Beyond the Knudson's hypothesis in von Hippel–Lindau (VHL) disease—proposing vitronectin as a “gene modifier”

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Von Hippel–Lindau (VHL) disease is an autosomal dominant hereditary cancer syndrome with a prevalence of 1 in 39,000 live births that results from a germline mutation of the *VHL* gene with over 90% penetrance by 65 years of age despite variable expression [1, 2]. *VHL* is a tumor suppressor gene located on the short arm of the chromosome 3 (3p25–26) that is made of three exons and widely expressed in both fetal and adult tissues [3, 4]. A patient with heritable VHL disease inherits a germline mutation of the gene from the affected parent and a normal gene from the unaffected parent (wild type). The Knudson's two-hit hypothesis of tumorigenesis applies to the initiation of the tumor growth when a somatic mutation affects the wild-type *VHL* allele [5]. Although germline *VHL* mutations are present in all the cells of the affected individuals who inherit the genetic trait, only the cells that are somatically mutated in the wild-type gene and are parts of a susceptible organ experience tumor growth [2]. In fact, patients with VHL disease are predisposed to develop specific central nervous system (CNS) and visceral lesions [1, 2, 4]. Affected individuals may develop retinal hemangioblastomas, endolymphatic sac tumors (ELST) of the labyrinth, or cranio-spinal hemangioblastomas in the cerebellum, brainstem, or spinal cord [2]. Furthermore, visceral involvement in some patients affects the kidney with renal cell carcinoma (RCC) or cysts, the adrenal glands with pheochromocytomas, the pancreas with tumors or cysts, and the epididymus or the broad ligament with cystadenomas [2]. Clinical criteria or genetic tests help in diagnosing VHL disease [1]. Obviously,

patients with a family history of VHL and CNS lesions (hemangioblastomas), RCC, pheochromocytoma, pancreatic cysts or ELST will meet the criteria for diagnosis of VHL [1]. However, 20% of patients lack a family history, but meet the criteria for VHL, if they present two or more CNS hemangioblastomas, or one CNS hemangioblastoma and a visceral VHL-associated tumor [1]. A classification of the VHL disease in type 1 or 2A–C based on the *VHL* mutation, molecular defect, and clinical manifestations have been proposed [4]. Patients at high risk undergo testing for germline *VHL* mutations with 100% detection rate if they have a positive family history [1, 6]. On the contrary, patients with negative family history present with tissue mosaicism that may hamper the use of testing their peripheral blood leukocytes lacking the *VHL* mutation [7]. The somatic *VHL* mutations are present in approximately 50% of sporadic RCC, whereas 20% of cases present hypermethylated *VHL* gene in the same group of tumors [4]. Hypermethylation of the *VHL* gene has only been described in the RCC, while somatic mutations account for 30% of sporadic hemangioblastomas without any description of *VHL* gene hypermethylation in this group of tumors [4]. In the context of sporadic tumors, the two-hit model occurs exclusively somatically rather than in the germline [4].

The *VHL* gene is widely expressed in tissues, including the ones not affected by VHL-transformed phenotype. The *VHL* mRNA encodes a 213 amino acid residue-protein (pVHL) with an apparent molecular weight of ~24 to 30 kDa (VHL₃₀) [1, 4]. A second pVHL isoform of approximately 19 kDa (VHL₁₉) is the by-product of in-frame start codon (ATG) at codon 54 [4]. Both isoforms have tumor suppressor activity. pVHL shuttles between the nucleus and the cytoplasm, and the nuclear-cytoplasmic trafficking is required for the function of the VHL tumor suppressor protein [4]. Primarily, pVHL complexes with

