Molecular pathology of von Hippel–Lindau disease and the *VHL* tumour suppressor gene

Frances M. Richards

von Hippel–Lindau (VHL) disease is a dominantly inherited cancer syndrome characterised by predisposition to multiple tumours of the eyes and central nervous system (haemangioblastomas), kidneys (renal cell carcinoma; RCC), adrenal chromaffin cells (phaeochromocytoma), and other organs. The *VHL* gene was isolated in 1993 and mutations or deletions in the *VHL* gene have been identified in the germline of nearly all tested individuals with VHL disease. Genotype–phenotype correlations have been observed: individuals with missense mutations are more likely to develop phaeochromocytoma than those with deletions or protein-truncating mutations are, and specific missense mutations at certain codons might not predispose to RCC. In accordance with its role as a tumour suppressor gene, the normal allele of the *VHL* gene is deleted, mutated or silenced by promoter methylation in the tumours from VHL patients, and in a large proportion of sporadic tumours of the same histological types as observed in VHL disease. Thus, the *VHL* gene is of major importance in the development of RCC in the general population. Recent advances in understanding the structure and function of the VHL protein (pVHL) have revealed insights into the different phenotypes, with indications that some retention of function might be required for predisposition to phaeochromocytoma. pVHL interacts with many cellular proteins, mainly via one of two protein-binding domains (α and β). The best-characterised interaction is that of pVHL with elongin C, which forms a complex with elongin B and Cullin 2 proteins. This complex has E3 ubiquitin ligase activity and promotes ubiquitin-mediated proteasomal degradation of the hypoxia-inducible factor

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1α (HIF-1α) transcription factor under normal oxygen (normoxic) conditions. Loss of pVHL function leads to stabilisation of HIF-1 and expression under normoxic conditions of hypoxia-inducible genes including vascular endothelial growth factor (VEGF), which might explain the hypervascular phenotype of VHL tumours. Several other genes implicated in intra- and intercellular signalling and control of tumour growth are overexpressed in the absence of pVHL, but it is not yet clear which features of pVHL function are most significant for tumour suppression in different tissues. Further advances in understanding pVHL function might eventually enable development of specific therapies for prevention or treatment of VHL tumours and RCC.

von Hippel–Lindau (VHL) disease is a dominantly inherited, multisystem, family cancer syndrome predisposing to tumours of the eyes, central nervous system (CNS), kidneys and other organs. It has been estimated that the birth incidence of VHL disease is between 1 in 36 000 and 1 in 45 500 live births in the UK (Refs 1, 2). The tumours are of specific histological types: retinal, cerebellar and spinal haemangioblastomas, clear-cell type (non-papillary) renal cell carcinoma (RCC), phaeochromocytoma, and (in up to 10% of cases) pancreatic islet cell tumours and endolymphatic sac tumours of the inner ear (Fig. 1a). In addition, multiple renal, pancreatic and epididymal cysts occur. VHL disease was named after Eugen von Hippel, who first described angiomas in the eye in 1904, and Arvid Lindau, who recognised the association of retinal, cerebellar and spinal haemangioblastomas in

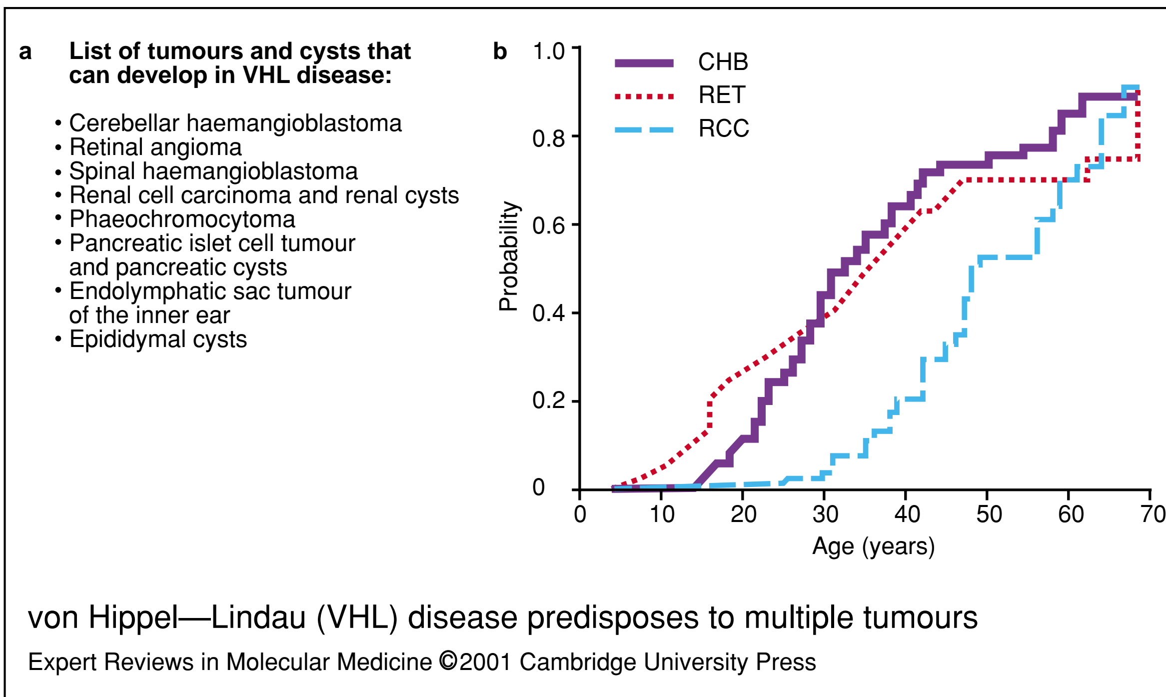

Figure 1. **von Hippel–Lindau (VHL) disease predisposes to multiple tumours.** (a) List of tumours and cysts that can develop in VHL disease; different patients develop various combinations of these tumour types and often develop multiple tumours of the same type. (b) The cumulative probability of developing a cerebellar haemangioblastoma (CHB), retinal angioma (RET) and renal cell carcinoma (RCC) in VHL disease increases with age (Ref. 6) **(fig001frb)**.

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1926. Haemangioblastomas are benign cystic tumours that appear as an overproliferation of blood vessels with a neoplastic stromal component, but as a result of their location in the eyes, brain stem or spine they cause considerable morbidity (Ref. 3). RCC, phaeochromocytoma and pancreatic tumours can all be malignant and therefore early detection is vital.

Identification of the *VHL* gene has enabled molecular diagnosis in affected individuals and at-risk relatives to identify gene carriers. Relatives who are shown not to carry the mutated gene can be reassured and excluded from screening protocols, while affected individuals and unaffected gene carriers require life-long surveillance to enable detection of tumours at a presymptomatic stage. Early detection and treatment of VHL complications, particularly RCC and retinal haemangioblastomas, can reduce morbidity and mortality from VHL disease. Thus, unlike some other genetic diseases, there are clear clinical benefits from predictive testing in VHL disease, and the uptake rate for genetic testing in affected families is high (>85%) (Refs 4, 5).

Large studies of the clinical features of VHL disease have demonstrated that the mean age at onset is 26.3 years, with almost complete penetrance such that 97% of patients have presented with symptoms by 60 years. Overall, 57–60% of patients develop cerebellar haemangioblastoma, 41–59% retinal angioma, 24–28% RCC, 13–14% spinal haemangioblastoma and 7–19% phaeochromocytoma (Refs 2, 6, 7). These frequencies depend to some extent on the particular germline mutation (see later). Not only might each patient have several different tumour types, but they often also develop multiple tumours of the same type, such as multiple retinal angiomas in the same eye or in both eyes (bilateral), or multiple RCC tumours in one or both kidneys. The earliest manifestation of VHL disease tends to be retinal or CNS haemangioblastoma, with RCC developing later (Fig. 1b). VHL disease can be split into several types, depending on the range of tumours detected within the family (Refs 8, 9) – in particular, the presence or absence of phaeochromocytoma (Table 1).

Very early on it was proposed that the *VHL* gene was a tumour suppressor gene, as defined by Knudson in 1971 (Refs 10, 11). Therefore, loss of function of both alleles would be required for tumour formation and, in an individual with a germline mutation in one allele, the probability of a 'second hit' somatic mutation occurring is much higher than the probability of two independent 'hits' in a cell from a normal individual (Fig. 2). This explains why VHL patients often have multiple tumours that develop at an earlier age than the equivalent sporadic tumours in the general population.

Table 1. VHL disease types and different germline *VHL* mutations (tab001frb)

VHL disease type	Tumour types observed in families			Germline *VHL* mutation types most commonly associated with phenotype
	HB	RCC	Phaeo	
Type 1	+	+	-	Deletions and truncations[a]
Type 2A	+	-	+	Missense Tyr98His[b] and Tyr112His
Type 2B	+	+	+	Missense[c]
Type 2C (familial phaeochromocytoma)	–	–	+	Missense Leu188Val, Val84Leu, Ser80Leu

[a] Also some missense mutations, usually of key structural amino acids in the hydrophobic core of the β-domain. Truncations include both frameshift and nonsense mutations.
[b] The 'Black Forest' founder mutation.
[c] Missense mutations in amino acids of the α-domain or in the surface patch of the β-domain.
Abbreviations: HB, retinal and central nervous system haemangioblastoma; phaeo, phaeochromocytoma; RCC, renal cell carcinoma; VHL, von Hippel–Lindau.

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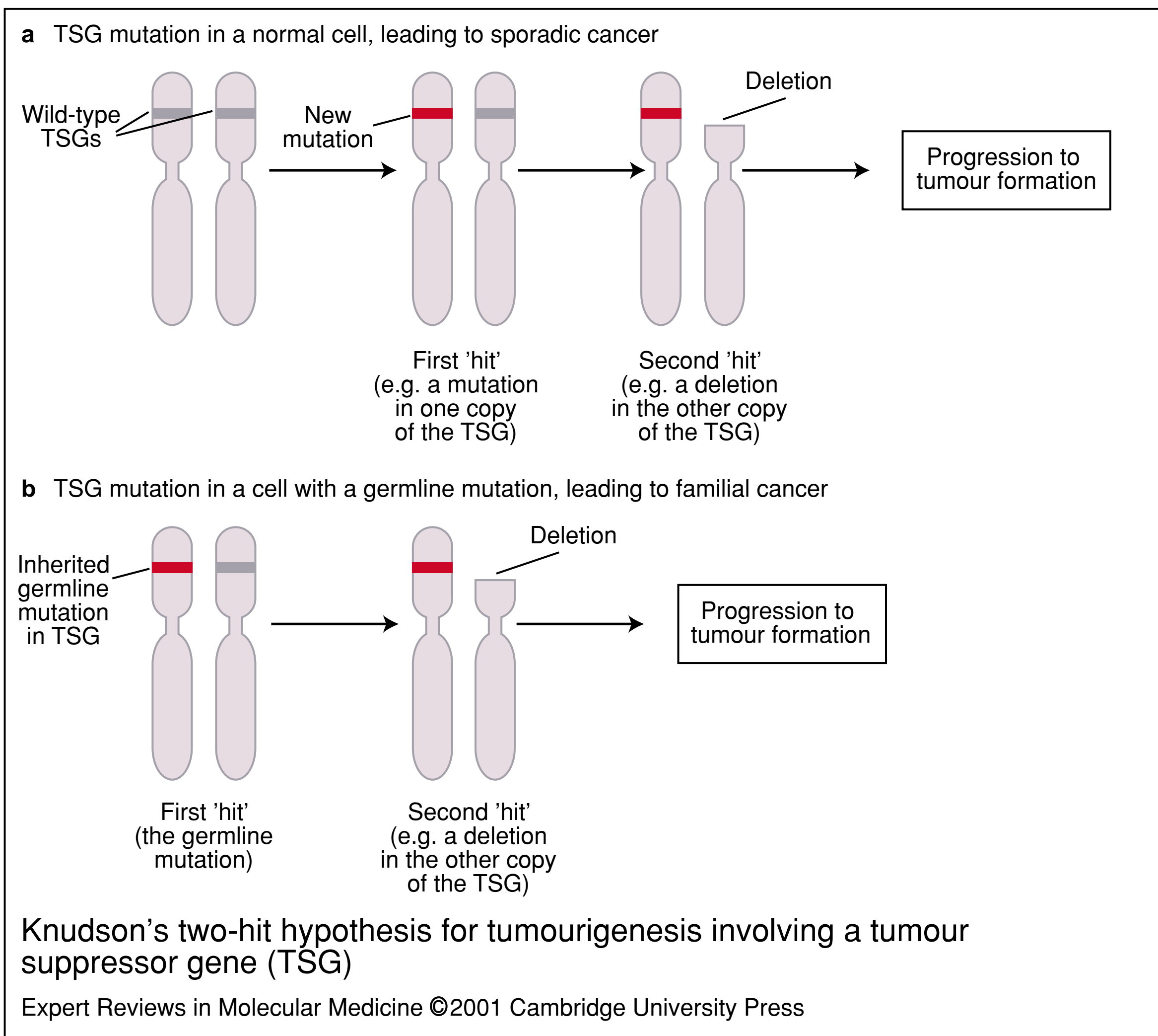

Figure 2. Knudson's two-hit hypothesis for tumourigenesis involving a tumour suppressor gene (TSG) (Ref. 10). One pair of chromosomes is depicted, with one TSG [the normal gene (grey), the mutated gene (red), and deletion of the gene (absence) are shown]. (a) Normal individuals have two normal copies of the TSG, so two independent 'hits' (mutations) are required in the same cell to initiate a cancer. (b) Individuals with a germline mutation of the TSG already have a first 'hit' in every cell and require only one subsequent 'hit' in a cell to initiate a cancer **(fig002frb)**.

The *VHL* gene

Mapping and identification of the *VHL* gene

Genetic linkage analysis was used to map the *VHL* gene in affected families; in 1988 it was localised to chromosome 3p (Ref. 12) and subsequent analysis localised it to a small region of 3p25-p26 (Refs 13, 14, 15, 16, 17, 18, 19). Physical mapping was then used to characterise the region (Refs 20, 21, 22, 23, 24, 25, 26, 27, 28), and analysis of germline DNA from VHL patients by pulsed-field gel electrophoresis and Southern blotting identified a small number of cases who had large [>50 kilobases (kb)] deletions (Refs 24, 26); these deletions helped to pinpoint the position of the *VHL* gene, which was finally identified in 1993 (Ref. 21).

