Von Hippel-Lindau Disease

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Abstract: Germline mutations in the VHL tumour suppressor gene may cause a variety of phenotypes including von Hippel-Lindau (VHL) disease, familial phaeochromocytoma and inherited polycythaemia. VHL disease is a multisystem familial cancer syndrome and is the commonest cause of familial renal cell carcinoma (RCC). VHL disease provides a paradigm for illustrating how studies of a rare familial cancer syndrome can produce advances in clinical medicine and important insights into basic biological processes. Thus the identification of the VHL gene has improved the diagnosis and clinical management of VHL disease and provided insights into the pathogenesis of sporadic clear cell RCC. Functional investigations of the VHL gene product have provided novel information on how cells sense oxygen and the role of hypoxia-response pathways in human tumourigenesis. Such information offers prospects of novel therapeutic interventions for VHL disease and common cancers including RCC.

INTRODUCTION

Von Hippel-Lindau (VHL) disease (MIM Number 193300) is a multisystem dominantly inherited characterised by susceptibility to retinal and central nervous system hemangioblastomas, clear cell renal cell carcinoma (RCC), pheochromocytoma, pancreatic endocrine tumours and endolymphatic sac tumours (ELSTs). In addition renal, pancreatic and epididymal cysts are frequent. VHL disease is the most frequent cause of RCC susceptibility and has a minimum incidence of 1/36000 live births [1]. Familial retinal angiomatosis (haemangioblastomas) was described by Collins and von Hippel at the end of the 19th and beginning of the 20th Centuries respectively, and the eponym of VHL disease was introduced in recognition of the contribution of Lindau who linked together the retinal, central nervous system and renal manifestations of the disease [2-5]. However for many years RCC was overlooked as a major feature of VHL disease. The VHL tumour suppressor gene was identified in 1993 [6]. Subsequently somatic VHL mutations were demonstrated to have a major role in the pathogenesis of sporadic clear cell RCC and the VHL gene product was found to have a critical role in the regulation of cellular hypoxic response mechanisms [7-9].

THE VHL TUMOUR SUPPRESSOR GENE: STRUCTURE AND EXPRESSION

The VHL gene consists of three exons and encodes two VHL proteins: a full length 213 amino acid protein (pVHL30) which migrates with an apparent molecular weight of ~28-30 Kda, and a shorter protein (~18-19 Kda; pVHL19), which is translated from an internal translation initiation site at codon 54 and produces a 160 amino acid protein. Two alternatively spliced VHL transcripts have been detected reflecting the presence (isoform I) or absence (isoform II) of exon 2 [10]. The latter isoform does not encode a functional gene product. A minimal promoter region of 106 bp has been defined and functional SP1 and AP2 binding sites defined [11,12]. Evolutionary conservation of VHL sequence is very strong over most of the sequence included in pVHL19, but the first 53 amino acids included in pVHL30 are much less conserved [13]. Thus the N-terminal sequence of pVHL30 contains 8 copies of a GXEEX acidic repeat motif in human and higher primates, but only 3 copies are present in the marmoset and one copy in rodent VHL genes.

The 4.7 kb mRNA is expressed widely in fetal and adult tissues and the organ-specific manifestations of VHL disease cannot be explained by differential tissue expression [6, 14-16].