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elongin B, elongin C, Rbx 1, and cullin 2 assembling an ubiquitin ligase that degrades the α subunit of hypoxia-inducible factor-1 α (HIF-1 α) [1, 4]. Under normal conditions, HIF-1 α regulates the cellular response to hypoxia through transcription regulation of factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor β chain (PDGF- β), erythropoietin, and transforming factor α (TGF α) [1, 4]. Contrarily, in conditions of absent or abnormal pVHL function, the HIF-dependent factors are constitutively expressed, particularly VEGF and PDGF- β , which explains the increased angiogenesis of the vascular VHL-associated tumors [1, 2, 4]. Therefore, overexpression of hypoxia-inducible mRNAs is the molecular hallmark of pVHL-defective cells [4]. Furthermore, VEGF-mediated increased tumor vascular permeability may cause the frequent condition of peritumoral edema and formation of cyst [1]. The induction of HIF-mediated expression of TGF α or erythropoietin and their respective receptors favors the establishment of autocrine or paracrine loops [1, 4]. The establishment and regulation of such functional loops may explain the pattern of growth of hemangioblastomas that may alternate tumor growth spurts with growth arrest as recently described [8]. Apart from HIF dysregulation, further conditions that favor tumorigenesis may be related to both non-functional or absent pVHL in the cell. VHL-deficient cells lose the ability to exit the cell cycle, making it the initial step in VHL tumorigenesis [1]. Ultimately, cells lacking pVHL are deficient in assembly of an extracellular fibronectin matrix or in regulating growth arrest mediated by cell-extracellular matrix signaling [9, 10].

Cranio-spinal hemangioblastomas are the most common tumors associated with VHL; 60–80% of patients developing hemangioblastoma of the cerebellum, brainstem, or spinal cord [1]. Almost 90% of these patients present with multiple CNS hemangioblastomas, and despite their benign histology these tumors may cause significant morbidity and mortality in patients affected with VHL [1]. Complete resection of hemangioblastomas is curative and most of these intracranial lesions can be resected safely by reserving surgery until symptoms arise [1, 2]. The occurrence of the tumor is not influenced by the type of the underlying *VHL* mutation as shown by Webster et al. at the end of the 1990s [11]. In 183 individuals with germline *VHL* gene mutations, the prevalence of ocular hemangioblastomas did not increase with age and their distribution in the gene carriers was statistically significantly different from the expected stochastic distributions [11]. Individuals with ocular tumors had significantly higher incidence of cerebellar hemangioblastomas and RCC with hazard ratio (HR) of 2.3 and 4.0, respectively [11]. In families with VHL disease, the number of ocular tumors was significantly correlated in individuals of closer rather than more distant relatedness. This model implies that the development of VHL tumors is determined at

an early age and is influenced by genetic and/or environmental “modifier effects” acting at multiple levels [11]. Webster et al. failed to show any association between the polymorphism of genes such as glutathione-S-transferase M1 gene (*GSTM1*) or the cytochrome P450 2D6 gene (*CYP2D6*) and the severity of ocular/renal tumorigenesis [11].

Over a decade later, Huang et al. in this issue describe the genetic polymorphism of vitronectin (VN) as a potential candidate for a “genetic modifier” that may affect the risk of occurrence of hemangioblastomas in individuals with *VHL* gene mutations [12]. In their study, the authors analyzed by 2D plasma electrophoresis samples from individuals affected by *VHL* gene mutations from both familial and non-familial cases compared to negative controls [12]. Healthy controls and a patient with *VHL* gene mutations presenting sporadic non-recurrent hemangioblastoma showed an intact band of VN in their plasma [12]. As described earlier by Kubota