VHL gene structure and sequence

The *VHL* gene consists of three exons encoding a 4.7 kb mRNA (messenger RNA), covering less than 20 kb of genomic DNA on chromosome 3p25-p26 (Fig. 3). The 642 nucleotide coding region encodes a polypeptide of 213 amino acids from the first methionine codon, with a second methionine codon (with a better Kozak consensus sequence for translation initiation) (Ref. 29) at

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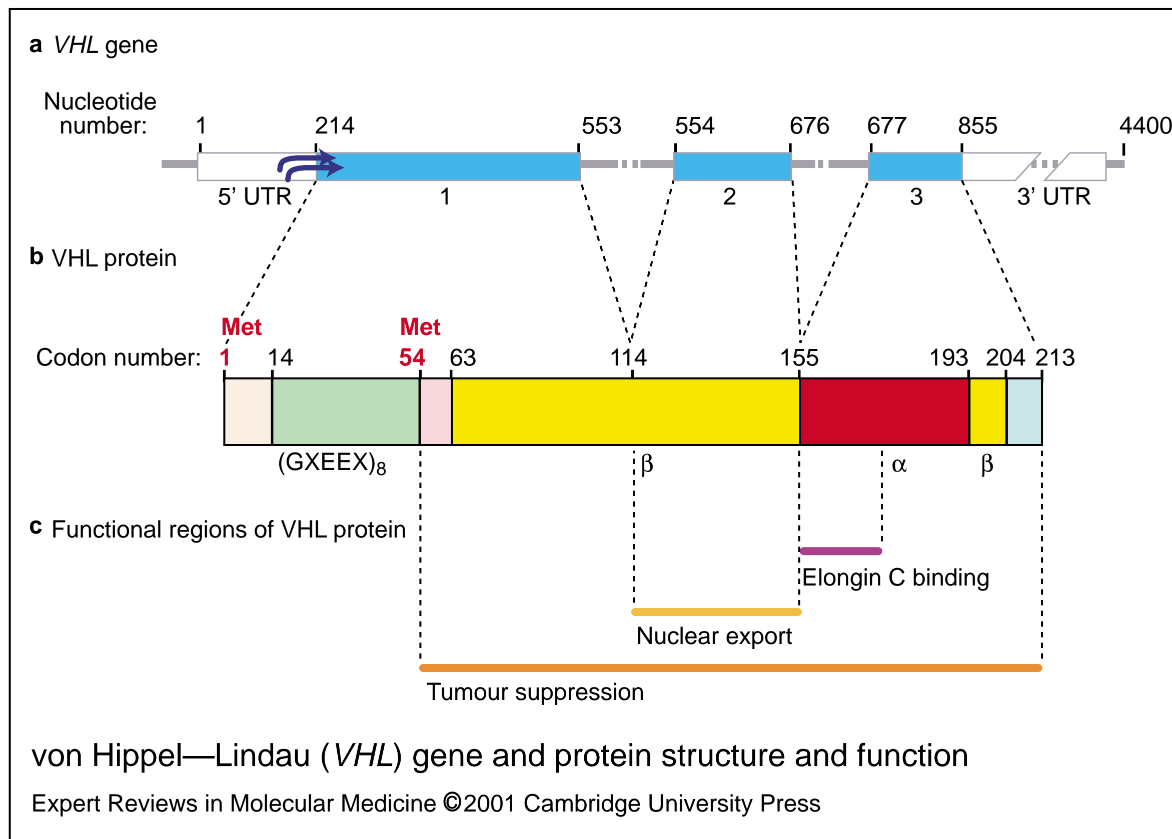

Figure 3. von Hippel–Lindau (*VHL*) gene and protein structure and function. (a) The *VHL* gene structure (nucleotides 1–4400) comprises three exons (blue). 5' and 3' untranslated regions (UTRs), and several transcription start sites (curved arrows), are shown. (b) The α- and β-domain structure of the VHL protein (codon numbers 1–213), and the two methionine (Met) start codons (at codons 1 and 54), are shown; the $(GXEEX)_8$ region is a pentameric repeat motif with unknown functional significance. (c) Regions of the protein required for different functions are indicated **(fig003frb)**.

codon 54 giving a protein of 160 amino acids. Both codons 1 and 54 appear to be used as start codons (see later). There is also alternative splicing: a small proportion of VHL mRNA lacks exon 2 (isoform 2; see later), which is predicted to produce an in-frame deletion of 41 amino acids, if translated. There was initial confusion over the numbering system for the amino acid sequence, because of uncertainty over the N-terminus when the gene was first cloned; some groups numbered from the first codon of the apparent open reading frame in the cloned cDNA (GenBank Accession No. L15409), which is now known to be 71 codons before the first methionine (ATG) codon and is therefore not translated. The nucleotide numbers used in this article are as described in GenBank sequence L15409; codon 1 is the first ATG, at nucleotide 214.

The *VHL* gene sequence is highly conserved in primates and rodents (Refs 30, 31), and has homologues in the nematode worm *Caenorhabditis elegans* (Ref. 30) and the fruit fly *Drosophila melanogaster* (Refs 32, 33). Sequence conservation is particularly high across regions known to be involved in binding to other proteins or in maintaining the VHL protein (pVHL) structure, and conservation of function has recently been confirmed in *Drosophila* (Ref. 33).

Codons 14 to 53 in human *VHL* encode eight copies of an acidic pentameric repeat [Gly-X-Glu-Glu-X; $(GXEEX)_8$] with homology to a procyclic surface membrane protein of *Trypanosoma brucei*. However, *VHL* sequence conservation is poor before codon 54 (the second methionine), and this repeat is not present in the rodent *VHL* genes, so the functional significance of this region is unclear.

The *VHL* gene promoter has been sequenced (GenBank Accession No. AF010238); it is a GC-rich, TATA-less and CCAAT-less promoter,

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with transcription initiating around a putative Sp1-binding site approximately 60 bp upstream from the first methionine codon (Ref. 34). The promoter contains numerous predicted binding sites for transcription factors but, as yet, functional analysis has not revealed how *VHL* gene expression is controlled. The 3' untranslated region (UTR) has also been characterised; part of the sequence is conserved in rodents, and the human 3' UTR contains 11 Alu repeats (Ref. 35).

Distribution of germline mutations

Germline mutations are those mutations that are present in all the cells of an individual, including the germ cells, and are therefore heritable. Germline *VHL* mutations have now been identified in more than 500 VHL families worldwide, and these show considerable heterogeneity in both their type and their location within the *VHL* gene. Large deletions of at least one exon [detected by Southern blotting or more recently by fluorescence in situ hybridisation (FISH)] occur in up to 40% of cases (Refs 24, 26, 36, 37), and intragenic mutations have been identified in approximately 60% of cases (Refs 8, 21, 38, 39, 40, 41, 42). These include frameshift, nonsense, missense and splice junction mutations, as well as in-frame deletions, and some mutations have been observed in more than one family (Fig. 4). Most recurrent mutations result from de novo mutations at hypermutable sequences ('hot spots') (Ref. 43), although a founder effect has been reported for the 'Black Forest' Tyr98His mutation reported in German families and American families of German descent (Refs 8, 9). The hot spots include delPhe76, Asn78Ser/His/Thr, Pro86Leu, Arg161Ter, Cys162Tyr/Phe/Trp, Arg167Gln/Trp and Leu178Pro (Ref. 40).

No mutations have been detected in codons 1 to 54 (i.e. before the second methionine start codon), suggesting that codons 1 to 54 might not be required for tumour suppressor function. A database of germline *VHL* mutations is accessible at http://www.umd.necker.fr:2005/ (Ref. 44).

As many as 15% of VHL cases have no family history of the disease and appear to represent cases of de novo mutation (Ref. 43). However, careful analysis of their clinically unaffected parents can in some cases reveal mosaicism in a parent, which will affect counselling of the family (Ref. 45).

A significant proportion (35–50%) of cases of familial or bilateral phaeochromocytoma have a germline *VHL* mutation (particularly missense mutations; see below) (Refs 46, 47), as do up to 3% of patients with apparently sporadic phaeochromocytoma (Refs 47, 48, 49, 50). Similarly, 3–11% of individuals with a sporadic haemangioblastoma under the age of 50 years have a germline *VHL* mutation, although some of these are missense variants of uncertain pathological significance (Refs 41, 51).

Genotype–phenotype correlations

Germline deletions, or protein-truncating mutations (frameshift and nonsense mutations), are observed more frequently in VHL families without phaeochromocytoma (i.e. with Type 1 VHL disease, presenting with haemangioblastoma and RCC), whereas missense mutations are observed more frequently in families with Type 2B VHL disease (presenting with haemangioblastoma, RCC and phaeochromocytoma) (Refs 8, 38, 52) (Table 1). Codon 167 missense mutations are particularly associated with Type 2B VHL disease, with a high incidence of phaeochromocytoma within the families. Two specific missense mutations are associated with Type 2A VHL (presenting with phaeochromocytoma and haemangioblastoma, but no RCC); these are Tyr98His (the Black Forest mutation) and Tyr112His (Refs 9, 40, 53). Interestingly, a different missense mutation at codon 112 (Tyr112Asn) in one family has given rise to VHL disease that has so far resulted in RCC, retinal and cerebellar haemangioblastoma and one case of phaeochromocytoma (Type 2B) (Ref. 54).

Specific missense mutations have also been observed in families with phaeochromocytoma but none of the other features of VHL (Type 2C VHL disease, or familial phaeochromocytoma); these are Leu188Val (Refs 55, 56), Val84Leu (Ref. 57) and Ser80Leu (Ref. 46). Individuals with these mutations are unlikely to develop haemangioblastomas or RCC. However, some patients with apparently isolated phaeochromocytoma have mutations described previously in Type 2B VHL disease (e.g. Arg167Trp) (Ref. 57); these individuals are at risk of developing the other tumour types.

The lack of protein-truncating mutations in Type 2 VHL disease suggests a bias against complete loss-of-function mutations for susceptibility to phaeochromocytoma; this has been confirmed by mapping of the mutations onto the structure of pVHL (Ref. 58) (see below).

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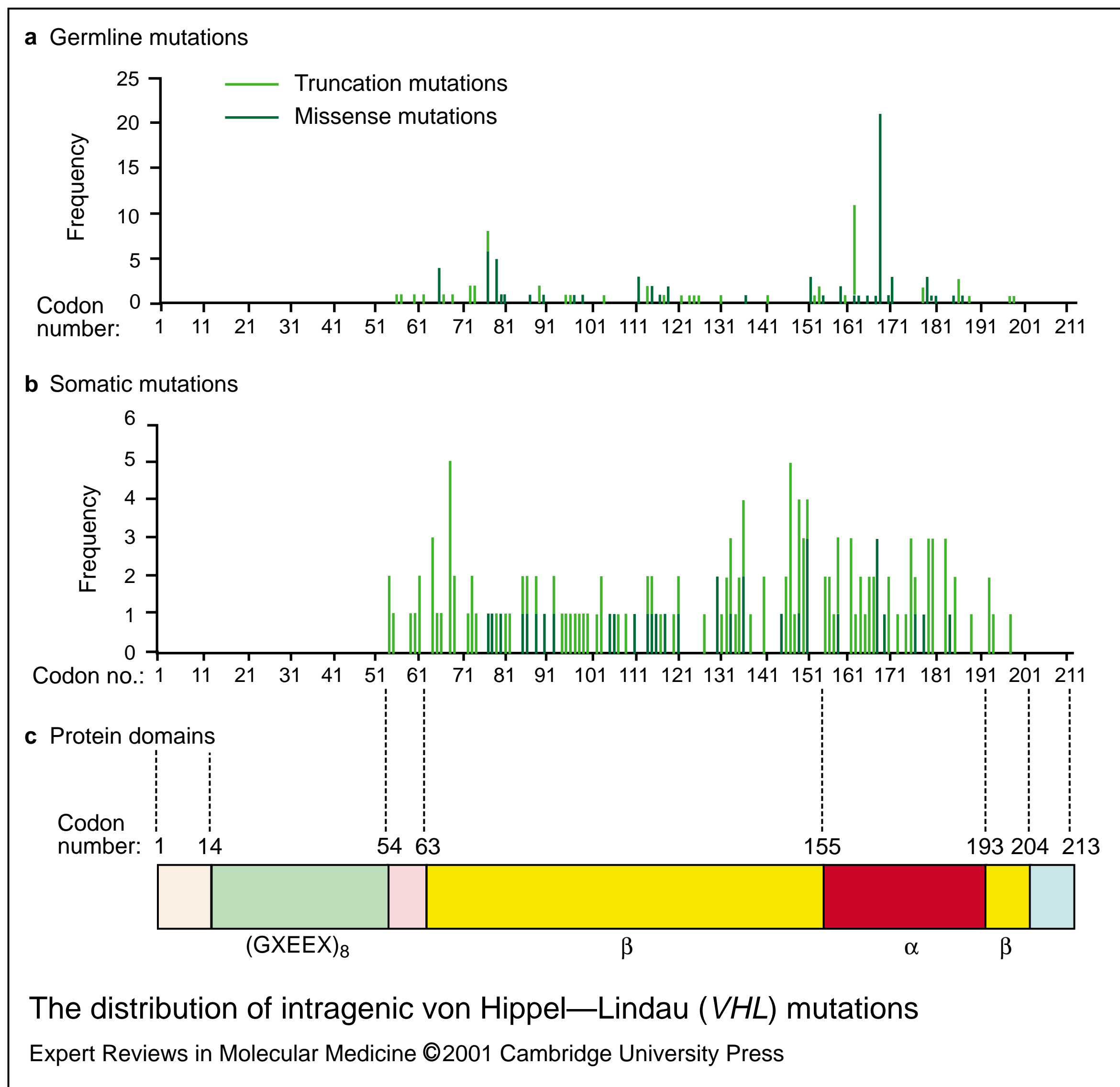

Figure 4. The distribution of intragenic von Hippel–Lindau (*VHL*) mutations. Truncating mutations (frameshift and nonsense) are shown with light-green lines; missense mutations are indicated with dark-green lines. (a) Germline *VHL* mutations in a panel of 120 VHL families from the UK (Refs 38, 40; and E.R. Maher, University of Birmingham Section of Medical and Molecular Genetics, UK, pers. commun.). Note that not all of the mutation 'hotspots' were detected in this panel of families. (b) Somatic *VHL* mutations detected in 152 renal cell carcinomas (RCCs), from the *VHL* mutation database created by C. Beroud *et al.* (Ref. 44), at http://www.umd.necker.fr:2005/. (c) The protein domains encoded by the *VHL* gene aligned to illustrate that most mutations are in the α- and β-domains **(fig004frb)**.

Modifier genes?

The incidence of retinal angioma does not appear to be correlated with specific germline mutations. The development of retinal angioma might be determined at an early age and be influenced by genetic and/or environmental modifier effects. However, no such modifier genes have yet been identified (Ref. 59).

Somatic mutations in VHL tumours

In contrast to germline mutations, somatic mutations are confined to somatic cells of an individual (i.e. any cell type except germ cells), and are therefore not heritable. Somatic mutations that occur in tumour suppressor genes in relevant tissues might initiate cancer development (Fig. 2). Tumours from VHL patients generally show

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deletion, mutation or methylation of the wild-type allele such that there is loss of the normal function of *VHL* in tumours. This is characteristic of a tumour suppressor gene.

