von Hippel-Lindau Syndrome: Target for Anti-Vascular Endothelial Growth Factor (VEGF) Receptor Therapy

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Key Words. *von Hippel-Lindau Syndrome · Vascular endothelial growth factor · Angiogenesis · Hypoxia-inducible factors*

ABSTRACT

von Hippel-Lindau (VHL) syndrome is a familial cancer syndrome caused by germline mutations in the VHL tumor suppressor gene. Mutations in the VHL gene result in the constitutive stabilization of transcription factors hypoxia-inducible factors 1α and 2α, which bind to specific enhancer elements in the vascular endothelial growth factor (VEGF) gene and stimulate angiogenesis. This increase in angiogenesis under normoxic conditions in key target organs such as the brain, kidney, and eye leads to high morbidity and reduced life expectancy. Drugs designed to block the VEGF signaling pathway may prevent the long-term complications of the disease. To test this hypothesis, a clinical study was initiated to evaluate the effect of the VEGF tyrosine kinase receptor inhibitor SU5416 in patients with VHL syndrome. Preliminary data on SU5416 indicate that it is well tolerated when administered chronically in such patients. However, since little is known about the long-term use of such inhibitors, patients will need careful monitoring. Data obtained from monitoring these patients will provide valuable information for adjuvant treatment trials in cancer patients. *The Oncologist* 2000;5(suppl 1):32-36

INTRODUCTION

von Hippel-Lindau (VHL) syndrome is an autosomal dominant familial cancer syndrome caused by germline mutations in the VHL tumor suppressor gene. This disease is characterized by abnormalities of vascular proliferation and an increased risk of certain cancers including clear cell renal carcinomas, pheochromocytomas, endolymphatic sac tumors, central nervous system hemangioblastomas, cysts of the kidney, liver, and pancreas, epididymal cystadenomas, neuroendocrine tumors of the pancreas, and retinal angiomas (Table 1) [1-4]. The age of onset for VHL varies depending on the manifestation, for example, retinal angiomas can develop from age 5 on, whereas renal cancers are not common until the mid-20s [5]. The cloning of the VHL gene in 1993 was a major step in understanding the disease [6]. Identification of the VHL gene on chromosome 3p25 revealed that it possesses a unique sequence and has characteristics of a tumor suppressor gene.

Families with VHL syndrome are classified as having either type 1 (without pheochromocytomas) or type 2 (with pheochromocytomas) disease. Type 2 disease is further classified into three subgroups: group A, patients with renal cancer; group B, patients without renal cancer and group C, patients with only pheochromocytoma. Genetic analyses of VHL kindreds have shown that patterns of mutation in the VHL gene correlate with the distinct clinical phenotypes [7-9]. For example, mutations in codon 167 and missense mutations correlate with type 2 disease [10-13]. Mutations leading to truncations of the gene are associated with a higher risk of renal cancer and are more common in sporadic renal cancer.

Screening programs aimed at detecting and treating complications at an early stage have improved both detection and life expectancy of patients with VHL [14, 15]. Early detection makes it possible to treat retinal angiomas with laser ablation, to remove renal cysts 3 cm in size (risk of malignancy at this size is much greater, but metastasis is of very low frequency), and to treat cerebellar or spinal cord hemangioblastomas neurosurgically. However, many patients still develop severe disease complications, including blindness from untreatable damage due to retinal neovascularization and the occurrence or recurrence of inoperable central nervous system (CNS) hemangioblastomas that require bilateral nephrectomy and transplantation. Thus, a well-tolerated

Table 1. Incidence of tumors associated with VHL syndrome

Lesion	%	Median age of onset (yr)	Age range (yr)
Retinal angioma	58	25	4-68
Cerebellar hemangioblastoma	57	29	13-61
Spinal cord hemangioblastoma	14	34	11-60
Renal cell carcinoma	25	44	23-68
Pheochromocytoma	13	20	12-58

chronic drug therapy that could effectively block the effects of the VHL mutation would be of great value to patients with VHL syndrome. Recent biochemical studies of the VHL protein (pVHL) support a model that it plays a critical role in modulating transcription factors that regulate vascular endothelial growth factor (VEGF) expression. This finding has helped highlight the potential of antiangiogenic agents as rational targets for the treatment of VHL.