Fig. 1 Tumor suppressor pVHL complexes with elongin B, elongin C, Rbx 1, and cullin 2, assembling an ubiquitin ligase that degrades the α subunit of hypoxia-inducible factor-1 α (HIF-1 α). Under normal conditions, HIF-1 α regulates the cellular response to hypoxia through transcription regulation of factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor β chain (PDGF- β), erythropoietin, and transforming factor α (TGF α). In conditions of absent or abnormal pVHL function, the HIF-1 α -dependent factors are constitutively expressed, particularly VEGF and PDGF- β , which explains the increased angiogenesis of the vascular VHL-associated tumors. Vitronectin (VN) T381C is a protein that is easily cleaved by proteases as compared to the wild-type VN (wtVN). VN is a multifunctional protein that mainly serves as a ligand for receptors like integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$. Despite the conflicting roles of $\alpha_v\beta_3/\alpha_v\beta_5$ in angiogenesis, one possible role of the integrins may be the regulation of VEGF receptor-2 (VEGFR-2) expression/function and the increase in the sensitivity of endothelial cells to VEGF-A. A mutated VN T381C that is prone to be cleaved and being kept together by a disulfide bond may function differently than wtVN in terms of $\alpha_v\beta_3/\alpha_v\beta_5$ -receptor activity. The mutated VN may also interfere with both its heparin-binding capacity and interaction with anti-angiogenic proteins as pointed out by Huang et al. [12]. VN-T381C may interfere with phosphorylation-mediated activity of VN in the regulation of cAMP response element-binding protein (CREB) that may induce complex formation of CREB-binding protein (CBP) in the HIF-1 α transcriptome

et al. [13], VN presents a polymorphism at codon 381 (T→C) that is easily detected by polymerase chain reaction (PCR) following restriction enzyme Pml digestion. Individuals carrying this T381C vitronectin polymorphism have no detectable VN band by 2D plasma electrophoresis due to in vivo cleavage of the protein [12]. The novelty of Huang's study is based on two major findings: (a) no correlation between the type of *VHL* mutations and the severity, and the earlier onset of the tumor lesions in patients; (b) the presence of T381C significantly increases the overall risk (OR 5, CI, 0.9–28.9) of developing VHL disease in families or individuals (non-familial) carrying the *VHL* gene defect [12]. The idea behind Huang's study is that tumor induction due to mutated *VHL* gene depends on the balance between pro-angiogenic (HIF-1 α dysfunction, increased VEGF levels, etc.) and anti-angiogenic factors. VEGF is excluded as a "genetic modifier" in Huang's study [12]. Although the number of patients analyzed is small, Huang's study shows that the frequency of VN polymorphism significantly correlates with a more aggressive form of VHL disease [12]. How does the biology of VN fit in this model? Although Huang et al. do not address the mechanistic aspect of the biology, their study certainly sets the ground for generation of new hypotheses on the relevance of VN in the pathogenesis of VHL disease (Fig. 1). Briefly, VN is a multifunctional protein that mainly serves as a ligand for receptors like integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ [14]. Despite the conflicting roles of $\alpha_v\beta_3/\alpha_v\beta_5$ in angiogenesis, one possible role of the integrins may be the regulation of VEGF receptor-2 (VEGFR-2) expression/function and the increase in the sensitivity of endothelial cells to VEGF-A [14, 15]. A mutated VN T381C that is prone to be cleaved and being kept together by a disulfide bond may function differently than the wild type in terms of $\alpha_v\beta_3/\alpha_v\beta_5$ -receptor activity. Furthermore, the mutated VN may also interfere with both its heparin-binding capacity and interaction with anti-angiogenic proteins as pointed out by Huang et al. (Fig. 1) [12]. Lastly, the T381C mutation may interfere with phosphorylation-mediated activity of VN in the regulation of cAMP response element-binding protein (CREB) that may induce complex formation of CREB-binding protein (CBP) in the HIF-1 α transcriptome (Fig. 1) [12, 16].

In conclusion, Huang's study is an interesting exploratory study in terms of population genetics that opens the door for a validation phase in a larger population study of patients with both familial and non-familial VHL disease. It has potential for both diagnostic and translational relevance: once validated, the T381C restriction fragment polymorphism (RFLP) by PCR might be used as a diagnostic tool for risk assessment in this peculiar population of patients. Furthermore, investigating the

role of VN in the pathogenesis of VHL disease might open new avenues in the treatment of VHL patients.

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