THE VHL TUMOUR SUPPRESSOR GENE: GERMLINE VHL MUTATIONS

Germline VHL gene mutations have been reported in >500 VHL disease kindreds [6, 17-20, http://www.umd.necker.fr]. In classical non-mosaic CHL disease patients mutation detection rate may be up to 100% [21-23]. However, no mutations have been reported in the first 53 amino acids of pVHL30. Germline VHL mutations may be divided into three groups: (a). large deletions which account for ~40% of all mutations, (b). intragenic missense mutations (~30%) and (c). protein truncating mutations (nonsense, frameshift insertions and deletions, splice site mutations) (~30%). Recurrent VHL gene mutations (e.g. 694C>T, 712C>T, 713G>A) mostly result from de novo mutations at mutation “hot-spots” [24], but the T505C (Y98H) missense substitution is a founder mutation originating in South-West
Germany and also found in North American kindreds of German origin [25]. In addition to VHL disease, germline VHL gene mutations have also been detected in patients presenting with apparently isolated familial pheochromocytoma (see genotype-phenotype correlations later). Remarkably, homozygous missense VHL mutations have been described in individuals with recessively inherited congenital polycythemia syndromes without VHL disease [26,27]. The best characterized of these mutations is the c.598C>T mutation which is found in a patients with Chuvash polycythemia and is thought to have arisen as a founder effect 14,000 to 62,000 years ago [28]. Patients with Chuvash polycythemia homozygous for a VHL 598C>T mutation are reported to have polycythemia, vertebral hemangiomas, varicose veins and elevated serum VEGF concentrations, but not an increased risk of spinocerebellar hemangioblastomas, RCC or pheochromocytoma [29].

THE VHL TUMOUR SUPPRESSOR GENE: SOMATIC VHL INACTIVATION

Statistical analysis of the age at onset of cerebellar haemangioblastomas and RCC in VHL disease and sporadic cases was consistent with a one- and two rate-limiting steps model as for familial and sporadic retinoblastoma [30]. Tumours from VHL patients demonstrate inactivation of the wild-type VHL allele by loss, mutation or methylation [31]. Furthermore allele loss may be detected in very early lesions [32,33]. Somatic VHL mutations and allele loss occur in up to 60% of clear cell RCC tumours and cell lines [7,8]. Furthermore transcriptional silencing by promoter hypermethylation occurs in RCC cell lines and ~15% of sporadic primary clear cell RCC [34,35]. These findings are consistent with VHL inactivation representing an early and frequent step in the development of many sporadic clear cell renal cancers. Yao et al [36] found that somatic VHL alterations in sporadic RCC were associated with better cancer-free survival and cancer-specific survival. Long-term exposure to high doses of trichloroethylene is associated with an increased risk of RCC and tumours from such cases have been reported to be preferentially associated with a specific somatic VHL mutation at nucleotide 454 [37]. Somatic VHL inactivation is also frequent in sporadic haemangioblastomas [38]. However, somatic VHL mutations are uncommon in sporadic pheochromocytoma [39]. Those which have been detected are missense mutations, suggesting that specific mutations are required to instigate a pheochromocytoma, analogous to the missense mutations predominantly associated with the development of pheochromocytoma in type 2 VHL disease. Although chromosome 3p allele loss is a feature of many human cancers, tumour types which display frequent chromosome 3 allele loss but which do not occur in VHL disease (e.g. lung, ovarian, head and neck and breast cancers) do not generally demonstrate somatic VHL gene mutations, although a subset of colorectal cancers can harbour changes [40].

MODEL ORGANISMS AND VHL

Transgenic experiments in mice and other model organisms may provide important insights into the function of human disease genes. However mice heterozygous for vhl (+/-) disruption were reported to have a normal phenotype. In contrast, homozygous VHL (/-) null mice developed normally until E9.5 to E10.5, but died in utero at E10.5 to E12.5 days with placental dysplasia and absent placental vasculogenesis [41]. In order to avoid embryonic lethality, Haase et al [42] generated a conditional mouse vhl knockout. Targeted inactivation of mouse VHL in the liver resulted in severe steatosis, multiple blood-filled vascular cavities, and foci of increased vascularity within the hepatic parenchyma. Another conditional mouse model was characterised by multiple hepatic hemangiomas, angiectasis and angiogenesis in multiple organs and defective spermatogenesis [43]. This each of these models implicated pVHL in normal vascular development. In Drosophila, knock-down of a pVHL homologue caused abnormal tracheal development [44].