Allelic deletions

In keeping with the role of *VHL* as a tumour suppressor, deletion of the *VHL* gene, manifested as loss of heterozygosity (LOH) of polymorphic markers on chromosome 3p25-p26, is common in tumours from VHL patients (51% of tumours), including RCC, phaeochromocytoma, pancreatic islet cell tumours and haemangioblastomas (Refs 60, 61). Loss of the normal *VHL* allele is likely to be an early event in tumour development, because it can be detected in the cystic and benign renal lesions in VHL patients that are thought to represent the early stages of neoplastic transformation to RCC (Ref. 62). In haemangioblastomas of the cerebellum (Ref. 63) or retina (Ref. 64), which are of mixed cell types, the LOH is confined to the stromal component; these stromal cells also express vascular endothelial growth factor [VEGF; also known as vascular permeability factor (VPF) – see later] and might therefore be the true neoplastic component, responsible for abundant neovascularisation in haemangioblastomas (Ref. 64). LOH has also been detected in the pancreatic microcystic adenomas that occasionally occur in VHL patients (Ref. 65); these are not likely to be benign precursors of the malignant pancreatic cancers that occur in VHL patients as they have a different histology, but this underlines the importance of the *VHL* gene in the control of cell growth in the pancreas.

Intragenic mutations

Somatic mutations in the *VHL* coding region (i.e. protein-truncating frameshift mutations) have been detected in 20% of haemangioblastomas from VHL patients (Ref. 61), but only a few intragenic somatic mutations have been detected in RCCs from VHL patients (Ref. 61). Somatic intragenic mutations in VHL phaeochromocytoma also appear to be rare (Ref. 47).

Methylation

The *VHL* gene can also be inactivated by hypermethylation of the normally unmethylated CpG (cytosine–guanine dinucleotide) islands in the 5' region of the *VHL* gene (Refs 61, 66, 67, 68), a phenomenon that has been detected in a number of tumour suppressor genes (Ref. 69). Hypermethylation has been detected in 33% of VHL tumours that do not demonstrate LOH, including RCC and haemangioblastoma (Ref. 61). Cis-specific local features are pivotal in both maintaining and perpetuating aberrant methylation of the *VHL* CpG island, because changes in the methylation status are not induced by transfer of unmethylated *VHL* transgenes or a single chromosome 3 into cells with a hypermethylated endogenous *VHL* gene, or by fusion between cells with unmethylated and hypermethylated *VHL* genes (Ref. 70). However, the contribution of some trans-acting factor to the generation of the initial aberrant *VHL* hypermethylation pattern cannot be excluded.

Somatic mutations in sporadic tumours

In keeping with its role as a tumour suppressor gene, loss of normal VHL function is also common in sporadic tumours of the same types as occur in VHL disease.

RCC

Intragenic *VHL* mutations have been observed in 56–69% of sporadic clear-cell (non-papillary type) RCCs (Refs 66, 71, 72, 73, 74, 75, 76), accompanied by loss of the other *VHL* allele (i.e. LOH) in 84–98% of those samples (Refs 71, 72, 73, 75). Deletions of a *VHL* allele have also been detected in 69% of sporadic clear-cell RCCs by FISH, although there was considerable intra-tumoural heterogeneity (Ref. 77).

Like the germline mutations, the somatic intragenic *VHL* mutations are distributed across much of the gene, downstream of the second methionine at codon 54 (Fig. 4). The mutations are also heterogeneous in type, and include missense and splice site mutations as well as frameshift and nonsense mutations. A mutation hotspot has been identified in RCC from individuals exposed to trichloroethylene (C454T; Pro81Ser), but this is not found in sporadic cases (Ref. 78). Another mutation hotspot – deletion of a T at a poly-T tract (nucleotides 653–657; frameshift at codon 148) – was detected in RCC in German patients but not elsewhere (Ref. 75), suggesting that a specific carcinogen might be involved. The *VHL* mutations are thought to be an early event in renal tumourigenesis as they are observed in localised (Stage I/II) tumours as well as in advanced or metastatic (Stage III/IV) RCC (Ref. 71). Other 3p loci, especially 3p14-p21, also seem to be important for

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malignant transformation of renal cells (Ref. 67), with or without *VHL* gene inactivation. However, *VHL* gene inactivation is usually confined to the clear-cell type of RCC (which is the same histological type as occurs in VHL disease), and few mutations have been observed in other histological types such as papillary (chromophilic) RCC (Refs 71, 73, 75). Epigenetic inactivation of the *VHL* gene (i.e. silencing by promoter methylation) is also observed in sporadic clear-cell RCC tumours: 15–19% have promoter hypermethylation (Refs 66, 67, 68, 75), which results in silencing of this allele. Some RCCs show methylation of one allele and deletion of the other, and occasionally both alleles are methylated (Ref. 66).

Haemangioblastomas

LOH at the *VHL* locus has been detected in the stromal cell component of 53% of sporadic cerebellar haemangioblastomas and somatic missense mutations have been detected in 10% (Ref. 79). Somatic intragenic missense and truncating mutations have also been detected in at least 23% of sporadic CNS haemangioblastomas (Ref. 80).

Phaeochromocytoma

Analysis of the *VHL* gene in 48 apparently sporadic phaeochromocytomas revealed only two somatic missense mutations (4%) (Ref. 47). Thus, other genes are probably more important for the development of most cases of phaeochromocytoma.

Other tumour types

LOH at the *VHL* locus has been detected in sporadic cases of pancreatic microcystic adenoma (Ref. 65) and endolymphatic sac tumours (Ref. 81). Mutations in the *VHL* gene are very rare in tumour types that are not associated with VHL disease – lung, breast, ovarian, testicular, cervical, endometrial, prostate, colon and bladder cancers, melanoma, mesothelioma, oral squamous cell carcinoma and follicular thyroid carcinoma (Refs 71, 74, 82, 83, 84) – although some *VHL* gene deletions have been reported in colon cancer (Ref. 85).

VHL gene expression and function

VHL expression in different tissues

The *VHL* gene appears to be expressed in most adult human tissues (Ref. 21). Two alternatively spliced mRNA isoforms (with and without exon 2) are detectable but, since patients with deletion of the whole of exon 2 have VHL disease (Ref. 36), the full-length isoform is likely to encode the active tumour suppressor. Transcription starts upstream of both methionine codons in exon 1 and so it is not possible to distinguish from the mRNA which of the two protein translation start sites is used.

During human embryogenesis, *VHL* mRNA is expressed in virtually all tissues from as early as six weeks of gestation, with particularly high levels in the urogenital system, brain, spinal cord, sensory ganglia, eyes and bronchial epithelium (Ref. 86). Thus, the areas of highest expression do not completely correlate with the tissues that are involved in VHL disease. In the developing metanephric kidney, *VHL* is expressed in the proximal tubule (from where RCC originates) (Refs 86, 87), but it is also abundant in the loops of Henle (which are not associated with RCC in VHL disease). There is also no difference in the relative amounts of the two differentially spliced mRNA isoforms in the different fetal tissues (Ref. 86). *VHL* gene expression is similarly widespread during mouse embryogenesis (Ref. 87).

Antibody staining of normal human adult tissues demonstrates that pVHL is expressed in: epithelial cells covering the body surface, the alimentary canal, and the respiratory and genitourinary tracts; secretory cells of the exocrine and endocrine organs; parenchymal cells of visceral organs; cardiomyocytes; neurons in nervous tissue; lymphocytes in lymphoid tissue; and macrophages (Refs 88, 89, 90). The tissue specificity of VHL disease therefore cannot be entirely explained by tissue-specific expression during either fetal development or adulthood. However, the antibodies used would not distinguish between the different sized pVHLs generated by alternative translation initiation sites (see later) or by alternative splicing.

pVHL might play a role in the development of the kidney: its expression is confined to tubular components in the fetal kidney, and *VHL* expression inhibits hepatocyte growth factor/scatter factor-induced branching morphogenesis and invasion in RCC cells in vitro (Ref. 91). Thus, pVHL might limit branching morphogenesis in renal tubules in vivo.

Knockout mice and other animal models

Homozygous $Vhl^{-/-}$ knockout mice die in utero at 10.5–12.5 days of gestation, as a result

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of defective placental vasculogenesis (Ref. 92). By contrast, heterozygous *Vhl*$^{+/-}$ mice appear phenotypically normal, without any evidence of spontaneous tumour development up to the age of 15 months; thus, there is not yet a good animal model of VHL disease. It has yet to be established whether tumours would develop at a higher rate than normal in heterozygous mice treated with carcinogens, or against a different genetic background. Conditional knockout rodents (i.e. where the *VHL* gene is inactivated only in specific cell types or tissues) are required to define the true role of *VHL* in development of tissues such as the kidney and brain. However, it is known that expression of pVHL correlates with neuronal differentiation in rodent CNS cells, and *VHL* transduction induces neuronal differentiation (Ref. 93). Furthermore, nitrosamine treatment of normal rats induces clear-cell RCC, and *VHL* mutations were detected in 38% of these rat tumours, suggesting that the tumour suppressor function of *VHL* is conserved in rodents (Ref. 94).

A reduction of *Drosophila Vhl* expression by RNA interference methodology causes breakage of the main vasculature and excessive looping of smaller vascular branches in the trachea, while overexpression causes a general lack of vasculature (Ref. 32). The authors of this study propose that pVHL is involved in halting cell movement at the end of vascular tube outgrowths. This is a useful model for the mammalian vasculature because branching morphogenesis in mammals is regulated in a similar way to that in *Drosophila* trachea.

The VHL protein

Size

As well as the full-length pVHL of 213 amino acids with an apparent molecular mass of 24–30 kDa [pVHL(30)] there is a second major product, of 18–19 kDa [pVHL(19)], that appears to result from translation initiation at the second AUG codon (codon 54). Both pVHL(30) and pVHL(19) are functional in tumour suppression (Refs 95, 96, 97). Analysis of various cell lines suggests that pVHL(30) protein is the more abundant species, although pVHL(19) translated from the second methionine is also detectable (Refs 98, 99). However, the first 53 amino acids from pVHL(30) are less well conserved than the sequence after codon 54, and no pathogenic mutations have been identified in codons 1–53, suggesting that this region might not be important for the tumour suppressor function of VHL. There is, as yet, no evidence for expression of pVHL from the alternatively spliced mRNA lacking exon 2 (isoform 2) in normal individuals. Post-translational modification of pVHL has not yet been thoroughly investigated, but it is possible that pVHL is phosphorylated because there are several kinase consensus sequences within the protein. pVHL can self-associate via amino acids 96–122 when overexpressed in cells (Ref. 100), which might cause dominant negative effects for *VHL* mutations outside this region. However, it has not been shown that pVHL is able to self-associate when expressed at physiological levels.

Structure

pVHL has two major structural domains, as identified from the three-dimensional structure of its heterotrimeric complex with elongins B and C (Ref. 58) (see below). Details of the crystal structure are available from the Protein Data Bank at http://www.rcsb.org/pdb/ (PDB ID: 1vcb) (Ref. 101). The β-domain consists of a seven-stranded β sandwich (amino acids 63–154) and one α-helix (amino acids 193–204). The smaller, α-domain (amino acids 155–192) consists of three α-helices, and binds to elongin C (Fig. 3). The α-domain is a hot spot for missense mutations in *VHL* (e.g. Arg167), and these amino acids have been identified as being involved in direct interaction with residues in elongin C or in interactions with other pVHL residues to stabilise the structure of the α-domain. Another region of pVHL in which missense mutations are frequently observed (Trp88, Asn90, Gln96, Tyr98, Tyr112) is an area on the surface of the β-domain opposite the binding site for elongin C; this surface patch might represent another macromolecule-binding site (Ref. 58). Analysis of different *VHL* mutations with respect to the type of VHL disease (Type 1 or Type 2) has revealed that most Type 2 mutations map to the binding site for elongin C or to the surface patch in the β-domain, or are predicted to cause relatively localised effects if a structural residue is involved. By contrast, the few missense mutations that cause Type 1 VHL disease tend to map to residues in the β-domain hydrophobic core and are predicted to cause complete unravelling of the pVHL structure. This suggests a bias against complete loss-of-function mutations in Type 2 VHL disease.

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Subcellular localisation

In cell cultures, pVHL is localised with a punctate (dotted) nuclear pattern, a diffuse cytoplasmic pattern, or as both in the same cell (Ref. 102). This is because pVHL engages in nucleocytoplasmic shuttling (Ref. 103), which is required for its function. This shuttling is transcription dependent (inhibition of transcription causes pVHL to localise to the nucleus), and is mediated by sequences within exon 2 (amino acids 114–154; Fig. 3) because pVHL with exon 2 deleted localises preferentially to the nucleus. Nuclear export of pVHL does not require binding to cullin 2 (CUL2; see later), but is dependent on adenosine triphosphate (ATP) and is mediated by Ran (Ref. 104), an abundant multifunctional GTPase that mediates nuclear import and export of many proteins (Ref. 105). There are also reports that pVHL shuttling is cell-cycle dependent: pVHL is both nuclear and cytoplasmic in subconfluent cells, but is more nuclear in confluent cells (Ref. 106); and cell-cycle analyses have shown exclusively nuclear staining in cells in G0/G1, with more diffuse cytoplasmic staining in cells in S phase (Ref. 106). However, another report showed that pVHL was nuclear in subconfluent cells and more cytoplasmic in confluent cells (Ref. 107). These contrasting findings might indicate artefacts resulting from cell fixation and antibody-staining protocols. Finally, there is a further report (using green fluorescent protein-tagged pVHL) suggesting that pVHL is located in the mitochondria (Ref. 108).

Suppression of tumourigenesis and effects on the cell cycle

Human *VHL*$^{-}$ RCC cells are tumourigenic in nude mice (mutant hairless athymic mice that are immunodeficient and thus do not reject tumour transplants from other species) and tumourigenesis is suppressed by re-introduction of the *VHL* gene (Refs 96, 98, 109). The effect of re-expression of *VHL* on the growth rate and cell-cycle profile of RCC cells grown in vitro is variable and might depend on the cell line: some lines show no effect (Refs 98, 109), while others demonstrate in vitro RCC cell growth inhibition by *VHL* (Refs 110, 111). *VHL* expression can restore the ability of RCC cell lines to exit the cell cycle and enter G0 quiescence upon serum withdrawal in culture (Ref. 112). The mechanism of these effects of pVHL on the cell cycle is not clear, but it might be associated with induction of the cyclin-dependent kinase inhibitor $p27^{Kip1}$ at both RNA and protein levels (Ref. 111). When *VHL*$^{-}$ RCC cells are grown as multicellular three-dimensional spheroids in vitro (rather than as a monolayer), introduction of a wild-type *VHL* gene suppressed growth and induced features of cell differentiation, including deposition of fibronectin in the extracellular space (Ref. 113). This indicates that pVHL does inhibit features of tumourigenesis in RCC cells, confirming that *VHL* is a true tumour suppressor gene. Recent experiments in an RCC cell line suggest that pVHL protects cells from UV-induced apoptosis (programmed cell death), possibly by inducing growth arrest via elevation of the levels of the cyclin kinase inhibitors p21 and p27 (Ref. 114). It seems counter-intuitive that cells lacking the functional pVHL tumour suppressor are more sensitive to apoptotic stimuli, but perhaps increased apoptosis provides selective pressure for cells that can escape death under these conditions, resulting in clonal outgrowth of tumourigenic cells following mutations in other genes.

pVHL-binding proteins

The function of a novel protein can often be elucidated by identification of the cellular proteins with which it interacts. Immunoprecipitation experiments reveal a large number of proteins that bind to pVHL (either directly or indirectly), not all of which have yet been characterised (Refs 102, 115).

