von Hippel–Lindau (VHL) disease is an autosomal dominant disorder that is associated with various tumors and cysts in the central nervous system (CNS) and other visceral organs. Inactivation of the VHL tumor suppressor protein with loss of function of the VHL protein, and Elongin B, C complex results in a dysfunction of the ubiquitination of hypoxia-inducible factor, which is an important step in the development of highly vascular tumors. The most frequent tumors are hemangioblastoma in the CNS and retina, pheochromocytoma in the adrenal gland, renal cell carcinoma and pancreatic neuroendocrine tumors. In this review, we summarize the recent literatures on the pathogenesis, clinical characteristics, diagnosis and treatment of VHL disease. Progress in molecular diagnosis and molecular targeting therapy is expected to improve the diagnosis and treatment of this disease. Medical, psychological and societal supports for patients with VHL disease and their families and supportive communication among the VHL families are also very important. They have proved to be of benefit for patients with this disease to overcome various social and psychological problems in the US and Europe. Since some drugs targeting the vascular endothelial growth factors or its receptor are undergoing clinical trials, a better prognosis of the tumor in VHL disease can be expected.

Key words: VHL disease – diagnosis and treatment – pathogenesis of tumor development

INTRODUCTION

von Hippel–Lindau (VHL) disease is an autosomal dominant disorder that is characterized by the development of various benign and malignant tumors and cysts. The major tumors and cysts are hemangioblastoma (HB) in the central nervous system (CNS), retinal hemangioblastoma (RA), pheochromocytoma (Pheo), renal cell carcinoma (RCC), cystadenoma and pancreatic neuroendocrine tumors (Fig. 1). The VHL tumor suppressor gene, which is located on chromosome 3p25–26, is responsible for this disease. The VHL gene was identified in 1993 by Zbar et al. by positional cloning (1). Following identification of the VHL gene, there has been remarkable progress in molecular genetics and molecular diagnosis of VHL disease and also in the understanding of the molecular basis of the pathogenesis of VHL-associated disorders. The major cause underlying the development of the disease is inactivation of the VHL tumor suppressor protein and subsequent loss of the function of the VHL protein, and Elongin B, C (VBC) complex. This results in the dysfunction of the ubiquitination of hypoxia-inducible factors (HIF) and other proteins for VBC complex. The failure in the degradation of HIFs is an important step in the development of highly vascular tumors (2). In this review, we summarize the recent views on the molecular pathogenesis, classification and principles of clinical diagnosis and treatment of VHL disease.

HISTORY: THE RECOGNITION OF AN AUTOSOMAL DOMINANT DISEASE AND IDENTIFICATION OF THE VHL GENE

The VHL disease was described in von Hippel’s literature in 1911 and Lindau’s literature in 1926 (3,4). Melmon and Rosen established the notion of the VHL disease in 1964 (5).
The positional cloning for the disease was started by Latif et al. with the accumulation of DNA in VHL families. Identification of the VHL disease gene was published in 1993. The responsible gene was named as ‘VHL tumor suppressor gene’, which is located on the chromosome 3p25–26 (1). Further studies showed that the VHL gene is also inactivated in sporadic RCC, HB and Pheo (2).

MOLECULAR BASIS OF THE PATHOGENESIS OF TUMOR AND CYST IN VHL DISEASE

The VHL tumor suppressor gene has 3 exons and 639 nt. The size of its mRNA is 4.5 kb. This gene encodes 213 amino acids. There are two starting codons at the first exon in the VHL gene. This gene is widely conserved in organisms ranging from Caenorhabditis elegans to humans. The development of VHL-associated disorders is explained by the classic theory of Knudson’s two-hit hypothesis, which postulated that both VHL alleles are inactivated by mutation and deletion of the VHL gene that result in functional loss of the tumor suppressor gene (6). A recent functional study of the VHL gene revealed that mRNA is transcribed from both starting codons (7).