FUNCTION OF THE VHL PROTEIN

Inspection of the pVHL sequence did not provide insights into likely function [6]. Although the identification of pVHL-interacting proteins, elongins B and C [45,46], suggested a possible role in transcriptional regulation, subsequent studies have suggested that this is not an important mechanism of pVHL-mediated tumour suppression. However, the finding that Cul-2, a member of the cullin protein family, associated with pVHL, elongin B and elongin C to form a tetrameric VCBC complex [47,48], did provide insights into possible pVHL functions. Thus structural and sequence motif homologies were noted between the VCBC complex and the yeast SCF (Skp1-Cdc53/Cul1-E-box) complex that was implicated in targeting specific proteins for ubiquitylation and proteosomal degradation [48]. According this hypothesis, pVHL was predicted to have a "F-box function" and would bind to the target protein(s). This model was supported by two subsequent reports. Firstly, the Rbx-1 protein was demonstrated to associate with the VCBC complex and to be an essential component of SCF complexes [49]. Secondly, the elucidation of the crystal structure of the pVHL/elonginB/elonginC complex demonstrated that pVHL has two principal domains: an ~100 residue amino-terminal domain rich in β-sheet (the β-domain) and a smaller carboxy-terminal α-helical domain (the α-domain). A large portion of the α-domain surface interacts with elongin C and significantly, a large proportion of disease-causing VHL mutations map to the α-
Figure 1. Regulation of HIF function.

Panel A: in normoxia the $\alpha$ HIF subunit is captured by the pVHL ubiquitin E3 ligase complex (elongins B and C, Cul2 and Rbx1), ubiquitylated and destroyed in the proteasome. Hence the HIF heterodimer does not form and there is no transcription of the hypoxia responsive target genes.

Panel B: with VHL inactivation the HIF-$\alpha$ subunit accumulates and dimerises with the $\beta$ subunit. The HIF heterodimer then binds to the hypoxic response element (HRE) of the target genes and activates transcription.

Panel C: In the presence of oxygen specific prolyl residues in HIF-1$\alpha$ are converted to hydroxyproline enabling pVHL to bind to HIF-1$\alpha$. However in hypoxia this reaction does not occur and pVHL is unable to bind HIF-1$\alpha$. Hence the HIF-$\alpha$ subunit accumulates and dimerises with the $\beta$ subunit. The HIF heterodimer then binds to the hypoxic response element (HRE) of the target genes and activates transcription.
domain and its residues that contact elongin C. A structural analogy was also noted between the F-box protein in SCF complexes and the elongin C binding site in pVHL. Elongin B binds pVHL indirectly through elongin C. Many other VHL mutations map to the β-domain, specifically to a patch not implicated in elongin C binding [50]. This suggested that two intact and distinct macromolecular binding sites are required for pVHL function, and the possibility that the β-domain may be involved in targetting proteolytic substrates to the general ubiquitylation complex which is bound via the α-domain. Subsequent studies demonstrated that the VCBC complex promoted ubiquitin ligase activity and that promotion of ligase activity was only associated with wild-type pVHL and not disease-causing pVHL mutants [51,52].

Although pVHL appears to have multiple functions, the best characterised is the role in regulating proteolytic degradation of the α subunits of the HIF-1 and HIF-2 transcription factors (see Fig. 1). The HIF transcription factors have a major role in co-ordinating cellular responses to hypoxia and regulate transcription of a wide range of target genes implicated in angiogenesis, proliferation and metabolism (e.g. VEGF, PDGFβ, TGFα and erythropoietin). HIF-1 and HIF-2 are heterodimers: the β-subunits of HIF-1 and HIF-2 are constitutively expressed, but under normoxic conditions the α-subunits are degraded rapidly by the proteasome. Hemangioblastomas and other VHL-associated tumours such as clear cell RCC and pheochromocytoma are highly vascular tumours and, under normoxic conditions, overexpress a wide range of hypoxia-inducible mRNAs, including VEGF [9,53,54]. Furthermore in sporadic clear cell RCC cell lines with VHL inactivation, the re-introduction of wild-type pVHL into the VHL null RCC cell lines restored normal oxygen-dependent regulation of VEGF and other hypoxia-inducible RNAs [54,55]. Maxwell et al [9] demonstrated that pVHL plays a critical role in the regulating proteosomal degradation of HIF-1 and HIF-2 α-subunits. Thus in normoxia, pVHL binds to HIF-1α and HIF-2α and targets them for polyubiquitylation and proteosomal degradation [9,56]. In VHL disease and sporadic tumours with VHL inactivation, absence of a functioning pVHL leads to normoxic overexpression of HIF-1 and HIF-2 and activation of downstream HIF-target genes. The ability of pVHL to bind HIF-1α is dependent of the hydroxylation status of specific proline residues in the HIF-1α protein. Hydroxylation of these prolines is oxygen dependent and so under hypoxic conditions the modification does not occur and pVHL is unable to bind HIF-1α [57,58].