Elongin C

pVHL binds a 9–14 kDa protein, which was identified as elongin C, and a 14–18 kDa protein (elongin B) binds to elongin C in this same complex (Refs 102, 115, 116, 117). Elongins B and C were originally identified as subunits of the heterotrimeric transcription elongation factor elongin (SIII). pVHL binds directly to elongin C mainly via amino acids 157–172 (Refs 99, 115, 117, 118) – the α-domain of pVHL (Ref. 58). This has been demonstrated by the following: when compared with wild-type pVHL, a mutant pVHL deleted from amino acids 157 to 213 (the C-terminus) showed greatly reduced ability to bind to elongin C/B, and mutant pVHLs with missense mutations Arg167Gln, Arg167Trp, and Leu158Pro (in the α-domain) also showed impaired binding; a synthetic peptide of amino acids 157–172 competed with pVHL for binding; and mutant pVHLs with missense mutations Tyr98His, Tyr98Asn, Gly93Asp, Ser111Asn,

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Tyr112His and Phe119Leu (in the β-domain) retained elongin C/B binding (Refs 115, 116, 117).

Folding and assembly of pVHL into a trimeric complex with elongin C and elongin B (the VCB complex; Fig. 5a) is directly mediated by association of pVHL with the chaperonin protein TriC (Ref. 119). The VCB complex is resistant to proteasomal degradation, and pVHLs harbouring mutations that disrupt elongin binding are unstable and rapidly degraded (Ref. 120).

Because elongins B and C are regulatory components of the transcription elongation heterotrimer elongin (SIII) (elongins A + B + C) (Ref. 121), it was originally suggested that pVHL sequestered elongins B and C away from elongin A and thus might inhibit transcriptional elongation of target genes (Refs 116, 122). However, the high cellular levels of elongin B and C and low levels of pVHL suggested that this is not the mechanism. Indeed, elongins B and C were subsequently shown to have other binding partners, including CUL2 (see below) and proteins containing a 50 amino acid domain referred to as a SOCS-box [because it was first identified in the cytokine-inducible suppressors of cytokine signalling (SOCS) proteins] (Ref. 123). Recently, however, pVHL was shown to repress transcriptional elongation of the gene encoding tyrosine hydroxylase (TH) in rat phaeochromocytoma cells, and this elongation pause at a region downstream of the *TH* gene was released by hypoxia (a physiological stimulus for TH mRNA expression) (Ref. 124). It is not clear whether pVHL-dependent regulation of transcription elongation occurs in any other genes, or whether this function is relevant to tumour suppressor activity.

CUL2

The VCB complex binds to a 70–76 kDa CUL2 protein (Refs 99, 125), a human member of the cullin gene family, which is involved in cell-cycle control in yeast (Ref. 126). CUL2 does not bind to pVHL in the absence of elongins B and C (Ref. 127), and while there is some evidence for elongin C binding to CUL2 in the absence of pVHL (Ref. 99), mutations that disrupt pVHL binding to elongin C (Arg167Trp and 157del) reduce the interaction with CUL2 (Ref. 127). The VCB–CUL2 complex inhibits accumulation of hypoxia-induced mRNAs such as VEGF (Ref. 99) by ubiquitin-mediated proteolysis of hypoxia-inducible factor 1 (HIF-1) (see below). VCB–CUL2 is an important component of the ubiquitin-mediated proteolysis system, which has been shown to be involved in a wide range of cellular functions, including cell-cycle progression and signal transduction (Box 1). The VCB–CUL2 complex exhibits E3 ubiquitin ligase activity, with Ubc5 a, b and c as the ubiquitin-conjugating E2 enzyme, and with another protein (Rbx1) enhancing the ligase activity (Refs 128, 129, 130, 131). Indeed, it was the sequence and structural similarity between VCB–CUL2 and components of the yeast SCF complex (Box 1) that led to the idea that the VCB complex might also be involved in ubiquitination; elongin C has homology to Skp1, CUL2 to Cdc53/CUL1, and elongin B to ubiquitin. pVHL does not share sequence homology with the other component of SCF complexes, the F-box proteins (Box 1), but there is structural similarity (Refs 58, 127). To summarise, pVHL might be the substrate recognition subunit of the VCB–CUL2 E3 ubiquitin ligase, a macromolecular complex comprising VCB, CUL2 and Rbx1 that is able to ubiquitinate proteins such as HIF-1 (see below and Fig. 5a).

CUL2 is covalently modified at Lys689 by NEDD8, a ubiquitin-like protein; pVHL complex formation promotes this conjugation (Refs 132, 133), and the NEDD8–CUL2 conjugates are part of the VCB–CUL2 complex in vivo. Although the functional significance of this covalent modification is unclear, one hypothesis is that NEDD8 attachment protects CUL2 from self-ubiquitination and degradation.

As it encodes a pVHL-binding protein, *CUL2* makes a good candidate tumour suppressor gene. However, no pathogenic mutations were detected by screening the *CUL2* gene in sporadic RCC, although LOH in the *CUL2* region of chromosome 10p11.1-p11.2 was detected in 24% of informative cases (Ref. 134). Similarly, no *CUL2* mutations were detected in sporadic phaeochromocytomas that lack *VHL* mutations, although two polymorphic variants in *CUL2* were over-represented in the phaeochromocytoma patients compared with a control population (Ref. 135). Thus, unless *CUL2* is inactivated by epigenetic events, it is not a major tumour suppressor gene in RCC or phaeochromocytoma.

Studies in RCC cells suggest that lack of pVHL reduces the rate of elimination of abnormally processed proteins (because of a lower rate of proteolysis after ubiquitination), so that RCC cells lacking pVHL are more sensitive to

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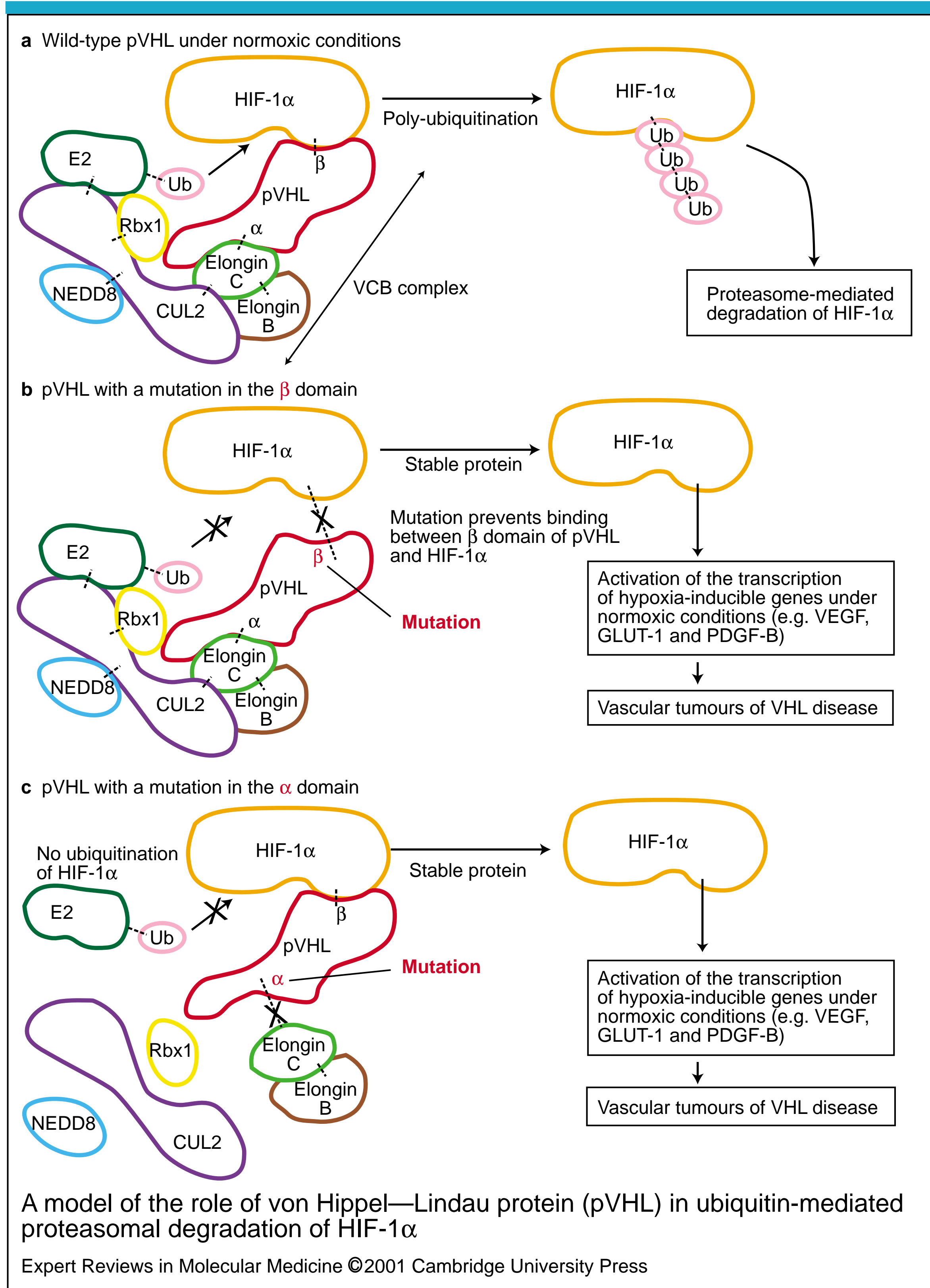

Figure 5. A model of the role of von Hippel–Lindau protein (pVHL) in ubiquitin-mediated proteasomal degradation of HIF-1α *(see next page for legend)* **(fig005frb).**

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Figure 5. A model of the role of von Hippel–Lindau protein (pVHL) in ubiquitin-mediated proteasomal degradation of HIF-1α. (a) Wild-type pVHL under normal oxygen (normoxic) conditions binds via its α-domain to elongin C and forms a complex with elongin B and CUL2. Assisted by Rbx1 and possibly NEDD8, this complex acts as an E3 ubiquitin ligase, transferring ubiquitin from the E2 ubiquitin-conjugating enzyme onto the substrate, the HIF-1α transcription factor, which is bound to the β-domain of pVHL. Ubiquitinated HIF-1α is then targeted for degradation by the proteasome. (b) pVHL with a mutation in the β-domain is unable to bind to HIF-1α, which does not become ubiquitinated and is not degraded. Elevated levels of HIF-1α protein then activate transcription of target genes that are normally induced only by hypoxia, including VEGF, which might play a role in development of the vascular tumours of VHL disease. (c) pVHL with a mutation in the α-domain is unable to bind to elongin C, so the E3 ubiquitin ligase complex does not form and is unable to ubiquitinate HIF-1α. HIF-1α levels increase and transcription of target genes is induced. Abbreviations: CUL2, cullin 2; E2, E2 ubiquitin-conjugating enzyme (e.g. Ubc5a); GLUT-1, glucose transporter 1; HIF-1α, hypoxia-inducible transcription factor 1α; PDGF-B, platelet-derived growth factor B chain; Ub, ubiquitin; VCB complex, trimeric complex of pVHL, elongin C and elongin B; VEGF, vascular endothelial growth factor **(fig005frb)**.

cytotoxicity caused by specific stresses such as glucose deprivation (Ref. 136). This supports the hypothesis of a role for pVHL in ubiquitin-mediated proteolysis of cellular proteins and suggests that other proteins in addition to HIF-1 might be targets for the VCB–CUL2 complex.

HIF-1

One target of the VCB–CUL2 complex is HIF-1, which plays a key role in regulating genes involved in energy metabolism, angiogenesis and apoptosis in response to hypoxia (Refs 137, 138). HIF-1 is a heterodimeric transcription factor consisting of HIF-1α and HIF-1β [also known as arylhydrocarbon-receptor nuclear translocator (ARNT)] subunits (Ref. 138). HIF-1 activity is primarily determined by stability of HIF-1α, which is rapidly degraded by ubiquitin-mediated proteolysis under normal conditions but becomes stabilised by hypoxia (Refs 139, 140). HIF-1α binds to the surface binding site (β-domain) of pVHL and is ubiquitinated and degraded in the presence of oxygen via the VCB–CUL2 complex (Refs 131, 141, 142, 143) (Fig. 6a). HIF-2α [also known as endothelial PAS domain protein 1 (EPAS-1), HIF-related factor (HRF), or HIF-like factor (HLF)] is a homologue of HIF-1α and is also a ubiquitination target of pVHL (Refs 142, 143). The mechanism whereby VCB–CUL2-mediated proteolysis of HIF-1α is inhibited in normal cells under hypoxic conditions has not been established, but might involve nuclear translocation and an intranuclear event (Ref. 144).

Box 1. Ubiquitin-mediated proteolysis

Ubiquitin-mediated proteolysis is involved in controlling the levels of many different proteins within the cell, including cell-cycle regulators, transcription factors and signalling proteins (e.g. IκBα, β-catenin, E2F-1, cyclin-dependent kinase inhibitor $p27^{Kip1}$, cyclins and p53) (Refs 130, 178, 179). Protein substrates are targeted for degradation following covalent attachment of the small protein ubiquitin, catalysed by a cascade of ubiquitin transferase enzymes (E1, E2, E3).

First, ubiquitin is activated by adenosine triphosphate (ATP) to form a high-energy thiol ester intermediate with the E1 ubiquitin-activating enzyme. Activated ubiquitin is then transferred from E1 to an E2 ubiquitin-conjugating enzyme. In the presence of an E3 ubiquitin protein ligase, E2 transfers ubiquitin to an ε-amino group of a lysine residue in the specific protein substrate. Polyubiquitin chains are then formed by generating isopeptide bonds between the C-terminal glycine and Lys48 of conjugated ubiquitin molecules. Polyubiquitinated proteins are subsequently captured and degraded by the 26S proteasome, an abundant protease particle.

The crucial substrate recognition step in ubiquitin-mediated proteolysis is mediated by the diverse family of E3 ubiquitin ligases. One well-characterised type of E3 is the Skp1–CUL1–F-box (SCF) protein complexes in yeast. The Skp1 subunit links any one of a set of adaptor F-box proteins to a core ubiquitination complex that is composed of the scaffold protein Cdc53/CUL1, the RING-H2 finger protein Rbx1/ROC1 (Refs 180, 181) and typically the E2 enzyme Cdc34 (Ref. 179).

