mammary tumors. Again, the necessity of such accessory proteins in tumor progression offers novel avenues for therapeutic intervention.

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pVHL's kryptonite: E2-EPF UCP

E2-EPF ubiquitin carrier protein (UCP) is a member of an E2 family of enzymes that catalyzes the ligation of ubiquitin to proteins targeted for destruction by the proteasome. UCP is overexpressed in common human cancers, suggesting its involvement in oncogenesis, but a physiologic target of UCP has not been identified. In a recent report published in *Nature Medicine*, Jung et al. identified von Hippel-Lindau (VHL) tumor suppressor protein, which targets the α subunit of hypoxia-inducible factor (HIF) for ubiquitin-mediated destruction, as a bona fide substrate of UCP and demonstrated a potential pVHL-HIF pathway-dependent role for UCP in cancer development.

von Hippel-Lindau disease (OMIM 193300) is a rare hereditary cancer syndrome that is characterized by the development of hypervascular tumors in multiple, and yet specific, organs, including the cerebellum, retina, adrenal gland, and kidney. VHL disease is caused by the inheritance of a faulty *VHL* gene, and the tumors arise when the remaining wild-type *VHL* allele is lost or inactivated via mutation, deletion, or promoter methylation in a susceptible cell. Biallelic inactivation of *VHL* has also been associated with the development of sporadic clear cell renal cell carcinoma (CC-RCC), the most common form of kidney cancer (Kaelin, 2002).

pVHL is a substrate-recruiting component of an E3 ubiquitin ligase called ECV (Elongins/Cul2/pVHL) that is structurally and functionally analogous to the SCF (Skp1/Cdc53/F box protein) complex. Crystal structure of the pVHL/elongin B/elongin C complex revealed two functional domains on pVHL: α and β (Stebbins et al., 1999). The α domain binds elongin C, which acts as a bridge connecting pVHL to the scaffold component Cul2, which binds Rbx1 and a cognate E2 ubiquitin-conjugating enzyme, Cdc34 or UbcH5. The β domain acts as a substrate-docking site. The majority of tumor-associated *VHL* mutations map to the surface residues on either domain, suggesting that these domains are functionally important for the tumor suppressor activity of pVHL.

To date, several cellular proteins have been identified as pVHL binding proteins that are subjected to ECV-dependent ubiquitylation. However, the most convincing substrate that continues to shed significant insight into the tumor suppressor function of pVHL is HIFα (see Figure 1). HIF is a major heterodimeric transcription factor consisting of α and β subunits that transactivates 60 or more hypoxia-inducible genes, including *vascular endothelial growth factor* (*VEGF*; also known as *vascular permeability factor*), *erythropoietin* (*EPO*), and *glucose transporter-1* (*GLUT1*) to promote angiogenesis, oxygen-carrying erythrocyte production, and anaerobic metabolism, respectively, in adaptation to compromised oxygen availability. While the HIFβ subunit (also known as aryl-hydrocarbon receptor nuclear translocator [ARNT]) is abundantly expressed irrespective of oxygen tension, the HIFα subunit is oxygen labile. In the presence of oxygen, HIFα is hydroxylated on conserved prolines within the oxygen-dependent degradation (ODD) domain by prolyl hydroxylase domain-containing enzymes (PHDs). Prolyl hydroxylation is both necessary and sufficient for the binding of HIFα by pVHL and subsequent ubiquitylation via ECV. Accordingly,

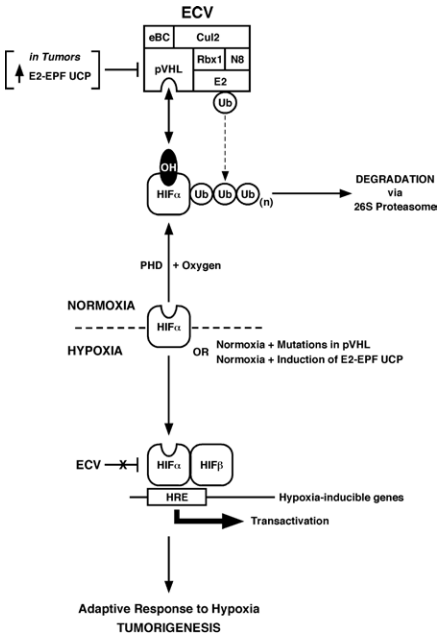

Figure 1. The UCP-pVHL-HIF pathway in cancer

See text for details. eBC, elongins B and C; N8, NEDD8; Ub, ubiquitin; OH, hydroxyl group.

hypoxia or mutation in pVHL leads to the stabilization of HIFα. HIFα then dimerizes with HIFβ to form an active transcriptional complex, which engages the 5′-RCGTG-3′ hypoxia-responsive elements (HREs) within the promoter/enhancer of hypoxia-

inducible genes, triggering a myriad of adaptive responses to hypoxia (Kaelin, 2002).