ROLE OF VHL IN UBIQUITINATION

Ubiquitination is well defined and involves activation of ubiquitin by a ubiquitin-activating enzyme, transfer of ubiquitin to protein substrate by a ubiquitin-conjugating enzyme, and ligation of ubiquitin to the substrate by the E3 ligase, followed by capture and degradation of the ubiquinated protein by the 26S proteasome [16]. E3 ligases are arranged in a complex modular pattern as seen in the stem cell factor (SCF) (Skp1-Cdc53/CUL-1/F-box protein) ligase. In the SCF E3 ligase, the F-box protein serves as an adaptor that recognizes and recruits target substrates to the ubiquitin ligase. Although SCF ligase complex was originally described in regulating the degradation of cyclins in yeast, the human homolog (hSKP1-human cullin-1-SKP2) is also involved in regulating the cell cycle [17].

The VHL gene encodes a protein with two major domains, which have recently been elucidated by crystallography [18, 19]. One of these domains, the α-domain, interacts with ElonginB-ElonginC-cullin-2 (hCUL-2) complex to form a multiprotein complex, CBC^{VHL}. The structure and function of the CBC^{VHL} complex resemble that of the SCF E3 ubiquitin ligase family (Fig. 1) [16, 18, 20-22]. In the CBC^{VHL} complex, VHL functions as the recruitment protein for the substrates to the ligase. A further component of the complex is the ubiquitin-like protein NEDD8, and conjugation of NEDD8 to hCUL-2 is linked to pVHL activity [20]. The overall complex requires functional pVHL and

key biochemical evidence for the role of VHL and also links VHL to mechanisms involved in cell cycle regulation. Indeed, normal functioning of VHL is needed for cells to exit the cell cycle after serum starvation [23, 24].

Recent reports indicate that other proteins also interact with VHL, including VHL-binding protein 1 [25]; Rbx-1 [26] (an evolutionary conserved RING-H2 finger protein that interacts with Cullins and activates ubiquitin ligase); and atypical protein kinase C isoforms pKCσ and λ (that interact with the β domain of VHL) [27]. There are three exons in the VHL gene and two splice variants generating proteins of 24 and 18 kDa, both of which can interact in these complexes [28-30]. ElonginB-ElonginC complex was initially identified as a positive regulator of RNA polymerase II and then as a component of the VHL complex. In addition to binding to VHL, it also interacts with other multiprotein complexes involved in regulation of cytokine signaling (suppressor of cytokine signaling-1, SOCS-1) [31].

HYPOXIA-INDUCIBLE FACTORS 1α AND 2α

The key to clarifying the role of VHL in the ubiquitin activation complex is to determine the target substrates for CBC^{VHL} complex and where they bind to the complex. At least two substrates are now known—the hypoxia-inducible factors 1α and 2α (HIF-1α and HIF-2α) [32, 33]. These transcription factors are unstable in intact cells under normoxia but are stabilized under hypoxic conditions. Under hypoxic conditions, HIF-1α and HIF-2α translocate to the nucleus where they form a heterodimer with the aryl hydrocarbon nuclear translocator. As shown in Figure 2, these heterodimers bind to specific hypoxia-regulated response elements and activate genes such as VEGF and isozymes of the glycolytic pathway, as well as the transferrin and urokinase receptors and inducible nitric oxide synthase [34]. Thus in hypoxia, several genes of potential importance in tumor growth, invasion, or metastasis are regulated by this pathway. In normoxia, the α-subunits of HIF are rapidly degraded by ubiquitin-mediated proteolysis that is mediated by pVHL [32]. Recent studies indicate that mutations in the VHL gene result in the constitutive stabilization of HIF-α subunits and the activation of HIF-1 in normoxia [32]. Consequently, VEGF as well as other HIF-responsive pathways are constitutively upregulated to levels that normally only occur under hypoxic conditions [33]. The association of mutations in VHL with familial cancer demonstrates that this is a key pathway in tumorigenesis.