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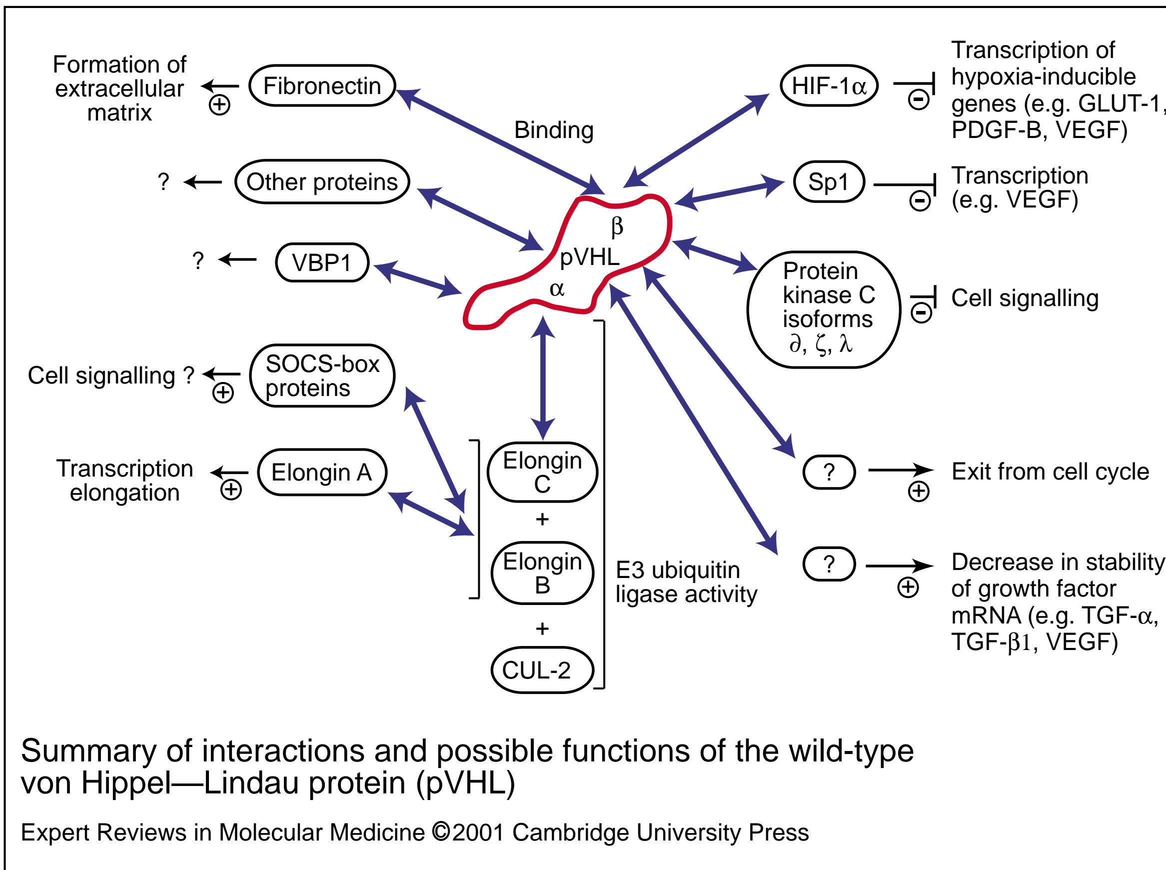

Figure 6. Summary of interactions and possible functions of the wild-type von Hippel–Lindau protein (pVHL). Double-headed, blue arrows indicate interactions between proteins. Single-headed, black arrows indicate events that occur as a result of pVHL binding to its partner. Black T-shaped lines indicate events that are inhibited by pVHL binding to its partner. Question marks indicate components of the network that are yet to be identified. Fibronectin, HIF-1α, Sp1 and protein kinase C isoforms have been reported to bind to the β-domain of pVHL; elongin C and probably VBP1 bind to the α-domain of pVHL. pVHL promotes correct formation of the fibronectin extracellular matrix, and might promote cell cycle exit and destabilisation of growth factor mRNAs via unknown mechanisms. pVHL inhibits HIF-1α- and Sp1-induced transcription of genes, and might inhibit cell signalling by protein kinase C. The pVHL complex with elongins and CUL2 has E3 ubiquitin ligase activity that is responsible for the inhibition of HIF-1α; it is not yet clear whether E3 activity is also responsible for the other functions of pVHL. Elongins C and B bind to other cellular proteins (elongin A and proteins containing a SOCS-box motif) independently of pVHL; there might be competition between these different pathways. Abbreviations: CUL2, cullin 2; GLUT-1, glucose transporter 1; HIF-1α, hypoxia-inducible transcription factor 1α; PDGF-B, platelet-derived growth factor B chain; SOCS, suppressor of cytokine signalling; TGF, transforming growth factor; VBP1, VHL-binding protein 1; VEGF, vascular endothelial growth factor **(fig006frb)**.

Tumour-associated missense mutations in the β-domain of pVHL reduce or abrogate binding and degradation of HIF-1 (Refs 131, 142, 143, 144) (Fig. 5b). Mutation in the α-domain of pVHL also stabilises HIF-1 since it prevents the formation of the VCB complex (Fig. 5c). Lack of wild-type pVHL results in stabilised HIF-1 and overexpression under normoxic conditions of genes containing a hypoxia-response element (HRE) in their promoter, including VEGF and glucose transporter 1 (GLUT-1). Thus, constitutive HIF-1 activation might underlie the hypervascular phenotype of VHL-associated tumours. Indeed, immunohistochemistry has shown elevated levels of HIF-1α and HIF-2α proteins in VHL-associated RCC and haemangioblastoma (Ref. 145).

It should be noted that the p53 tumour suppressor protein also promotes ubiquitin-

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mediated proteasomal degradation of HIF-1α (Ref. 146), and overexpression of HIF-1α protein is a feature of many tumour types (possibly because of hypoxia) (Ref. 147). Thus, the tissue-specificity of tumour types associated with *VHL* mutation might not be related to its interaction with HIF-1α. Furthermore, pVHL might recognise target proteins for protein degradation in addition to HIF-1α and HIF-2α. Several other proteins have been shown to bind to the β-domain of pVHL (see below), but their functional significance has not yet been determined.

Fibronectin

The extracellular adhesive glycoprotein fibronectin is a multifunctional protein that binds to cell-surface integrins and plays a role in the control of tumour cell migration and metastasis. Fibronectin has been shown to bind to wild-type but not to mutant pVHLs (including those with missense mutations in the β-domain) (Refs 143, 148); binding probably occurs with a fraction of pVHL associated with the endoplasmic reticulum (ER) during ER-mediated export of fibronectin to the cell surface. Assembly of the fibronectin extracellular matrix is grossly defective in *VHL*$^{-/-}$ RCC cells and can be partially restored by re-introduction of wild-type pVHL (Ref. 148). Furthermore, *Vhl*$^{-/-}$ mouse embryo fibroblasts are also impaired in fibronectin extracellular matrix assembly compared with wild-type counterparts (Ref. 148). This suggests a direct role for pVHL in fibronectin matrix assembly.

Sp1 transcription factor

One group has reported that the transcription factor Sp1 interacts with pVHL, and that pVHL represses Sp1-mediated activation of the VEGF promoter in a reporter construct (Ref. 149). pVHL might partially downregulate VEGF transcription by directly binding and inhibiting Sp1 (Ref. 100), and also by inhibiting protein kinase C ζ (PKC-ζ) binding to and phosphorylation of Sp1 (Ref. 150). pVHL amino acids 96–122 (in the β-domain) are sufficient for binding to the zinc fingers of Sp1, thereby interfering with the ability of Sp1 to bind DNA (Ref. 100). The interaction of pVHL with Sp1 has yet to be confirmed by other groups.

PKC

Two groups have reported that part of the β-domain of pVHL (amino acids 114–122) interacts directly with the regulatory domain of several PKC isotypes (δ, ζ and λ) and inhibits kinase activity (Refs 151, 152); these PKC isotypes have been implicated in regulation of cell growth and apoptosis (Ref. 152). Binding of pVHL to PKC-δ and PKC-ζ might prevent PKC translocation to the cell membrane and thus prevent signalling steps [including mitogen-activated protein kinase (MAPK) activation] that result in VEGF overexpression (Ref. 151). Furthermore, pVHL inhibits PKC-ζ binding to (and phosphorylating and activating) the Sp1 transcription factor and thus might prevent activation of VEGF promoter expression (Ref. 150). Finally, pVHL inhibition of PKC-δ interaction with insulin-like growth factor 1 (IGF-1) receptor might inhibit IGF-1-mediated signalling (Ref. 153).

Other pVHL-binding proteins

Yeast two-hybrid analysis using *VHL* cDNA has identified seven polypeptides that bind to pVHL (Ref. 154), including elongin C and a novel protein named pVHL-binding protein 1 (VBP1). VBP1 binds to full-length pVHL (213 amino acids), but does not bind pVHL truncated at amino acid 187. This result is the same as for elongin C in this assay, suggesting involvement of α-domain binding. The *VBP1* gene was investigated as a candidate gene in sporadic RCC but no mutations were found (Ref. 134), and the function of this protein is unknown.

Imidopeptidase bound to a region of pVHL within the N-terminal amino acids 1–57, as did two unidentified proteins. Human immunodeficiency virus (HIV) Tat-binding protein 1 and the actin-binding protein filamin bound to a region contained within pVHL amino acids 1–187. The significance of these interactions has yet to be investigated.

pVHL target genes

Interaction of pVHL with its various binding partners results in changes in gene expression that inhibit tumour cell growth, angiogenesis, and other functions. Identifying these pVHL target genes is crucial to understanding the mechanism of tumourigenesis resulting from *VHL* mutation and should ultimately enable the development of specific treatments for VHL disease.

VEGF

Known pVHL target genes include VEGF (Ref. 155), which is a hypoxia-inducible gene that is highly expressed in many tumours, including

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VHL-associated and sporadic RCC, and stimulates neoangiogenesis in growing solid tumours. Hypoxia regulates the production of VEGF in normal tissues at both transcriptional and post-transcriptional levels, by activating the HIF-1 transcription factor, leading to increased VEGF transcription, and by increasing VEGF mRNA stability.

RCC cells with inactivated endogenous *VHL* overexpress VEGF mRNA and protein in vitro; re-expression of wild-type VHL inhibits VEGF expression (Refs 109, 156, 157) and restores its hypoxia inducibility. Several studies have shown that inhibition by pVHL does not affect VEGF transcription initiation or elongation (as was originally suggested because of the pVHL–elongin association) (Ref. 109), but does decrease the half-life of VEGF mRNA (Ref. 156), suggesting that pVHL regulates VEGF expression at a post-transcriptional level (i.e. independent of HIF-1 and Sp1 transcription factor activity). pVHL might control VEGF mRNA stability by decreasing the formation of a novel hypoxia-inducible protein complex bound to the VEGF 3' UTR (Ref. 158). However, one group has shown that pVHL does indeed inhibit Sp1-mediated transcription from the VEGF promoter by pVHL binding to Sp1 and to PKC-ζ (Refs 149, 150). Also, since HIF-1, which is known to activate VEGF transcription, is destablised by pVHL it seems likely that pVHL does inhibit VEGF trancription. Taken together, these results suggest that loss of pVHL function might lead to overexpression of VEGF via both transcriptional and post-transcriptional mechanisms, at least in cultured cells.

In vivo, alteration of the *VHL* gene is associated with upregulation of VEGF. Primary clear-cell RCC tumours show overexpression of VEGF mRNA and protein (Ref. 159), particularly in those with reduced *VHL* expression (Ref. 160). Elevated serum VEGF levels have also been detected in patients with RCC (Ref. 161). In addition, VEGF is overexpressed in the stroma of VHL-associated and sporadic CNS haemangioblastomas (Refs 162, 163) and in retinal angiomas (Ref. 64), whereas the vascular endothelial-cell component of haemangioblastomas expresses the two homologous VEGF receptors Flt-1 (Fms-like tyrosine kinase) and KDR (kinase insert domain-containing receptor) (Ref. 162). Recent studies have shown that the VEGF overexpression correlates with elevated levels of expression of the HIF-2α transcription factor in haemangioblastomas (Ref. 164). Elevated VEGF mRNA was also detected in epididymal cystadenoma cells from a VHL patient (Ref. 163). It is not known whether human phaeochromocytomas express VEGF, but VEGF mRNA is expressed by the rat phaeochromocytoma cell line PC12 (Ref. 165).

In summary, pVHL suppresses VEGF gene expression under normoxic conditions (probably via both transcriptional and post-transcriptional mechanisms), and loss of pVHL leads to overexpression of VEGF (at least partially by stabilisation of HIF-1). Overexpression of VEGF is a consistent feature of tumours with *VHL* mutations, which might explain the hypervascular phenotype of most VHL tumours.

Other hypoxia-inducible genes

As well as overexpressing VEGF, $VHL^{-/-}$ RCC cells overexpress GLUT-1 and the platelet-derived growth factor B chain (PDGF-B), which are two other genes that are normally induced by hypoxia. Re-introduction of wild-type pVHL inhibited the production of these mRNAs under normoxic conditions and restored their normal hypoxia inducibility (Ref. 156). Similarly, erythropoeitin is a hypoxia-inducible gene that is overexpressed in VHL tumours, including haemangioblastomas (Ref. 166), which can occasionally lead to excessive production of red blood cells (secondary polycythaemia or paraneoplastic erythrocytosis) (Ref. 167) in VHL patients. Two carbonic anhydrase genes (CA12 and CA9) are overexpressed in some RCC cell lines and are inhibited by re-introduction of wild-type pVHL (Ref. 168), and it has recently been demonstrated that CA9 and CA12 are hypoxia-inducible genes directly regulated by the HIF/pVHL system (Ref. 169). Carbonic anhydrase enzymes produce carbonic acid from CO_2 and might acidify the immediate extracellular milieu surrounding cancer cells, creating a microenvironment conducive to tumour growth and spread. As mentioned earlier, another hypoxia-inducible mRNA – TH – is also regulated by pVHL in rat phaeochromocytoma cells (Ref. 124).

Urokinase-type plasminogen activator (uPA)

Urokinase-type plasminogen activator (uPA) mRNA and protein levels are higher, and plasminogen activator inhibitor 1 (PAI-1) levels are lower, in RCC cells with wild-type pVHL compared with RCC cells lacking pVHL or expressing mutant pVHL. This suggests that pVHL might play a role in regulation of

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angiogenesis by regulation of plasmin-mediated proteolysis of the extracellular matrix (Ref. 170).

