis revealed that on average 60% of the analyzed primary human gastric tumors exhibited reduced RUNX3 levels rising to nearly 90% among the late stage, representing highly metastatic tumors. Upon examination of 119 tumor samples, Li et al. were able to identify only a single nucleotide transition causing an arginine-to-cytosine conversion within the conserved Runt domain, which did not strengthen the tumor suppressor argument much. However, the high CpG nucleotide content triggered analysis of DNA methylation status, and interestingly *RUNX3* hypermethylation in a large number of primary tumor samples was found to correlate with gene silencing, which indicates an unusual strong prevalence for epigenetic gene silencing. To further establish a causal link between RUNX3 expression and oncogenesis, Li et al. injected gastric cancer cells engineered to overexpress either wild-type or Runt domain mutated RUNX3 into nude mice. While RUNX3 mediated a significant reduction in tumorigenicity, the Runt domain mutant aggravated tumor formation. Moreover, gastric epithelial cells immortalized by loss of p53 only were able to form tumors in nude mice when Runx3 was also deleted. Together, these results underline the role of RUNX3 as a bona fide tumor suppressor.

It is now clear that all three Runx family members play important roles in normal developmental processes as well as in cancers (Table 1). RUNX1, perhaps better known as AML1, plays a critical role in hematopoietic development, and genetic ablation of either *Runx1* or *Cbf*β results in embryonic