The role of HIF-1 in nonfamilial cancer has been evaluated using the mouse xenograft of hepatoma cell line Hepa-1, which was deficient in hypoxia signaling pathway [35]. The tumor cells with a normal hypoxia signaling pathway entered
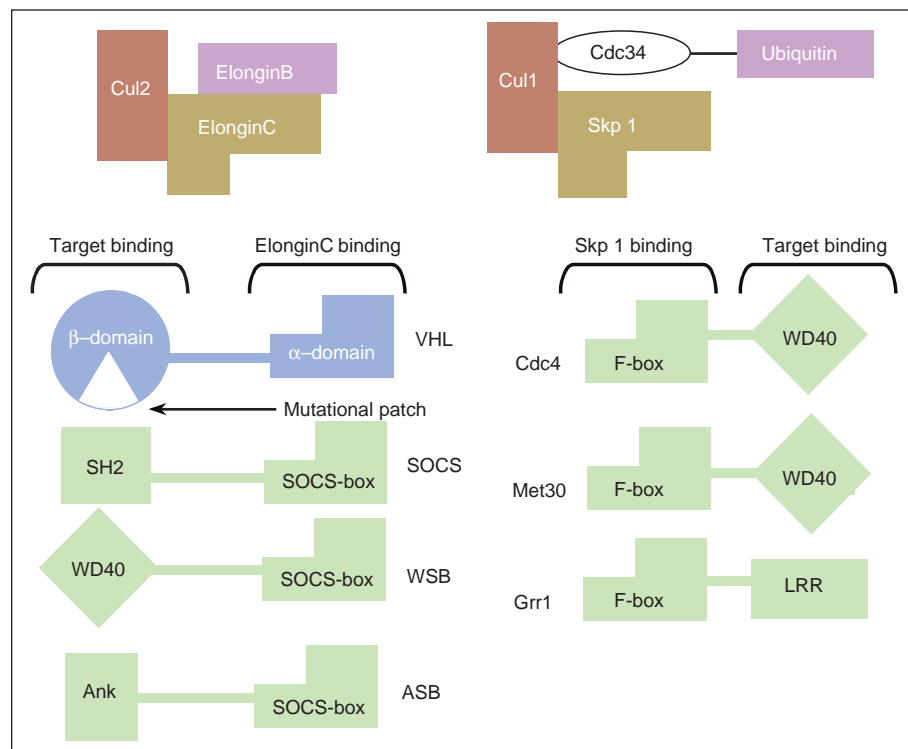
Figure 1. Structure of von Hippel-Lindau (VHL)-ElonginB-ElonginC complex and the stem cell factor (SCF) ligase complex. The similar structure of the VHL-ElonginB-ElonginC and SCF complexes support the hypothesis that the VHL-ElonginB-ElonginC complex may function to regulate the ubiquitin-mediated degredation of target proteins in a manner analogous to that of the SCF complex. Reproduced with permission [18].

cells deficient for hypoxia signaling did not. Hypoxia deficient cells also showed much slower growth in vivo with less angiogenesis and failed to induce VEGF [35]. These results demonstrated the important role of HIF pathway in tumor angiogenesis and growth, even in a fully transformed cell line. Further evidence for the role of HIF-1 in tumorigenesis is derived from studies using immunohistochemical techniques to assess HIF expression in tumor specimens. Using monoclonal antibodies specific for HIF-1, we were able to show that all common tumors switch on this pathway, whereas it is hardly detectable in normal tissues [36]. Therefore, HIF-1α and HIF-2α are key targets for developing new therapy against cancer, and genes downstream in the HIF pathway are also potential targets.