Various tumors, such as HB in the CNS, RA, Pheo, RCC, neuroendocrine tumors in the pancreas, and renal and pancreatic cysts, have been found to develop through the same process as that in the inactivation of the VHL gene and subsequent functional loss of the VBC complex. Molecular structural studies revealed that the VHL protein has an α- or β-domain in the 3' portion or in the middle portion of this gene. The α-domain is functionally important for binding other proteins such as Elongin B, C. The β-domain is necessary for binding target proteins for VBC complex which are further ubiquitinated. Since the VBC complex acts as the E3 ligase for ubiquitination and further proteosomal degradation of target proteins, loss of functional VHL protein results in the accumulation of target proteins, e.g. HIF1α, HIF2α (Fig. 2a) and atypical protein kinase λ (8). HIFs are major transcription factors under the hypoxic condition. They are bound to the β-domain of the VHL protein of the VBC complex. They are usually ubiquitinated and degraded by the VBC complex under the normoxic (= normal O2 pressure) condition. They are not degraded under hypoxic conditions. As this mechanism is turned off with the loss of functional VHL protein, a high level of non-degraded HIF causes increased transcription of VEGF, PDGF and TGF-α (Fig. 2b). These findings explain cell growth and the development of microvascular vessels and accelerated growth in HB, RCC or other VHL-related tumors (2,9).

Clinical review of VHL disease

HIF results in the increased production of tyrosine hydroxylase and subsequent overproduction of catecholamine in VHL-related Pheo (10). Recently, it was shown that dysfunction of the VBC complex also results in the accumulation of atypical protein kinase λ. It also results in the overproduction of B-jun for the inhibition of apoptosis neural crest cells, in the medulla in adrenal gland (Fig. 2c). This is considered to be one of the causes of the development of Pheo (11).

CLINICAL CLASSIFICATION OF VHL DISEASE

VHL was clinically classified into two types of diseases with or without Pheo in the initial classification of this disease (2,12). Those without Pheo are categorized as VHL type 1 disease. Those with Pheo are categorized as VHL type 2 disease. VHL type 2 disease is further classified into three categories: type 2A, type 2B and type 2C. Type 2A VHL has Pheo...
with other HB in the CNS, but not with RCC. Type 2B exhibits Pheo, RCC and other CNS tumors. A recent notion is that type 2C disease has only Pheo, with no other disease (Table 1). Only a few mutations for VHL type 2C have been identified. Chuvash polycythemia is a rare type of the disease, which is caused by VHL gene inactivation at a specific point of the VHL protein, and does not result in a tumor, but rather in polycythemia (13).

**CLINICAL CRITERIA FOR THE DIAGNOSIS OF VHL DISEASE**

The following criteria are used for the diagnosis of VHL disease:

(i) Patients with a family history of developing HB in the CNS or RA, RCC, Pheo or pancreatic tumors or cysts, epididymal cystadenoma.

(ii) Patients without a family history of VHL disease, but who develop HB or RA in combination with other tumors, such as RCC, Pheo, pancreatic tumors or cysts, or epididymal cystadenoma.

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**Figure 2.** (a) VBC complex and its mode of action. Normoxic condition; normal O2 pressure, Ub; ubiquitin, HIF; Hypoxia-inducible factor, pVHL; VHL protein, α: Elongin C binding domain in pVHL, β: β domain (substrate binding domain) in pVHL, CUL2; Cullin2 forms a complex with Elongin B, C and pVHL. (b) Mutation of the VHL gene and resulting upregulation of HIF, VEGF, PDGF and TGF-α. (c) Mutation of the VHL gene and upregulation of aPKCα; aPKCα, Atypical protein kinase C.

**Table 1. Clinical classification in the VHL disease**

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<td>Retinal HB</td>
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Retinal HB, retinal hemangioblastoma; CNS HB, central nervous system hemangioblastoma; RCC, renal cell carcinoma; Pheo, pheochromocytoma.
GENETIC TESTING FOR VHL DISEASE

Once diagnosed, an investigation to identify the mutation in the VHL gene can benefit family members. If the proband’s mutation can be identified, its presence or absence in family members at risk can then define their status. Genetic testing for mutations in the VHL gene requires complete sequencing of the coding regions, Southern blot analysis and fluorescent in situ hybridization (FISH), which has ~70% sensitivity in our laboratory. The remaining 30% is regarded as a large deletion of the exon or the 3’ end of this gene.