pVHL has also been implicated in a variety of cellular processes including cell cycle control, regulation of mRNA stability, fibronectin metabolism and microtubule stability [59-62]. The tumour suppressor and biological effects of pVHL might result from the effects of HIF targets such as TGFα, Cyclin D1, CXCR4, VEGF etc. overproduction of VEGF and PDGF can be implicated in the angiogenesis associated with VHL tumours. TGFα is a renal cell mitogen that promotes serum-independent growth of renal cancer cells and CXCR4 is a chemokine receptor that has been implicated in organ-specific metastasis and renal cancer prognosis. Alternatively pVHL targeting of non-HIF target proteins for ubiquitylation and proteolysis might be involved. Additional pVHL targets have been suggested and include an atypical protein kinase C (PKClambda) and a novel deubiquitinating enzyme [63,64]. However, the precise relevance of these targets to VHL tumour suppressor activity remains to be defined. Although the role of pVHL in HIF-1 and HIF-2 regulation provides a plausible explanation for the angiogenic phenotype of VHL-associated tumours, it was unclear to what extent HIF dysregulation might be oncogenic per se. To investigate this conundrum, HIF-1α or HIF-2α proteins resistant to pVHL-mediated degradation were transfected into RCC cell lines. Intriguingly, Kondo et al. [65] found evidence that HIF-2 might be oncogenic per se, whereas Maranchie et al [66] found no such evidence for HIF-1. One explanation for these findings is that HIF-1 and HIF-2 have different roles and targets. Interestingly, there is increasing evidence that HIF-1 and HIF-2 may have distinct repertoires of target genes [67,68].

**VON HIPPEL-LINDAU DISEASE: DIAGNOSTIC CRITERIA, CLINICAL FEATURES AND SCREENING**

The identification of a pathogenic germline VHL gene mutations provides a specific and reliable basis for the diagnosis of VHL disease. However if a molecular diagnosis is not possible, less sensitive clinical diagnostic criteria are employed. Thus, in the presence of a confirmed family history of VHL disease, the finding of a single typical VHL tumour (e.g. retinal or central nervous system hemangioblastoma, clear cell RCC, pheochromocytoma, pancreatic endocrine tumour or endolymphatic sac tumour) in an at risk relative enables a clinical diagnosis of VHL disease to be made [69]. However in isolated cases without a family history, the presence of two tumours (e.g. two hemangioblastomas or a hemangioblastoma and a visceral tumour) are required for the diagnosis. The new mutation rate in VHL is ~ 2-4 x10⁻⁶ [1] and the necessity for two tumours to have occurred in isolated cases means that diagnosis is often delayed in such cases. A diagnosis of VHL disease should be considered in all cases of retinal and central nervous system haemangioblastomas, but also in patients with familial, multicentric or young onset pheochromocytoma and RCC. Molecular genetic analysis enables an early diagnosis of VHL disease in patients who do not satisfy conventional clinical diagnostic criteria. Thus ~40% of patients with apparently isolated familial or bilateral pheochromocytoma have germline VHL gene mutations [70].
unpublished observations) and ~4% of patients with an isolated central nervous system haemangioblastoma (and a negative family history and no evidence of subclinical features of VHL disease) have a germline VHL gene mutation [71]. Estimates of the risk of VHL disease in patients presenting with apparently isolated retinal angioma are available [72]. For patients with familial RCC and no evidence of VHL disease, a diagnosis of familial papillary RCC and familial clear cell RCC which is not allelic with VHL disease should also be considered [73-75].