OTHER GENES REGULATED BY pVHL

Several genes important in cancer growth are regulated by mutant and wild-type pVHL. Transforming growth factor β [37], hepatocyte growth factor receptor [38], PAI-1 [39], and carbonic anhydrases [40] are downregulated by wild-type pVHL and upregulated by mutant pVHL. The pVHL also binds fibronectin intracellularly and is necessary for proper assembly of fibronectin in the extracellular matrix [41]. Thus, there is a pleiotropic response to VHL mutations, and whether this is common to all cell lines with VHL mutations is not known. In normal tissues, erythropoietin is regulated by the HIF pathway and is aberrantly

VEGF: TARGET FOR THERAPY IN VHL SYNDROME

Although many genes seem to be regulated by the VHL gene, the clearest role is in regulation of the hypoxia response pathway. Among these genes, VEGF is particularly important because it is responsible for many of the complications associated with VHL syndrome [43], including angiomas and hemangioblastomas [42-45].

The clinical evaluation of current antiangiogenic therapy in cancer is problematic due to the short treatment time and difficulty in determining the optimal dose. In phase I trials, patients are treated only for a few weeks with antiangiogenic agents at what may be less than the optimal dose. Additionally, many patients have bulky tumors that progress rapidly while on treatment. Since antiangiogenic agents tend to stabilize disease rather than produce regression and may induce less normal tissue toxicity than conventional agents, their optimal dose is harder to evaluate. While antiangiogenic therapy will probably be most successful as long-term adjuvant treatment, protocols are difficult to develop without knowing long-term toxicity.

Patients with the VHL syndrome provide an opportunity to study the long-term effects of antiangiogenic therapy while preventing the progression of their disease; these patients can be treated for many years unlike patients with advanced cancers who may only have a few months to live. Optimal drug doses will be easier to determine because the main pathway for renal proliferation seems to be VEGF,

Figure 2. Pathways of regulation of HIF-1α and von Hippel-Lindau (VHL). The labile transcription factor HIF-1α is stabilized in the cytoplasm by hypoxic stress via a sensor and translocates to the nucleus where it heterodimerizes with aryl hydrocarbon nuclear translocator (ARNT) to bind to specific hypoxia response elements (see text for details). There are three members of the HIF-1α family and three of the ARNT family. The VHL gene product is involved in targeting HIF-1α to the proteasome where it is degraded in normoxia.
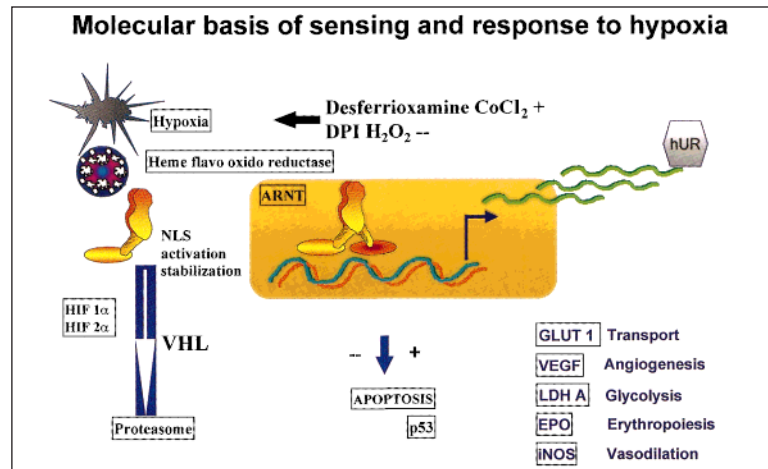
show an effect in VHL compared with cancers that may have multiple angiogenic pathways. Patients could be treated for years with long-term therapy to prevent lesion progression as well as to help define long-term toxicity of adjuvant treatment in cancer trials.

To test this hypothesis, we have initiated a multicenter trial to evaluate the effect of chronic administration of a VEGF-Flk-1/KDR tyrosine kinase inhibitor (SU5416) in patients with VHL who had complications or risks of complications, but in whom existing conventional modalities could no longer be used. These complications included progressive loss of vision not amenable to laser therapy, progressive CNS lesions not amenable to surgery, and progressive renal cysts <3 cm in size, the removal of which would necessitate renal dialysis. End points include reduction of retinal edema and vascular permeability, prevention of lesion progression, and analysis of a range of serum and plasma markers including VEGF. The results of this study will provide valuable information on the benefits of antiangiogenic therapy in VHL patients and on the long-term effects of antiangiogenic therapy.

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