Transforming growth factor β1 (TGF-β1)

pVHL represses TGF-β1 mRNA and protein levels in RCC cells by decreasing the mRNA half-life. Overexpression of TGF-β1 is a feature of some patients with RCC and loss of pVHL might allow overexpression of TGF-β1. This cytokine acts in a paracrine fashion to aid metastasis and angiogenesis (Ref. 171).

Transforming growth factor α (TGF-α)

TGF-α is an important growth factor for RCC and is overexpressed in many RCC tumours and cell lines. Introduction of pVHL decreases TGF-α mRNA and protein levels in RCC cells by decreasing mRNA stability (Ref. 172). It is possible that pVHL modulates the activity of certain RNA-binding proteins that are involved in mRNA stability, as has been found for VEGF (Ref. 158).

The future: functional aspects

In conclusion, it is clear that inactivation of the *VHL* gene (by deletion, mutation or methylation) is a key event in the development of tumours in VHL disease and also in the development of a large proportion of sporadic RCCs and haemangioblastomas. Wild-type pVHL has multiple interactions with other cellular proteins and multiple effects on target genes (Fig. 6). Key features are the binding of pVHL to elongin C, forming a complex with elongin B and CUL2 that has E3 ubiquitin ligase activity. pVHL might act as the substrate recognition subunit of this complex, to recruit proteins such as HIF-1α for ubiquitination and subsequent proteasome-mediated degradation. Loss of pVHL leads to increased levels of HIF-1α under normoxic conditions, resulting in overexpression of hypoxia-inducible genes such as VEGF. This is likely to be one of the key events that results in tumour growth, but other pVHL functions (perhaps involving its interaction with fibronectin) might also play a role in tumourigenesis. More genes with expression affected by pVHL will certainly be identified in the near future, particularly using microarray technology to investigate the cell signalling pathways involved. Therefore, there is still considerable work to do to elucidate clearly the key functions of pVHL that are critical for tumour suppression.

Little is known about how expression of *VHL* is regulated, so experiments are under way to identify the transcription factors that control VHL mRNA expression. Similarly, analysis of pVHL is required to determine whether its levels are regulated under different conditions, and whether its function is controlled by post-translational modifications such as phosphorylation.

Analysis of many different *VHL* mutations is under way to determine whether the different VHL phenotypes are related to the ability of the mutant proteins to retain certain protein-binding functions. Much of the research to date has concentrated on RCC (because of the availability of cell lines), but there might be tissue-specific functions that relate to phaeochromocytoma and haemangioblastoma that have yet to be identified.

The future: clinical aspects

Identification of the *VHL* gene and genotype–phenotype correlations might allow predictions of the risk of developing particular tumour types, so that personalised screening protocols can be designed for each patient once his or her mutation is known. The screening techniques, including magnetic resonance imaging (MRI), computed tomography (CT) and ultrasound, enable early detection of tumours and cysts in brain and abdomen. Treatment is becoming more sophisticated: nephron-sparing surgery tends to be used where feasible now, instead of radical nephrectomy, to maintain as much kidney function as possible for as long as possible in VHL patients. Once radical nephrectomy becomes inevitable, renal dialysis or transplant is required (Ref. 173). Similarly, laparoscopic adrenal-sparing surgery is being developed for phaeochromocytoma, to delay the necessity for lifelong steroid replacement therapy after total adrenalectomy. There are also advances in treatment of CNS haemangioblastomas, such as stereotactic radiosurgery, which allows ablation of some tumours that were previously untreatable because of their location (Ref. 3). Peripheral retinal angiomas can be successfully treated by cryotherapy or laser treatment at an early stage, but others, such as those close to the optic disk, are more difficult to treat without affecting visual acuity (Ref. 174). Thus, although current surgical techniques aim to reduce morbidity and mortality in VHL patients, they are limited in their effectiveness, and the generation

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of specific biological and/or chemical therapies for VHL tumours would be beneficial.

Phase I and II clinical trials of a specific VEGF inhibitor are under way in VHL patients with kidney and brain tumours (results not yet published), and many other anti-angiogenic drugs are under evaluation in other cancer patients (Refs 175, 176). If effective, these might inhibit the growth of hypervascular VHL tumours. Attempts are also being made to develop gene therapy for treatment of VHL tumours (and sporadic RCC in which pVHL function has been lost), initially using recombinant adenovirus to express the normal *VHL* gene (Ref. 177). Such methods are in their infancy, but there is hope that further advances in understanding the function of the pVHL tumour suppressor will lead ultimately to effective therapies for the prevention or treatment of VHL tumours.

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References

1 Maher, E.R. et al. (1991) Von Hippel-Lindau disease: a genetic study. J Med Genet 28, 443-447, PubMed ID: 91374477

2 Maddock, I.R. et al. (1996) A genetic register for von Hippel-Lindau disease. J Med Genet 33, 120-127, PubMed ID: 97083374

3 Richard, S. et al. (1998) Haemangioblastoma of the central nervous system in von Hippel-Lindau disease. French VHL Study Group. J Intern Med 243, 547-553, PubMed ID: 98345182

4 Evans, D.G. et al. (1997) Uptake of genetic testing for cancer predisposition. J Med Genet 34, 746-748, PubMed ID: 97462977

5 Levy, M. and Richard, S. (2000) Attitudes of von Hippel-Lindau disease patients towards presymptomatic genetic diagnosis in children and prenatal diagnosis. J Med Genet 37, 476-478, PubMed ID: 20381982

6 Maher, E.R. et al. (1990) Clinical features and natural history of von Hippel-Lindau disease. Q J Med 77, 1151-1163, PubMed ID: 91110873

7 Lamiell, J.M, Salazar, F.G. and Hsia, Y.E. (1989) von Hippel-Lindau disease affecting 43 members of a single kindred. Medicine (Baltimore) 68, 1-29, PubMed ID: 89082313

8 Chen, F. et al. (1995) Germline mutations in the von Hippel-Lindau disease tumor suppressor gene: correlations with phenotype. Hum Mutat 5, 66-75, PubMed ID: 95245342

9 Brauch, H. et al. (1995) Von Hippel-Lindau (VHL) disease with pheochromocytoma in the Black Forest region of Germany: evidence for a founder effect. Hum Genet 95, 551-556, PubMed ID: 95278911

10 Knudson, A.G. (1971) Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci U S A 68, 820-823, PubMed ID: 71153958

11 Knudson, A.G. (1985) Hereditary cancer, oncogenes, and antioncogenes. Cancer Res 45, 1437-1443, PubMed ID: 85151778

12 Seizinger, B.R. et al. (1988) Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. Nature 332, 268-269, PubMed ID: 88156962

13 Hosoe, S. et al. (1990) Localization of the von Hippel-Lindau disease gene to a small region of chromosome 3. Genomics 8, 634-640, PubMed ID: 91115319

14 Seizinger, B.R. et al. (1991) Genetic flanking markers refine diagnostic criteria and provide insights into the genetics of Von Hippel Lindau disease. Proc Natl Acad Sci U S A 88, 2864-2868, PubMed ID: 91187892

15 Maher, E.R. et al. (1990) Mapping of von Hippel-Lindau disease to chromosome 3p confirmed by genetic linkage analysis. J Neurol Sci 100, 27-30, PubMed ID: 91210849

16 Maher, E.R. et al. (1991) Mapping of the von Hippel-Lindau disease locus to a small region of chromosome 3p by genetic linkage analysis. Genomics 10, 957-960, PubMed ID: 92009933

17 Crossey, P.A. et al. (1993) Genetic linkage between von Hippel-Lindau disease and three microsatellite polymorphisms refines the localisation of the VHL locus. Hum Mol Genet 2, 279-282, PubMed ID: 93271986

18 Richards, F.M. et al. (1993) Detailed genetic mapping of the von Hippel-Lindau disease tumour suppressor gene. J Med Genet 30, 104-107, PubMed ID: 93187971

19 Vance, J.M. et al. (1990) Confirmation of linkage in von Hippel-Lindau disease. Genomics 6, 565-567, PubMed ID: 90228974

20 Szymanski, S. et al. (1991) Physical mapping the putative Vhl-gene region. Cytogenet Cell Genet 58, 1882

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

21 Latif, F. et al. (1993) Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260, 1317-1320, PubMed ID: 93262488
22 Liu, W. et al. (1993) The isolation of a yeast artificial chromosome (YAC) contig extending for 2 megabases in the vicinity of the von Hippel Lindau disease gene. Hum Mol Genet 2, 1177-1182, PubMed ID: 94004857
23 Phipps, M.E. et al. (1993) Physical mapping of chromosome 3p25-p26 by fluorescence in situ hybridisation (FISH). Hum Genet 92, 18-22, PubMed ID: 93374393
24 Richards, F.M. et al. (1993) Mapping the Von Hippel-Lindau disease tumour suppressor gene: identification of germline deletions by pulsed field gel electrophoresis. Hum Mol Genet 2, 879-882, PubMed ID: 93372848
25 Szymanski, S.C. et al. (1993) Long range restriction map of the von Hippel-Lindau gene region on human chromosome 3p. Hum Genet 92, 282-288, PubMed ID: 94010864
26 Yao, M. et al. (1993) von Hippel-Lindau disease: identification of deletion mutations by pulsed-field gel electrophoresis. Hum Genet 92, 605-614, PubMed ID: 94085961
27 Kuzmin, I. et al. (1994) One-megabase yeast artificial chromosome and 400-kilobase cosmid-phage contigs containing the von Hippel-Lindau tumor suppressor and Ca(2+)-transporting adenosine triphosphatase isoform 2 genes. Cancer Res 54, 2486-2491, PubMed ID: 94215191
28 Liu, W. et al. (1994) Isolation of a contig of overlapping Yac clones in the vicinity of the Vonhippel-Lindau disease gene and a candidate Vhl gene. Cytogenet Cell Genet 65, 42
29 Kozak, M. (1978) How do eucaryotic ribosomes select initiation regions in messenger RNA? Cell 15, 1109-1123, PubMed ID: 79084168
30 Woodward, E.R. et al. (2000) Comparative sequence analysis of the VHL tumor suppressor gene. Genomics 65, 253-265, PubMed ID: 20313892
31 Gao, J. et al. (1995) Cloning and characterization of a mouse gene with homology to the human von Hippel-Lindau disease tumor suppressor gene: implications for the potential organization of the human von Hippel-Lindau disease gene. Cancer Res 55, 743-747, PubMed ID: 95153660
32 Adryan, B. et al. (2000) Tracheal development and the von Hippel-Lindau tumor suppressor homolog in Drosophila. Oncogene 19, 2803-2811, PubMed ID: 20309858
33 Aso, T. et al. (2000) Drosophila von Hippel-Lindau tumor suppressor complex possesses E3 ubiquitin ligase activity. Biochem Biophys Res Commun 276, 355-361, PubMed ID: 20462962
34 Kuzmin, I. et al. (1995) Identification of the promoter of the human von Hippel-Lindau disease tumor suppressor gene. Oncogene 10, 2185-2194, PubMed ID: 95303481
35 Renbaum, P. et al. (1996) Isolation and characterization of the full-length 3' untranslated region of the human von Hippel-Lindau tumor suppressor gene. Hum Genet 98, 666-671, PubMed ID: 97085563
36 Richards, F.M. et al. (1994) Detailed mapping of germline deletions of the von Hippel-Lindau disease tumour suppressor gene. Hum Mol Genet 3, 595-598, PubMed ID: 94348507
37 Pack, S.D. et al. (1999) Constitutional von Hippel-Lindau (VHL) gene deletions detected in VHL families by fluorescence in situ hybridization. Cancer Res 59, 5560-5564, PubMed ID: 20019463
38 Crossey, P.A. et al. (1994) Identification of intragenic mutations in the von Hippel-Lindau disease tumour suppressor gene and correlation with disease phenotype. Hum Mol Genet 3, 1303-1308, PubMed ID: 95078832
39 Kondo, K. et al. (1995) Germline mutations in the von Hippel-Lindau disease (VHL) gene in Japanese VHL. Clinical Research Group for VHL in Japan. Hum Mol Genet 4, 2233-2237, PubMed ID: 96177654
40 Zbar, B. et al. (1996) Germline mutations in the Von Hippel-Lindau disease (VHL) gene in families from North America, Europe, and Japan. Hum Mutat 8, 348-357, PubMed ID: 97114291
41 Olschwang, S. et al. (1998) Germline mutation profile of the VHL gene in von Hippel-Lindau disease and in sporadic hemangioblastoma. Hum Mutat 12, 424-430, PubMed ID: 99045377
42 Yoshida, M. et al. (2000) Germ-line mutation analysis in patients with von Hippel-Lindau disease in Japan: an extended study of 77 families. Jpn J Cancer Res 91, 204-212, PubMed ID: 20222345
43 Richards, F.M. et al. (1995) Molecular analysis of de novo germline mutations in the von Hippel-Lindau disease gene. Hum Mol Genet 4, 2139-2143, PubMed ID: 96154686
44 Beroud, C. et al. (1998) Software and database for the analysis of mutations in the VHL gene.