VHL disease may present in childhood or old age, but the usual age of symptomatic presentation is in the second and third decades. Prior to molecular genetic testing, penetrance was considered to be almost complete by age 60 years [76], but penetrance may be reduced with certain missense mutations [77]. Retinal (mean age at symptomatic diagnosis 24.5 years) and cerebellar haemangioblastomas (29 years) are the most common presenting features. Although the risk of pheochromocytoma and RCC may be influenced by allelic heterogeneity (see genotype-phenotype correlations later), in most cases the lifetime risk of retinal and cerebellar haemangioblastomas and RCC is high (see Fig. 2). In most families the lifetime risk of pheochromocytoma is low, but in some families pheochromocytoma is the most frequent complication. The earlier onset of retinal angioma and cerebellar haemangioblastoma compared to RCC (mean 40 years for symptomatic diagnosis) means that the risk of RCC is underestimated in cross sectional studies and the lifetime probability for each of these three major complications has been estimated as >70% [76], although tumour specific risks are influenced by allelic heterogeneity (see later).

In many cases, early diagnosis and treatment of tumours in VHL disease improves prognosis and reduces morbidity and mortality. Hence all VHL disease patients and their at risk relatives (unless excluded by molecular testing) should be offered regular surveillance. The multisystem involvement of VHL disease requires a complex multifaceted screening programme starting in childhood. Although surveillance programmes should be adjusted to individual circumstances (e.g. mutation-specific tumour risks if known), general guidelines are provided in Table 1. The co-ordination of such screening programmes can be challenging and requires the involvement of multiple health professionals. Further details of the site-specific clinical manifestations, screening and management are discussed below.

VON HIPPEL-LINDAU DISEASE AND THE KIDNEY

The major renal features of VHL disease are clear cell carcinoma and cystic disease. In cross-sectional studies the frequency of RCC in VHL disease is 25-45% and RCC is the first manifestation of VHL in <20% of cases [76,78]. However the lifetime risk is much higher in most cases (see Fig. 2). In contrast to non-familial disease, men and women are equally affected and there is a much earlier age of onset (mean age at clinical diagnosis is ~40 years). Individuals with, or at risk of, VHL disease are offered annual abdominal screening, usually by MRI or ultrasound, from 16 years (the earliest age at which renal tumours have been detected [79]. Such screening means that most renal tumours in VHL disease are detected at an early, presymptomatic, stage. Small renal tumours tend to enlarge slowly (mean <2 cm/year), and after establishing the growth

![Figure 2](image-url). Tumour risks in VHL disease.
apparently normal looking CA9 positive cells that are detectable by light microscopy, but also only the numerous CA9 expressing microfoci of early parenchyma with anti-CA9 antibody can reveal not only the numerous CA9 expressing microfoci of early renal tumours and tend to grow slowly and, if asymptomatic, surgical intervention is usually not required. Tumours with an associated cyst may become symptomatic as the cyst enlarges, but the results of surgery for single peripherally located cerebellar lesions are usually excellent. However, the surgical treatment of multicentric tumours, and particularly, brain stem and spinal tumours can be hazardous. Although the detection of an asymptomatic CNS hemangioblastoma does not usually lead to intervention, early detection can facilitate patient management and so VHL disease patients and art risk relatives may be offered MRI brain scans (± spine) every 24-36 months beginning in adolescence.