Tumor growth is inevitably challenged by the limited diffusional capacity of oxygen from the nearest blood vessel, generating a progressively more hypoxic tumor interior. Thus, the induction of HIFα observed in most solid tumors is in part a result of the general oxygen-sensing pathway. In addition, cancer-causing mutations in certain tumor suppressor genes have been identified to bypass the necessity of low-oxygen tension to initiate a "*pseudo*" hypoxic response to promote tumor development (Ohh, 2006). For example, mutations in *TSC2* tumor suppressor gene increase the level of HIF-1α via the mammalian target of rapamycin (mTOR)-dependent and -independent mechanisms that may involve chromatin remodeling. The loss of PTEN is correlated with increased HIF-1α levels, presumably via the Akt/protein kinase B signaling pathway. Mutations in *succinate dehydrogenase* (*SDH*) result in the cytosolic accumulation of succinate, which inhibits PHDs, leading to the stabilization and activation of HIF-1α. The loss of p53 is associated with enhanced HIF-1α expression, presumably due to the attenuation of Mdm2-mediated ubiquitylation of HIF-1α upon the absence of p53. Perhaps the most striking and direct mechanism of stabilizing HIFα involves mutations in pVHL, which permit HIFα to escape from ECV-dependent ubiquitin-mediated degradation.

In a recent article published in *Nature Medicine*, Jung et al. report that E2-EPF UCP, a gene associated with liver and gastric cancers that is capable of increasing the expression of the HRE-driven luciferase reporter gene, forms a complex with pVHL and catalyzes an E3-independent ubiquitylation of pVHL, promoting the destruction of pVHL via the 26S proteasome (Jung et al., 2006) (see Figure 1). Under a normal physiologic state, the expression of UCP and HIF-1α was undetectable in the mouse liver, but upon transduction with an adenovirus-encoded UCP, the level of pVHL decreased with the concomitant accumulation of HIF-1α. In numerous transformed cell lines, as well as primary and metastatic liver, colon, and breast tumors, an inverse relationship between UCP and pVHL was observed where a detectable UCP expression correlated with decreased pVHL and increased HIF-1α expressions. In addition, UCP depletion attenuated the growth rate and invasiveness of tumor cells, while UCP overexpression promoted tumor progression and lung metastasis of melanoma cells in a mouse xenograft model. Previously, Kondo et al. showed in a mouse xenograft model that the tumorigenic potential of 786-O (*VHL*$^{-/-}$;*HIF-1α*$^{-/-}$) CC-RCC cells is critically dependent on the expression of HIF-2α (Kondo et al., 2002). Importantly, Jung et al. demonstrate that, in 786-O cells lacking pVHL, overexpression or depletion of UCP neither had an effect on HIF-2α expression nor influenced the tumor growth rate of implanted 786-O cells in mice, suggesting that the effect of UCP on tumor growth is mediated by the pVHL-HIF pathway (Jung et al., 2006).

Although it is not known how UCP is upregulated in transformed cells, its oncogenic expression is predicted to result in the induction of HIFα via pVHL degradation in virtually any tissue type, since *VHL* is ubiquitously expressed. Curiously, however, germline inheritance of a mutated *VHL* allele causes tumor development in few select organs. This would suggest that a loss or functional inactivation of the remaining wild-type pVHL in certain tissues is insufficient for cellular transformation, assuming that the second "*hit*" is equally probable in all cell types throughout the body. Furthermore, there is compelling evidence to suggest that the functional loss of pVHL even in VHL disease-associated tumors is inadequate for malignant transformation. For example, a conditional inactivation of *VHL* in the renal proximal tubules resulted in glomerular and tubular renal cysts without the presentation of tumors (Rankin et al., 2006), recapitulating the human condition where the loss of *VHL* has been observed in preneoplastic cysts (Lubensky et al., 1996), and suggests that other genetic events are required for the progression of the premalignant cysts to CC-RCC. A certain mutation in pVHL is also associated with congenital polycythemia (Ang et al., 2002). However, these individuals are not at a higher risk of developing cancer, despite enhanced HIF activity as evidenced by the increased levels of EPO, VEGF, and GLUT1. Thus, UCP-mediated depletion of pVHL in non-VHL disease-associated or VHL disease-associated tissues will likely require additional genetic mutations/alterations for tumorigenesis.

Moreover, pVHL has other binding partners, some of which are subjected to ECV-dependent degradation while others are not subjected to the ubiquitin pathway (Ohh, 2006). For example, atypical protein kinase C, VHL-interacting deubiquitinating enzyme (VDU), and the seventh (Rpb7) and the large (Rbp1) subunits of RNA polymerase II are ubiquitylated via ECV. SP1 transcription factor, VHL-associated KRAB-A domain-containing protein (VHLaK) transcription repressor, microtubules, and fibronectin bind to pVHL without being targeted for the ubiquitin-mediated destruction. In addition, pVHL was unexpectedly shown to directly bind and stabilize p53 by suppressing Mdm2-mediated ubiquitylation, and to induce the acetylation of p53 upon genotoxic stress by promoting p53-p300 interaction, resulting in increased p53 transcriptional activity and p53-mediated cell cycle arrest and apoptosis (Roe et al., 2006). Thus, one must consider the possibility that UCP-mediated degradation of pVHL will influence the biological activities associated with one or more of these other pVHL binding proteins, which consequently aid in the process of tumorigenesis. Also, as noted by Jung et al., UCP may catalyze E3-dependent or -independent ubiquitin-mediated degradation of additional substrates, besides pVHL, whose loss will ultimately promote cancer development. These outstanding questions notwithstanding, the discovery of E2-EPF UCP is primed to ignite the next chapter of the molecular mechanisms in pVHL-mediated neoplasia.

Acknowledgments

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