CLINICAL CHARACTERISTICS OF THE TUMORS AND CYSTS, DIAGNOSIS AND TREATMENT

HEMANGIOBLASTOMA IN THE CNS

Hemangioblastoma in the CNS develops from childhood at an age of <10 years or early in the teens until the age of 30 years. It develops both as type 1 or type 2A and 2B VHL disease in ~70% of VHL patients. The most common sites for HB development are the cerebellum and spinal cord. The symptoms of this disease are largely explained by the expansion of the tumor in the cranial space or the spinal cord. Early signs and symptoms are back pain, headaches, numbness, dizziness, and weakness or pain in the arms and legs. Polycythemia with overproduction of erythropoietin is associated with a high level of HIFs in tumors. HB is diagnosed by MRI of the brain and the spinal cord (Fig. 3). MRI is recommended for patients over 10 years of age, at least once a year. If the tumors are small and asymptomatic, the patient’s clinical condition should be carefully watched until the appearance of any symptoms. The best treatment for this tumor is surgical resection. If the tumor is difficult to remove from its primary site, it can sometimes be treated with the gamma knife. The long-term outcome with the gamma knife treatment is still not certain, however. Most of the VHL patients have experienced operations for HB on several occasions (N. Shinohara, Personal communication). Recently, it is becoming clear that HB and its postoperative morbidity are major causes of physical disability in VHL disease (T. Shuin, in preparation).

RETINAL HEMANGIOBLASTOMA

Retinal hemangioblastoma develops from an age of <10 years until the age of 30 years. It shows a similar age of development as HB in the CNS. After this age, the frequency of development of RA decreases gradually. It occurs both as VHL type 1 and VHL type 2 diseases. Usually only one tumor develops in one eye. It does not show any symptoms in most of the patients (Fig. 4). In the US and Europe 70% of VHL patients have RA. Japanese VHL patients have a lower incidence of <40%
(T. Shuin, in preparation). RAs are usually detected by ophthalmoscopy or fluorescent angiography by experienced ophthalmologists. Screening examinations of the retina must be carried out at least once a year, beginning at an early age after birth. RA is pathologically the same as HB in the CNS. It is treated by laser photo-coagulation if small. In our recent survey, some patients lost their vision due to the late diagnosis of RA. Sometimes tumors are too large to be treated with any other methods than by entire removal of the affected eye.

PHEOCHROMOCYTOMA

Pheochromocytoma develops in the adrenal gland or paraganglia as the type 2 VHL disease, which accounts for 10% of VHL disease cases (Fig. 5). It developed at different ages ranging from <10 years to >40 years in our survey. Previous reports showed that it has specific point mutations in the VHL gene. Some are detected at exon 3 at a high frequency, often located at or near the binding region of Elongin C. Pheo should be removed surgically. Since it is possible to diagnose small size Pheo before the appearance of major symptoms depending on the medical technique employed, they may be removed by laparoscopic technique with low morbidity. Since Pheo in the adrenal gland is usually diagnosed as a single tumor, it is preferable to remove only the small tumor itself and to retain the rest of the normal adrenal gland in order to conserve the normal adrenal function. Follow-up of this tumor must be conducted in patients less than 10 years of age with CT scan once a year, especially for those with a family history of the type 2 VHL disease. Pheo is often observed at an early age less than 5 years (T. Shuin, in preparation).

RENAL CELL CARCINOMA AND RENAL CYST

RCC develops from around the age of 20 until 50 years as the type 1 and type 2B VHL disease. It rarely develops in those under 20 years of age. Tumors develop at multiple sites in the kidney (Figs 6 and 7). If the tumors are <3 cm in diameter, careful observation of tumors without surgery is preferable. Radical nephrectomy is not recommended for the RCCs in VHL disease. They usually grow 0.5 cm in diameter in a year (12). Patients with RCC must be examined by CT scanning or MRI once a year. If one of the tumors in the kidney is more than 3 cm in diameter, all tumors should be removed by enucleation or partial nephrectomy. Recently, percutaneous radiofrequency ablation or cryosurgery is often performed to ablate the tumors. Tumors are heated up to 60°C or frozen down to −180°C with these techniques. Functions in the affected kidneys are conserved more easily with percutaneous radiofrequency ablation or cryosurgery than the operations performed by open surgery with nephron-sparing techniques. There are still several problems related to the effectiveness of these techniques depending on the site of the tumors. Even though patients do not have RCC, if their relatives have it, they must be checked once a year with CT scan before the age of 20 years. Surgical resection is not recommended for any renal cyst with no tumor inside (N. Shinohara N, Personal communication).