Microscopically, haemangioblastomas consist of large polygonal stromal cells emeshed in a rich capillary network. The finding of VHL wild-type allele loss in the stromal cell component has defined these as the neoplastic element [88]. Thus VHL inactivation within the stromal cells is associated with dysregulation of the HIF transcription factors (see above) and overexpression of VEGF and other angiogenic HIF target genes promoting capillary

| Screen for Retinal Angioma: | Affected or at risk individuals should undergo careful ophthalmic examinations every 12 months beginning in infancy or early childhood. |
| Screen for Hemangioblastoma: | It is suggested that individuals with or at risk for VHL-associated tumors should have MRI scans of the head (± spine) every 12-36 months beginning in adolescence. |
| Screen for Renal Cell Carcinoma: | Individuals at risk for VHL-associated tumors should have ultrasound examinations or MRI scans of the abdomen every 12 months from age 16 years. |
| Screen for Pheochromocytoma: | At risk individuals should undergo yearly screening for pheochromocytoma beginning in early childhood. This consists of blood pressure monitoring and 24-hour urine studies to measure catecholamine metabolites. Measurement of plasma normetanephrine levels is reported to be the most sensitive test for detecting pheochromocytoma in VHL disease (Eisenhofer et al 1999) and this and annual adrenal ultrasound scan (from age 8 years) should be considered when there is a family history of pheochromocytoma. |

Table 1. Surveillance protocols in von Hippel-Lindau disease.

rate, small tumours may be monitored every 6 months [80]. Although computer tomography is the most sensitive method for detecting and monitoring renal tumours (particularly in the presence of renal cysts), but MRI or ultrasound scans are generally preferred for regular follow-up (so to avoid a large cumulative radiation load). It appears that the risk of distant metastasis from a renal tumour <3 cm is small. Hence solid renal lesions are usually monitored and managed conservatively a diameter of 3 cm is reached. At this stage a partial nephrectomy is performed and other accessible solid lesions are also removed [81]. This “nephron-sparing approach” appears to be associated with a low risk of distant metastasis, although further surgery is often required because of a high incidence of local recurrence from new primary tumours [82]. In contrast, 25% of VHL patients presenting with a RCC more than 3 cm diameter develop metastatic disease [81]. Multiple partial nephrectomies for recurrent RCC may eventually compromise renal function such that dialysis or renal transplantation becomes necessary. To date it appears that renal transplant associated immunosuppression does not affect adversely the underlying course of VHL disease and the prognosis of VHL patients after transplantation appears similar to that of other comparable groups [83].

RCC in VHL disease is invariably clear cell type, and RCC in VHL disease are macroscopically and microscopically indistinguishable from sporadic clear cell RCC. However the presence of multiple primary tumours and renal cysts are highly suggestive of VHL disease. Thus renal cysts are present in most VHL disease patients by middle age [84]. Renal cysts in VHL disease may be lined by atypical epithelium or contain small RCC [85], so that an apparently benign cyst can evolve into a complex cyst and an associated RCC. Microscopically, apparently normal kidney from VHL patients can contain many microfoci of RCC [86]. VHL associated clear cell RCC express highly hypoxia-inducible genes such as VEGF and carbonic anhydrase IX (CA9), and staining of apparently normal renal parenchyma with anti-CA9 antibody can reveal not only the numerous CA9 expressing microfoci of early RCC detectable by light microscopy, but also apparently normal looking CA9 positive cells that have lost pVHL expression and appear to represent the earliest stage of tumourigenesis [33].