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

Nucleic Acids Res 26, 256-258, PubMed ID: 98062440
45 Sgambati, M.T. et al. (2000) Mosaicism in von Hippel-Lindau disease: lessons from kindreds with germline mutations identified in offspring with mosaic parents. Am J Hum Genet 66, 84-91, PubMed ID: 20100610
46 Woodward, E.R. et al. (1997) Genetic predisposition to phaeochromocytoma: analysis of candidate genes GDNF, RET and VHL. Hum Mol Genet 6, 1051-1056, PubMed ID: 97358578
47 Eng, C. et al. (1995) Mutations in the RET proto-oncogene and the von Hippel-Lindau disease tumour suppressor gene in sporadic and syndromic phaeochromocytomas. J Med Genet 32, 934-937, PubMed ID: 96423317
48 van der Harst, E. et al. (1998) Germline mutations in the vhl gene in patients presenting with phaeochromocytomas. Int J Cancer 77, 337-340, PubMed ID: 98326725
49 Brauch, H. et al. (1997) Sporadic pheochromocytomas are rarely associated with germline mutations in the vhl tumor suppressor gene or the ret protooncogene. J Clin Endocrinol Metab 82, 4101-4104, PubMed ID: 98060968
50 Bar, M. et al. (1997) Sporadic phaeochromocytomas are rarely associated with germline mutations in the von Hippel-Lindau and RET genes. Clin Endocrinol (Oxf) 47, 707-712, PubMed ID: 98159313
51 Hes, F.J. et al. (2000) Cryptic von Hippel-Lindau disease: germline mutations in patients with haemangioblastoma only. J Med Genet 37, 939-943, PubMed ID: 20558899
52 Hes, F. et al. (2000) Genotype-phenotype correlations in families with deletions in the von Hippel-Lindau (VHL) gene. Hum Genet 106, 425-431, PubMed ID: 20289756
53 Chen, F. et al. (1996) Genotype-phenotype correlation in von Hippel-Lindau disease: identification of a mutation associated with VHL type 2A. J Med Genet 33, 716-717, PubMed ID: 97016544
54 Bradley, J.F. et al. (1999) Two distinct phenotypes caused by two different missense mutations in the same codon of the VHL gene. Am J Med Genet 87, 163-167, PubMed ID: 20004505
55 Neumann, H.P. et al. (1995) Consequences of direct genetic testing for germline mutations in the clinical management of families with multiple endocrine neoplasia, type II. Jama 274, 1149-1151, PubMed ID: 96011708
56 Ritter, M.M. et al. (1996) Isolated familial pheochromocytoma as a variant of von Hippel-Lindau disease. J Clin Endocrinol Metab 81, 1035-1037, PubMed ID: 96368564
57 Crossey, P.A. et al. (1995) Molecular genetic diagnosis of von Hippel-Lindau disease in familial phaeochromocytoma. J Med Genet 32, 885-886, PubMed ID: 96154868
58 Stebbins, C.E, Kaelin, W.G. and Pavletich, N.P. (1999) Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. Science 284, 455-461, PubMed ID: 99221829
59 Webster, A.R. et al. (1998) An analysis of phenotypic variation in the familial cancer syndrome von Hippel-Lindau disease: evidence for modifier effects. Am J Hum Genet 63, 1025-1035, PubMed ID: 98431786
60 Crossey, P.A. et al. (1994) Molecular genetic investigations of the mechanism of tumourigenesis in von Hippel-Lindau disease: analysis of allele loss in VHL tumours. Hum Genet 93, 53-58, PubMed ID: 94095202
61 Prowse, A.H. et al. (1997) Somatic inactivation of the VHL gene in Von Hippel-Lindau disease tumors. Am J Hum Genet 60, 765-771, PubMed ID: 97260402
62 Lubensky, I.A. et al. (1996) Allelic deletions of the VHL gene detected in multiple microscopic clear cell renal lesions in von Hippel-Lindau disease patients. Am J Pathol 149, 2089-2094, PubMed ID: 97110380
63 Vortmeyer, A.O. et al. (1997) von Hippel-Lindau gene deletion detected in the stromal cell component of a cerebellar hemangioblastoma associated with von Hippel-Lindau disease. Hum Pathol 28, 540-543, PubMed ID: 97302340
64 Chan, C.C. et al. (1999) VHL gene deletion and enhanced VEGF gene expression detected in the stromal cells of retinal angioma. Arch Ophthalmol 117, 625-630, PubMed ID: 99256891
65 Vortmeyer, A.O. et al. (1997) Allelic deletion and mutation of the von Hippel-Lindau (VHL) tumor suppressor gene in pancreatic microcystic adenomas. Am J Pathol 151, 951-956, PubMed ID: 97468663
66 Herman, J.G. et al. (1994) Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. Proc Natl Acad Sci U S A 91, 9700-9704, PubMed ID: 95024028
67 Clifford, S.C. et al. (1998) Inactivation of the von Hippel-Lindau (VHL) tumour suppressor gene and allelic losses at chromosome arm 3p in primary renal cell carcinoma: evidence for a

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

VHL-independent pathway in clear cell renal tumourigenesis. Genes Chromosomes Cancer 22, 200-209, PubMed ID: 98287627

68 Graff, J.R. et al. (1997) Mapping patterns of CpG island methylation in normal and neoplastic cells implicates both upstream and downstream regions in de novo methylation. J Biol Chem 272, 22322-22329, PubMed ID: 97413847

69 Jones, P.A. and Laird, P.W. (1999) Cancer epigenetics comes of age. Nat Genet 21, 163-167, PubMed ID: 99140765

70 Kuzmin, I. et al. (1999) Analysis of aberrant methylation of the VHL gene by transgenes, monochromosome transfer, and cell fusion. Oncogene 18, 5672-5679, PubMed ID: 99455247

71 Gnarra, J.R. et al. (1994) Mutations of the VHL tumour suppressor gene in renal carcinoma. Nat Genet 7, 85-90, PubMed ID: 94355983

72 Foster, K. et al. (1994) Somatic mutations of the von Hippel-Lindau disease tumour suppressor gene in non-familial clear cell renal carcinoma. Hum Mol Genet 3, 2169-2173, PubMed ID: 95187156

73 Shuin, T. et al. (1994) Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinomas. Cancer Res 54, 2852-2855, PubMed ID: 94243796

74 Whaley, J.M. et al. (1994) Germ-line mutations in the von Hippel-Lindau tumor-suppressor gene are similar to somatic von Hippel-Lindau aberrations in sporadic renal cell carcinoma. Am J Hum Genet 55, 1092-1102, PubMed ID: 95067971

75 Brauch, H. et al. (2000) VHL alterations in human clear cell renal cell carcinoma: association with advanced tumor stage and a novel hot spot mutation. Cancer Res 60, 1942-1948, PubMed ID: 20227251

76 Meyer, A.J. et al. (2000) Novel mutations of the von hippel-lindau tumor-suppressor gene and rare DNA hypermethylation in renal-cell carcinoma cell lines of the clear-cell type. Int J Cancer 87, 650-653, PubMed ID: 20384683

77 Moch, H. et al. (1998) Intratumoral heterogeneity of von Hippel-Lindau gene deletions in renal cell carcinoma detected by fluorescence in situ hybridization. Cancer Res 58, 2304-2309, PubMed ID: 98283382

78 Brauch, H. et al. (1999) Trichloroethylene exposure and specific somatic mutations in patients with renal cell carcinoma. J Natl Cancer Inst 91, 854-861, PubMed ID: 99270753

79 Lee, J.Y. et al. (1998) Loss of heterozygosity and somatic mutations of the VHL tumor suppressor gene in sporadic cerebellar hemangioblastomas. Cancer Res 58, 504-508, PubMed ID: 98117301

80 Kanno, H. et al. (1994) Somatic mutations of the von Hippel-Lindau tumor suppressor gene in sporadic central nervous system hemangioblastomas. Cancer Res 54, 4845-4847, PubMed ID: 94349292

81 Vortmeyer, A.O. et al. (2000) Somatic von Hippel-Lindau gene mutations detected in sporadic endolymphatic sac tumors. Cancer Res 60, 5963-5965, PubMed ID: 20535963

82 Sekido, Y. et al. (1994) Molecular analysis of the von Hippel-Lindau disease tumor suppressor gene in human lung cancer cell lines. Oncogene 9, 1599-1604, PubMed ID: 94239735

83 Foster, K. et al. (1995) Molecular genetic analysis of the von Hippel-Lindau disease (VHL) tumour suppressor gene in gonadal tumours. Eur J Cancer 31A, 2392-2395, PubMed ID: 96262884

84 Partridge, M. et al. (1999) Location of candidate tumour suppressor gene loci at chromosomes 3p, 8p and 9p for oral squamous cell carcinomas. Int J Cancer 83, 318-325, PubMed ID: 99426871

85 Zhuang, Z. et al. (1996) von Hippel-Lindau disease gene deletion detected in microdissected sporadic human colon carcinoma specimens. Hum Pathol 27, 152-156, PubMed ID: 96190933

86 Richards, F.M. et al. (1996) Expression of the von Hippel-Lindau disease tumour suppressor gene during human embryogenesis. Hum Mol Genet 5, 639-644, PubMed ID: 96311559

87 Kessler, P.M. et al. (1995) Expression of the Von Hippel-Lindau tumor suppressor gene, VHL, in human fetal kidney and during mouse embryogenesis. Mol Med 1, 457-466, PubMed ID: 96091369

88 Los, M. et al. (1996) Expression pattern of the von Hippel-Lindau protein in human tissues. Lab Invest 75, 231-238, PubMed ID: 96337195

89 Corless, C.L. et al. (1997) Immunostaining of the von Hippel-Lindau gene product in normal and neoplastic human tissues. Hum Pathol 28, 459-464, PubMed ID: 97258775

90 Sakashita, N. et al. (1999) Expression of von Hippel-Lindau protein in normal and pathological human tissues. Histochem J 31, 133-144, PubMed ID: 99343074

91 Koochekpour, S. et al. (1999) The von Hippel-Lindau tumor suppressor gene inhibits hepatocyte growth factor/scatter factor-induced

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

invasion and branching morphogenesis in renal carcinoma cells. Mol Cell Biol 19, 5902-5912, PubMed ID: 99384258
92 Gnarra, J.R. et al. (1997) Defective placental vasculogenesis causes embryonic lethality in VHL-deficient mice. Proc Natl Acad Sci U S A 94, 9102-9107, PubMed ID: 97404355
93 Kanno, H. et al. (2000) Role of the von Hippel-Lindau tumor suppressor protein during neuronal differentiation. Cancer Res 60, 2820-2824, PubMed ID: 20306594
94 Shiao, Y.H. et al. (1998) von Hippel-Lindau gene mutations in N-nitrosodimethylamine-induced rat renal epithelial tumors. J Natl Cancer Inst 90, 1720-1723, PubMed ID: 99043199
95 Iliopoulos, O, Ohh, M. and Kaelin, W.G. (1998) pVHL19 is a biologically active product of the von Hippel-Lindau gene arising from internal translation initiation. Proc Natl Acad Sci U S A 95, 11661-11666, PubMed ID: 98426210
96 Schoenfeld, A, Davidowitz, E.J. and Burk, R.D. (1998) A second major native von Hippel-Lindau gene product, initiated from an internal translation start site, functions as a tumor suppressor. Proc Natl Acad Sci U S A 95, 8817-8822, PubMed ID: 98337999
97 Blankenship, C. et al. (1999) Alternate choice of initiation codon produces a biologically active product of the von Hippel Lindau gene with tumor suppressor activity. Oncogene 18, 1529-1535, PubMed ID: 99200767
98 Iliopoulos, O. et al. (1995) Tumour suppression by the human von Hippel-Lindau gene product. Nat Med 1, 822-826, PubMed ID: 96071567
99 Lonergan, K.M. et al. (1998) Regulation of hypoxia-inducible mRNAs by the von Hippel-Lindau tumor suppressor protein requires binding to complexes containing elongins B/C and Cul2. Mol Cell Biol 18, 732-741, PubMed ID: 98107658
100 Cohen, H.T. et al. (1999) An important von Hippel-Lindau tumor suppressor domain mediates Sp1-binding and self-association. Biochem Biophys Res Commun 266, 43-50, PubMed ID: 20050048
101 Berman, H.M. et al. (2000) The Protein Data Bank. Nucleic Acids Res 28, 235-242, PubMed ID: 20063269
102 Duan, D.R. et al. (1995) Characterization of the VHL tumor suppressor gene product: localization, complex formation, and the effect of natural inactivating mutations. Proc Natl Acad Sci U S A 92, 6459-6463, PubMed ID: 95327666
103 Lee, S. et al. (1999) Transcription-dependent nuclear-cytoplasmic trafficking is required for the function of the von Hippel-Lindau tumor suppressor protein. Mol Cell Biol 19, 1486-1497, PubMed ID: 99108121
104 Groulx, I, Bonicalzi, M.E. and Lee, S. (2000) Ran-mediated nuclear export of the von Hippel-Lindau tumor suppressor protein occurs independently of its assembly with cullin-2. J Biol Chem 275, 8991-9000, PubMed ID: 20187624
105 Azuma, Y. and Dasso, M. (2000) The role of Ran in nuclear function. Curr Opin Cell Biol 12, 302-307, PubMed ID: 20263557
106 Ye, Y. et al. (1998) Subcellular localization of the von Hippel-Lindau disease gene product is cell cycle-dependent. Int J Cancer 78, 62-69, PubMed ID: 98389488
107 Lee, S. et al. (1996) Nuclear/cytoplasmic localization of the von Hippel-Lindau tumor suppressor gene product is determined by cell density. Proc Natl Acad Sci U S A 93, 1770-1775, PubMed ID: 96312487
108 Shiao, Y.H. et al. (2000) The von Hippel-Lindau tumor suppressor targets to mitochondria. Cancer Res 60, 2816-2819, PubMed ID: 20306593
109 Gnarra, J.R. et al. (1996) Post-transcriptional regulation of vascular endothelial growth factor mRNA by the product of the VHL tumor suppressor gene. Proc Natl Acad Sci U S A 93, 10589-10594, PubMed ID: 97008046
110 Chen, F. et al. (1995) Suppression of growth of renal carcinoma cells by the von Hippel-Lindau tumor suppressor gene. Cancer Res 55, 4804-4807, PubMed ID: 96046577
111 Kim, M. et al. (1998) Recombinant adenovirus expressing Von Hippel-Lindau-mediated cell cycle arrest is associated with the induction of cyclin-dependent kinase inhibitor p27Kip1. Biochem Biophys Res Commun 253, 672-677, PubMed ID: 99119067
112 Pause, A. et al. (1998) The von Hippel-Lindau tumor suppressor gene is required for cell cycle exit upon serum withdrawal. Proc Natl Acad Sci U S A 95, 993-998, PubMed ID: 98115862
113 Lieubeau-Teillet, B. et al. (1998) von Hippel-Lindau gene-mediated growth suppression and induction of differentiation in renal cell carcinoma cells grown as multicellular tumor spheroids. Cancer Res 58, 4957-4962, PubMed ID: 99025877
114 Schoenfeld, A.R. et al. (2000) The von Hippel-Lindau tumor suppressor gene protects cells from UV-mediated apoptosis. Oncogene 19, 5851-

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

5857, PubMed ID: 21013283

115 Kishida, T. et al. (1995) Cellular proteins that bind the von Hippel-Lindau disease gene product: mapping of binding domains and the effect of missense mutations. Cancer Res 55, 4544-4548, PubMed ID: 96006196

116 Duan, D.R. et al. (1995) Inhibition of transcription elongation by the VHL tumor suppressor protein. Science 269, 1402-1406, PubMed ID: 95389164

117 Kibel, A. et al. (1995) Binding of the von Hippel-Lindau tumor suppressor protein to Elongin B and C. Science 269, 1444-1446, PubMed ID: 95389173

118 Ohh, M. et al. (1999) Synthetic peptides define critical contacts between elongin C, elongin B, and the von Hippel-Lindau protein. J Clin Invest 104, 1583-1591, PubMed ID: 20056159

119 Feldman, D.E. et al. (1999) Formation of the VHL-elongin BC tumor suppressor complex is mediated by the chaperonin TRiC. Mol Cell 4, 1051-1061, PubMed ID: 20101246

120 Schoenfeld, A.R, Davidowitz, E.J. and Burk, R.D. (2000) Elongin BC complex prevents degradation of von Hippel-Lindau tumor suppressor gene products. Proc Natl Acad Sci U S A 97, 8507-8512, PubMed ID: 20359737

121 Aso, T. et al. (1995) Elongin (SIII): a multisubunit regulator of elongation by RNA polymerase II. Science 269, 1439-1443, PubMed ID: 95389172

122 Aso, T. et al. (1996) The inducible elongin A elongation activation domain: structure, function and interaction with the elongin BC complex. Embo J 15, 5557-5566, PubMed ID: 97051814