PANCREATIC CYSTS AND ISLET CELL (NEUROENDOCRINE) TUMORS IN THE PANCREAS

Patients with pancreatic cysts or cystadenomas are usually asymptomatic (Fig. 7). These cysts sometimes cause expansive pain inside the pancreas. Multiple cysts in the pancreas are often observed as a characteristic image in the CT scan. Islet cell tumors in the pancreas are often malignant and metastasize to regional lymph nodes. These tumors secrete various neuroendocrine substances. When their presence is diagnosed, partial resection of the pancreas is recommended depending on the site of the tumor. Even if they metastasize to the lymph nodes, the prognosis of this tumor is expected to be relatively good [(14), I. Yamasaki, manuscript in preparation].
The prognosis of the VHL disease was once regarded to be determined by the outcome of the treatment for RCC. RCC is the main tumor that metastasizes to other organs in VHL disease. Since the treatment of RCC in VHL disease has changed with the emergence of nephron-sparing surgery and other non-surgical techniques, such as radiofrequency ablation, it is becoming a curable condition with less invasive approach. Morbidity resulting from postoperative complications is caused by the treatment of (spinal cord) HB. Representative of those are several neurological complications, such as paraplegia, and sensory and motor disturbances.

**Recent Antiangiogenic Therapy for VHL-related Tumors**

Recently, clinical trials of antiangiogenic therapies have been performed for the treatment of RCC using new drugs. Thalidomide with interferon or SU5416 alone was shown to stabilize tumor growth in RCC, but not to reduce its size (15,16). Other molecular targeting drugs designed as inhibitors of VEGF-receptor kinase are undergoing clinical trials. These drugs are yielding very promising results in clinical studies, showing more than 40% cases of partial response and up to 90% cases of partial response plus stable disease (17,18). Some inhibitors designed specific for HIFs may also be developed in the future.

**Future Prospects and Need for Governmental Support**

(i) **Supportive Activity by the VHL Family Alliance in the World**

There is a growing trend to organize familial support groups for VHL disease. They constantly maintain mutual cooperation for both the VHL patients and their families. Familial support groups were small in number at the beginning. For example, the VHL Alliance in USA was composed of only a few families at the 1st VHL symposium held in 1994. This increased to more than 2000 members consisting of patients and their families at the time of the 4th VHL symposium in the Mayo Clinic, Rochester, USA, in 2000. The organization is now worldwide. VHL family alliances act in the US, Canada, England, France, Germany, Italy and Japan (19). They have homepages to show their activity for VHL disease (20). They provide a reference handbook for VHL patients. They also maintain a database of information about doctors in the world who understand the specific features of VHL disease. They also donate some of their surgically removed tumors for research designed to improve understanding of VHL disease.

(ii) **Need for Governmental Support**

VHL is a lifetime disease. Patients need to be constantly checked for the tumors and cysts that develop at various sites in the CNS and visceral organs throughout his/her
lifetime. Some patients even receive up to 20 surgical operations in their lifetime to remove tumors. The number of VHL patients in Japan is less than 1000, and the number of their families is less than 200. Each patient constantly suffers from problems caused by multiple tumors or cysts from various organs. Older patients who have received multiple operations have the serious problem of postoperative morbidity in the CNS and visceral organs. A hopeful prospect for this disease is the appearance of molecular targeting antiangiogenic drugs in the near future. They seemed to have shown considerable efficacy in the initial clinical trials (17,18). It is also highly recommended that this disease be included as one of the intractable disease (‘Nanbyo’) by the Welfare and Labor Ministry in Japan. Although this process may take a long time, inclusion will encourage VHL patients in Japan. Patients with this disease must be cared for by well-trained specialists and genetic counselors throughout their life to improve the prognosis and their psychological conditions caused by the above-mentioned conditions.

References