VON HIPPEL-LINDAU DISEASE AND THE CENTRAL NERVOUS SYSTEM

CNS hemangioblastomas are a cardinal feature in VHL disease and are the presenting feature in ~40% of cases [76]. Approximately 30% of all patients with cerebellar hemangioblastoma have VHL disease and the mean age at diagnosis of those with VHL disease (~30 years) is considerably younger than in sporadic cases (48 years) [30]. Lifetime risk of CNS hemangioblastomas in VHL disease is 60-80% [76]. By MRI scanning, the most common locations of are the cerebellum (38% of tumours detected by MRI), brainstem (10%), spinal cord (51%) and rarely supratentorialial [87]. However surgical intervention is most commonly performed for cerebellar lesions. About 20% of cerebellar and brain stem hemangioblastomas have an associated cysts and these tumours are more likely to become symptomatic [87]. Hemangioblastomas are benign tumours and tend to grow slowly and, if asymptomatic, surgical intervention is usually not required. Tumours with an associated cyst may become symptomatic as the cyst enlarges, but the results of surgery for single peripherally located cerebellar lesions are usually excellent. However, the surgical treatment of multicentric tumours, and particularly, brain stem and spinal tumours can be hazardous. Although the detection of an asymptomatic CNS hemangioblastoma does not usually lead to intervention, early detection can facilitate patient management and so VHL disease patients and art risk relatives may be offered MRI brain scans (± spine) every 24-36 months beginning in adolescence.
growth and cyst formation [53,89]. The microscopic appearance of capillary haemangioblastoma can resemble that of a clear cell RCC and occasionally this has caused a misdiagnosis of a cerebellar haemangioblastoma in a VHL patient with a cerebellar metastasis from a RCC [90]. As in the kidney, normal brain and spinal cord in VHL disease may contain numerous widespread “angiomesenchymal tumourlets” that resemble early hemangioblastoma. These lesions show 3p25 allelle loss suggesting that while VHL inactivation may be necessary for hamenagioblastoma formation, it is not sufficient [91].

Endolymphatic sac tumors (ELST) were recognized as a feature of VHL disease relatively recently, but the finding of bilateral ELSTs is pathognomic for VHL disease. In a large survey of VHL patients using MRI and CT scans, Manski et al [92] found that 11% patients with VHL disease had an ELST. Hearing loss is the most common symptom of an ELST, but tinnitus and vertigo are also occur in many cases. Mean age at onset of hearing loss was 22 years and in 62% of patients with ELSTs, hearing loss was the first manifestation of VHL.

VON HIPPEL-LINDAU DISEASE AND THE EYE

Retinal hemangioblastomas, (commonly referred to as retinal angiomas) are the most common presenting feature of VHL disease and are multiple and bilateral in many (~50%) cases. In one survey retinal angiomas (mean 1.85 lesions, range 0-15) were detected in almost 70% of VHL patients and the cumulative risk of visual loss was estimated as 35% in gene carriers and 55% in patients with retinal angiomas at age 50 years [93]. Potentially sight-threatening complications such as exudation, retinal traction or hemorrhage tend, on average, to be associated with larger angiomas. Patient management is directed towards identifying asymptomatic angiomas as early treatment reduces the risk of visual loss. Hence, screening for retinal angiomas by indirect ophthalmoscopy (and fluorescein angiography in some cases) every 12 months beginning in infancy or early childhood, should be offered to all affected or at risk individuals. Most retinal angiomas are peripherally situated and respond well to laser photocoagulation or cryotherapy. However about 15% have an optic disc angioma and these lesions are usually kept under surveillance until there is clear evidence of progression because of the complications of treatment (optic nerve damage). As with CNS hemangioblastomas, treatment with VEGF antagonists provide a possible treatment option for lesions which conventional therapy is contra-indicated [94].

VON HIPPEL-LINDAU DISEASE AND THE ADRENAL MEDULLA

The overall frequency of pheochromocytoma in VHL patients is 10-20% but there are wide interfamilial variations. In South-Western Germany 11% of patients with apparently sporadic pheochromocytoma had a germline VHL mutation [95], although the frequency in a UK series is approximately half of this (unpublished observations). Mean age at diagnosis of pheochromocytoma in VHL disease is 30 years and both adrenal and extra-adrenal pheochromocytomas can occur. However extra-adrenal pheochromocytomas are relatively more common in individuals with germline SDH subunit gene mutations than in those with germline VHL mutations [95,96]. Although the overall risk of malignancy in pheochromocytomas is generally considered to be ~10%, the rate in VHL disease appears to be less than this (~5%).

VON HIPPEL-LINDAU DISEASE AND THE PANCREAS

The most frequent pancreatic manifestation of VHL disease is multiple cysts, which are present in most older patients. However pancreatic cysts rarely impair pancreatic function and are therefore not usually a management concern. Pancreatic tumours occur in ~10% of cases and are usually non-secretory islet cell tumours. A high frequency of that malignancy has been reported in VHL associated islet cell tumours and surgery is indicated in tumours >3 cm while tumours <1 cm may be monitored [97].