123 Kamura, T. et al. (1998) The Elongin BC complex interacts with the conserved SOCS-box motif present in members of the SOCS, ras, WD-40 repeat, and ankyrin repeat families. Genes Dev 12, 3872-3881, PubMed ID: 99088036

124 Kroll, S.L. et al. (1999) von Hippel-Lindau protein induces hypoxia-regulated arrest of tyrosine hydroxylase transcript elongation in pheochromocytoma cells. J Biol Chem 274, 30109-30114, PubMed ID: 99445572

125 Pause, A. et al. (1997) The von Hippel-Lindau tumor-suppressor gene product forms a stable complex with human CUL-2, a member of the Cdc53 family of proteins. Proc Natl Acad Sci U S A 94, 2156-2161, PubMed ID: 97225922

126 Kipreos, E.T. et al. (1996) cul-1 is required for cell cycle exit in C. elegans and identifies a novel gene family. Cell 85, 829-839, PubMed ID: 96279828

127 Pause, A. et al. (1999) Studying interactions of four proteins in the yeast two-hybrid system: structural resemblance of the pVHL/elongin BC/hCUL-2 complex with the ubiquitin ligase complex SKP1/cullin/F-box protein. Proc Natl Acad Sci U S A 96, 9533-9538, PubMed ID: 99380552

128 Lisztwan, J. et al. (1999) The von Hippel-Lindau tumor suppressor protein is a component of an E3 ubiquitin-protein ligase activity. Genes Dev 13, 1822-1833, PubMed ID: 99350324

129 Iwai, K. et al. (1999) Identification of the von Hippel-lindau tumor-suppressor protein as part of an active E3 ubiquitin ligase complex. Proc Natl Acad Sci U S A 96, 12436-12441, PubMed ID: 20006252

130 Tyers, M. and Rottapel, R. (1999) VHL: a very hip ligase. Proc Natl Acad Sci U S A 96, 12230-12232, PubMed ID: 20006215

131 Kamura, T. et al. (2000) Activation of HIF1alpha ubiquitination by a reconstituted von Hippel-Lindau (VHL) tumor suppressor complex. Proc Natl Acad Sci U S A 97, 10430-10435, PubMed ID: 20442392

132 Wada, H, Yeh, E.T. and Kamitani, T. (1999) The von Hippel-Lindau tumor suppressor gene product promotes, but is not essential for, NEDD8 conjugation to cullin-2. J Biol Chem 274, 36025-36029, PubMed ID: 20054495

133 Liakopoulos, D. et al. (1999) Conjugation of the ubiquitin-like protein NEDD8 to cullin-2 is linked to von Hippel-Lindau tumor suppressor function. Proc Natl Acad Sci U S A 96, 5510-5515, PubMed ID: 99254071

134 Clifford, S.C. et al. (1999) Genomic organization and chromosomal localization of the human CUL2 gene and the role of von Hippel-Lindau tumor suppressor-binding protein (CUL2 and VBP1) mutation and loss in renal-cell carcinoma development. Genes Chromosomes Cancer 26, 20-28, PubMed ID: 99369715

135 Duerr, E.M. et al. (1999) Differences in allelic distribution of two polymorphisms in the VHL-associated gene CUL2 in pheochromocytoma patients without somatic CUL2 mutations. J Clin Endocrinol Metab 84, 3207-3211, PubMed ID: 99415629

136 Gorospe, M. et al. (1999) Protective function of von Hippel-Lindau protein against impaired protein processing in renal carcinoma cells. Mol Cell Biol 19, 1289-1300, PubMed ID: 99108102

137 Carmeliet, P. et al. (1998) Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

and tumour angiogenesis. Nature 394, 485-490, PubMed ID: 98361237

138 Dang, C.V. and Semenza, G.L. (1999) Oncogenic alterations of metabolism. Trends Biochem Sci 24, 68-72, PubMed ID: 99198474

139 Huang, L.E. et al. (1996) Activation of hypoxia-inducible transcription factor depends primarily upon redox-sensitive stabilization of its alpha subunit. J Biol Chem 271, 32253-32259, PubMed ID: 97113031

140 Salceda, S. and Caro, J. (1997) Hypoxia-inducible factor 1alpha (HIF-1alpha) protein is rapidly degraded by the ubiquitin-proteasome system under normoxic conditions. Its stabilization by hypoxia depends on redox-induced changes. J Biol Chem 272, 22642-22647, PubMed ID: 97426408

141 Maxwell, P.H. et al. (1999) The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 399, 271-275, PubMed ID: 99279691

142 Ohh, M. et al. (2000) Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel-Lindau protein. Nat Cell Biol 2, 423-427, PubMed ID: 20342796

143 Cockman, M.E. et al. (2000) Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. J Biol Chem 275, 25733-25741, PubMed ID: 20400511

144 Tanimoto, K. et al. (2000) Mechanism of regulation of the hypoxia-inducible factor-1 alpha by the von Hippel-Lindau tumor suppressor protein. Embo J 19, 4298-4309, PubMed ID: 20402336

145 Krieg, M. et al. (2000) Up-regulation of hypoxia-inducible factors HIF-1alpha and HIF-2alpha under normoxic conditions in renal carcinoma cells by von Hippel-Lindau tumor suppressor gene loss of function. Oncogene 19, 5435-5443, PubMed ID: 21010658

146 Ravi, R. et al. (2000) Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev 14, 34-44, PubMed ID: 20107031

147 Zhong, H. et al. (1999) Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res 59, 5830-5835, PubMed ID: 20047531

148 Ohh, M. et al. (1998) The von Hippel-Lindau tumor suppressor protein is required for proper assembly of an extracellular fibronectin matrix. Mol Cell 1, 959-968, PubMed ID: 98315379

149 Mukhopadhyay, D. et al. (1997) The von Hippel-Lindau tumor suppressor gene product interacts with Sp1 to repress vascular endothelial growth factor promoter activity. Mol Cell Biol 17, 5629-5639, PubMed ID: 97415639

150 Pal, S. et al. (1998) Activation of Sp1-mediated vascular permeability factor/vascular endothelial growth factor transcription requires specific interaction with protein kinase C zeta. J Biol Chem 273, 26277-26280, PubMed ID: 98434524

151 Pal, S. et al. (1997) The von Hippel-Lindau gene product inhibits vascular permeability factor/vascular endothelial growth factor expression in renal cell carcinoma by blocking protein kinase C pathways. J Biol Chem 272, 27509-27512, PubMed ID: 98010573

152 Okuda, H. et al. (1999) Direct interaction of the beta-domain of VHL tumor suppressor protein with the regulatory domain of atypical PKC isotypes. Biochem Biophys Res Commun 263, 491-497, PubMed ID: 99423491

153 Datta, K. et al. (2000) Inhibition of insulin-like growth factor-I-mediated cell signaling by the von Hippel-Lindau gene product in renal cancer. J Biol Chem 275, 20700-20706, PubMed ID: 20347162

154 Tsuchiya, H, Iseda, T. and Hino, O. (1996) Identification of a novel protein (VBP-1) binding to the von Hippel- Lindau (VHL) tumor suppressor gene product. Cancer Res 56, 2881-2885, PubMed ID: 96258200

155 Thomas, K.A. (1996) Vascular endothelial growth factor, a potent and selective angiogenic agent. J Biol Chem 271, 603-606, PubMed ID: 96132779

156 Iliopoulos, O. et al. (1996) Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. Proc Natl Acad Sci U S A 93, 10595-10599, PubMed ID: 97008047

157 Siemeister, G. et al. (1996) Reversion of deregulated expression of vascular endothelial growth factor in human renal carcinoma cells by von Hippel-Lindau tumor suppressor protein. Cancer Res 56, 2299-2301, PubMed ID: 96200310

158 Levy, A.P, Levy, N.S. and Goldberg, M.A. (1996) Hypoxia-inducible protein binding to vascular endothelial growth factor mRNA and its modulation by the von Hippel-Lindau protein. J Biol Chem 271, 25492-25497, PubMed ID: 96411767

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
ISSN 1462-3994 ©2001 Cambridge University Press

https://doi.org/10.1017/S1462399401002654 Published online by Cambridge University Press

159 Brown, L.F. et al. (1993) Increased expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in kidney and bladder carcinomas. Am J Pathol 143, 1255-1262, PubMed ID: 94056646

160 Brieger, J. et al. (1999) Inverse regulation of vascular endothelial growth factor and VHL tumor suppressor gene in sporadic renal cell carcinomas is correlated with vascular growth: an in vivo study on 29 tumors. J Mol Med 77, 505-510, PubMed ID: 99402050

161 Jacobsen, J. et al. (2000) Vascular endothelial growth factor as prognostic factor in renal cell carcinoma. J Urol 163, 343-347, PubMed ID: 20067876

162 Wizigmann-Voos, S. et al. (1995) Up-regulation of vascular endothelial growth factor and its receptors in von Hippel-Lindau disease-associated and sporadic hemangioblastomas. Cancer Res 55, 1358-1364, PubMed ID: 95188174

163 Leung, S.Y. et al. (1998) Expression of vascular endothelial growth factor in von Hippel-Lindau syndrome-associated papillary cystadenoma of the epididymis. Hum Pathol 29, 1322-1324, PubMed ID: 99039721

164 Flamme, I, Krieg, M. and Plate, K.H. (1998) Up-regulation of vascular endothelial growth factor in stromal cells of hemangioblastomas is correlated with up-regulation of the transcription factor HRF/HIF-2alpha. Am J Pathol 153, 25-29, PubMed ID: 98328398

165 Claffey, K.P, Wilkison, W.O. and Spiegelman, B.M. (1992) Vascular endothelial growth factor. Regulation by cell differentiation and activated second messenger pathways. J Biol Chem 267, 16317-16322, PubMed ID: 92355593

166 Krieg, M, Marti, H.H. and Plate, K.H. (1998) Coexpression of erythropoietin and vascular endothelial growth factor in nervous system tumors associated with von Hippel-Lindau tumor suppressor gene loss of function. Blood 92, 3388-3393, PubMed ID: 99005369

167 Messinezy, M. and Pearson, T.C. (1999) The classification and diagnostic criteria of the erythrocytoses (polycythaemias). Clin Lab Haematol 21, 309-316, PubMed ID: 20111542

168 Ivanov, S.V. et al. (1998) Down-regulation of transmembrane carbonic anhydrases in renal cell carcinoma cell lines by wild-type von Hippel-Lindau transgenes. Proc Natl Acad Sci U S A 95, 12596-12601, PubMed ID: 98445416

169 Wykoff, C.C. et al. (2000) Hypoxia-inducible expression of tumor-associated carbonic anhydrases. Cancer Res 60, 7075-7083, PubMed ID: 21028110

170 Los, M. et al. (1999) Regulation of the urokinase-type plasminogen activator system by the von Hippel-Lindau tumor suppressor gene. Cancer Res 59, 4440-4445., PubMed ID: 99413505

171 Ananth, S. et al. (1999) Transforming growth factor beta1 is a target for the von Hippel-Lindau tumor suppressor and a critical growth factor for clear cell renal carcinoma. Cancer Res 59, 2210-2216., PubMed ID: 99247570

172 Knebelmann, B. et al. (1998) Transforming growth factor alpha is a target for the von Hippel-Lindau tumor suppressor. Cancer Res 58, 226-231., PubMed ID: 98103634

173 Goldfarb, D.A. (1998) Nephron-sparing surgery and renal transplantation in patients with renal cell carcinoma and von Hippel-Lindau disease. J Intern Med 243, 563-567., PubMed ID: 98345184

174 Wittebol-Post, D., Hes, F.J. and Lips, C.J. (1998) The eye in von Hippel-Lindau disease. Long-term follow-up of screening and treatment: recommendations. J Intern Med 243, 555-561., PubMed ID: 98345183

175 Hagedorn, M. and Bikfalvi, A. (2000) Target molecules for anti-angiogenic therapy: from basic research to clinical trials. Crit Rev Oncol Hematol 34, 89-110., PubMed ID: 20261343

176 Cherrington, J.M., Strawn, L.M. and Shawver, L.K. (2000) New paradigms for the treatment of cancer: the role of anti- angiogenesis agents. Adv Cancer Res 79, 1-38, PubMed ID: 20278617

177 Kim, M. et al. (1999) Cancer gene therapy with a recombinant adenovirus expressing a von Hippel-Lindau tumor suppressor gene. Cancer Gene Ther 6, S1-S2

178 Ciechanover, A. et al. (1994) Degradation of the tumor suppressor protein p53 by the ubiquitin- mediated proteolytic system requires a novel species of ubiquitin- carrier protein, E2. J Biol Chem 269, 9582-9589., PubMed ID: 94193636

179 Hershko, A. and Ciechanover, A. (1998) The ubiquitin system. Annu Rev Biochem 67, 425-479, PubMed ID: 98431658

180 Kamura, T. et al. (1999) The Rbx1 subunit of SCF and VHL E3 ubiquitin ligase activates Rub1 modification of cullins Cdc53 and Cul2. Genes Dev 13, 2928-2933., PubMed ID: 20047893

181 Kamura, T. et al. (1999) Rbx1, a component of the VHL tumor suppressor complex and SCF ubiquitin ligase. Science 284, 657-661., PubMed ID: 99234320

Accession information: (01)00265-4a.pdf (short code: txt001frb); 19 March 2001
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Further reading, resources and contacts

Hodgson, S.V. and Maher, E.R. (1999) A Practical Guide to Human Cancer Genetics (2nd edn), Cambridge University Press, Cambridge, UK

Online sources of general information on cancer and specific information on VHL disease, directed at patients:

Oncolink (University of Pennsylvania Cancer Center)
http://cancer.med.upenn.edu

CancerNET (US National Cancer Institute) and CancerNET UK
http://cancernet.nci.nih.gov

http://www.graylab.ac.uk/cancernet.html

The VHL Family Alliance (Patient Support Group)
http://www.vhl.org

Relevant research databases:

Online Mendelian Inheritance In Man (all known human genetic diseases and the genes associated with them; OMIM Accession No. for VHL disease is 193300)
http://www3.ncbi.nlm.nih.gov/Omim

GenBank (human gene sequences)
http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html

VHL gene mutation database
http://www.umd.necker.fr:2005/

Protein Data Bank at Research Collaboratory for Structural Bioinformatics (protein structure database)
http://www.rcsb.org/pdb/

Online review on ubiquitin-dependent proteolysis
http://www.proteasome.com/proteolysis2000.htm

Institute home page
http://www.bham.ac.uk/ich/genetics.htm

Features associated with this article

Figures

Figure 1. von Hippel–Lindau (VHL) disease predisposes to multiple tumours (fig001frb).
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Figure 6. Summary of interactions and possible functions of the wild-type von Hippel–Lindau protein (pVHL) (fig006frb).

Tables

Table 1. VHL disease types and different germline *VHL* mutations (tab001frb).

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