OTHER MANIFESTATIONS OF VHL DISEASE

Epididymal cystadenomas have been reported in up to 60% of males with VHL disease and are often bilateral [98]. They are usually asymptomatic and do not require treatment, but may present as an intrascrotal mass or be detected during investigations for infertility. Broad ligament cystadenomas have been reported in a handful of VHL patients but appear to be rare in the general population and may be under-recognised in VHL disease [99].

GENOTYPE-PHENOTYPE CORRELATIONS IN VON HIPPEL-LINDAU DISEASE

Complex genotype-phenotype associations are an intriguing feature of VHL disease. Thus there are marked interfamilial variations in pheochromocytoma susceptibility and VHL disease kindreds have been divided into Type 1 (no pheochromocytoma) and Type 2 (pheochromocytoma) subsets accordingly. In Type 1 kindreds, the germline mutation is usually a large deletion or a truncating mutation. A minority of Type 1 families have a germline missense mutations, but many of these affect hydrophobic residues that are predicted to disrupt the structure of pVHL [17,19,50,100]. In contrast most Type 2 (pheochromocytoma present) families have a missense mutation in a pVHL surface amino acid, and there is a trend against hydrophobic core mutations suggesting that type 2 mutations
have a strong bias against total loss of function [50]. Type 2 families are further subdivided according to the presence or absence of RCC and haemangioblastomas. Thus in Type 2A kindreds haemangioblastomas and phaeochromocytoma occur but not (or rarely) RCC. Type 2B phenotype is characterised by the development of haemangioblastomas, RCC and phaeochromocytoma and Type 2C kindreds only develop phaeochromocytoma [25,70,101]. The genotype-phenotype observations are consistent with the hypothesis that total loss of function mutations are associated with a reduced risk of phaeochromocytoma. In vitro analysis of pVHL function demonstrates partial retention of pVHL binding to elongin C or HIF-1 with phaeochromocytoma associated missense mutations [102]. Furthermore, although Type 2A and Type 2B mutations demonstrate impaired ability to regulate HIF-1, Type 2C mutants retained the ability to regulate HIF-1 (although fibronectin binding was impaired) [102,103]. These findings suggest that HIF-1 dysregulation is not necessary for phaeochromocytoma development in VHL disease.

In addition to the phenotypic variability associated with allelic heterogeneity, genetic modifiers may influence the phenotypic expression of VHL disease (Webster et al, 1998). Thus Individuals with oculocerebellar hemangioblastomas were found to have a significantly increased incidence of cerebellar hemangioblastoma and RCC compared to those without retinal involvement and relative-pair analysis revealed a significant correlation between numbers of retinal angiomatas in close relatives but not distant relatives [104]. Allelic variants in the cyclin D1 gene have been reported to influence haemangioblastoma development [105].

CONCLUSION

Perhaps the ultimate validation of the “VHL tumour suppressor gene paradigm” [106] would be if knowledge of pVHL function led to novel treatments for VHL tumours and their sporadic counterparts. The finding that HIF-2 overexpression could promote RCC growth in vivo suggest that inhibition of HIF (or their downstream consequences) would be advantageous. There is preliminary evidence that VEGF inhibitors (e.g. SU5416) may, in some cases, stabilise haemangioblastomas by reducing peritumoural oedema [107]. In additional administration of a neutralising VEGF antibody to patients with metastatic RCC significantly prolonged time to progression of the disease [108]. The addition of complementary agents with PDGF and TGFα/EGFR inhibition would be expected to provide further benefits. Alternatively, direct inhibition of HIF would provide a further therapeutic option. However the efficacy of such approaches will depend on the extent to which HIF-independent pVHL functions contribute to pVHL tumour suppressor activity. Nevertheless there is a strong expectation that further understanding of the role of the VHL tumour suppressor gene in tumourigenesis will be translated into improvements in clinical oncology.

REFERENCES


