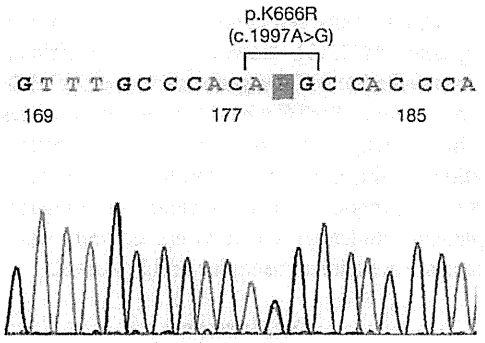

Fig. 2 *RET* mutation analysis
A missense mutation was identified in codon 666 (c.1997A>G, p.K666R).

the genotype-phenotype correlation is identified [2-3, 10]. To date, more than 200 *RET* mutations have been reported. In patients with MEN2A, the mutations occur mainly in codons 609, 611, 618, and 620 in exon 10 or in codon 634 in exon 11, whereas in those with MEN2B the mutations are seen in exon 16 (M918T) and less often in exon 15 (A883F). The mutations in individuals with FMTC affect extracellular cysteine codons in exon 10 or intracellular codons other than A883 and M918 [5]. Codon 666 is known to be related to intracellular juxtamembrane domain of RET and is a highly conserved residue among species [11]. Some missense mutations in this codon have been reported, as shown in Table 1. Ahmed *et al.* [12] identified a variant c.1996A>G (p.K666E) in a total of 15 individuals in three unrelated families. Among these, eight had clinical manifestations of either MEN2A or FMTC, such as pheochromocytoma, MTC, C cell hyperplasia, and positive pentagastrin stimulation test. Barrello *et al.* [13] found the same germ-line transition in heterozygosity in a 48-year-old woman and her sisters. *In vitro* transfection study to confirm the NIH3T3-transforming ability revealed that the variant had oncogenic potential, enhanced by the presence of the functional non-oncogenic polymorphism G691S. Romei *et al.* [14] reported a different variant, p.K666M, in 250 families with hereditary MTC followed up in 20 different Italian centers, with an overall prevalence of 0.4% (1/250). Muzza *et al.* [11] demonstrated a mutation, c.1998G>T (p.K666N), in a 65-year-old MTC patient without MEN2A features. *In vitro* assay using HEK-293 cells transfected with a plasmid carrying the *RET* variant p.K666N showed high kinase and transforming activities. Moreover, a significant alteration of the transmembrane α-helix was predicted by mapping of the mutant in the intracellular juxtamembrane domain related to codon 666. Mastroianno *et al.* [15] reported two germline mutations, K666M of *RET* and IVS4+1G>T, located in the splice donor site of *MEN1* Exon 4 in a 45-year-old proband. Four relatives carried the same *RET* mutation, eight had the same *MEN1* mutation, and four had both mutations. Three individuals with *RET* mutation alone developed C cell hyperplasia and one was asymptomatic. Among those with MEN1 mutation alone, six had PHPT, three had insulinoma, one had Zollinger–Ellison syndrome, one had glucagonoma, one had pituitary tumor, and two had skin tumors. In cases with both mutations, the following manifestations appeared: MTC (n=2), PHPT (n=3), gastrinoma (n=1), pituitary tumor (n=2), Cushing syndrome (n=1), carcinoid (n=2), papillary thyroid carcinoma (n=2), and skin tumors (n=1). Therefore, missense mutations in codon 666 are in the minority, but play a pivotal role in tumor development. To our knowledge, c.1997A>G (p.K666R) is a new novel mutation that is pathogenic in C cell transformation. While total thyroidectomy is strongly recommended in

Table 1 Missense mutation in *RET* codon 666

Mutation	Age/gender	C cell status	Comorbidity	Reference
c.1996A>G (p. K666E)	35y/M and 4 relatives	MTC	Pheochromocytoma, hypertension	[12]
c.1996A>G (p. K666E)	12 family members	MTC, C cell hyperplasia, positive pentagastrin test		[12]
c.1996A>G (p. K666E)	64y/M and 1 relative	MTC		[12]
c.1996A>G (p. K666E)	48y/F	MTC, C cell hyperplasia		[13]
c.1996A>T (p. K666M)				[14]
c.1998G>T (p. K666N)	65y/F	MTC		[11]
c.1996A>T (p.K666M)	45y/M and relatives	C cell hyperplasia, MTC	8 relatives: positive *MEN1* mutation 4 relatives: positive *RET* mutation Proband and 3 relatives: positive both mutations	[15]
c.1997A>G (p.K666R)	38y/F	MTC		Present case

patients harboring *RET* mutations, simultaneous parathyroidectomy with autotransplantation at the primary surgery of MTC is controversial. There were two reasons for the choice of total parathyroidectomy with autotransplantation in the present case. One is that the possibility of having MEN2A could not be excluded in the presence of SIPTH. Another is that identification of the parathyroid glands was anticipated to be difficult during operation for PHPT occurring after thyroid surgery for MTC or reoperation for locally recurrent MTC. Further discussion on whether this approach is proper or not may be required.

In the management guidelines, missense mutations in codon 666, which lead to the development of MEN2A or FMTC, are positioned at risk level A, or lowest risk of aggressive MTC [3]. However, it is often difficult to determine whether MTC is generated as part of MEN2-related disease or FMTC when it is a unique manifestation. In addition, it is still not clear whether all missense mutations in this codon will lead to the same clinical course and prognosis. Further careful observation of the patient's clinical presentation is absolutely required to elucidate the clinical behavior of this variant.

Disclosure

None of the authors have any potential conflicts of interest associated with this research.

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Review Article

Retinal Capillary Hemangioma in von Hippel-Lindau Disease: Current Concept, Diagnosis and Managements

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Abstract

von Hippel-Lindau (VHL) disease is caused by a mutation in the VHL gene, resulting in the functional disorder in VHL-encoded protein (pVHL). Recent advances in experimental and clinical studies on VHL gene/protein and VHL disease have provided novel concepts in molecular pathology and clinical managements. pVHL plays a critical role in the regulation of hypoxia inducible factor (HIF)-dependent as well as HIF-independent signaling pathways. These mechanisms should underlie the pathogenesis of VHL-related retinal vascular tumors. It is still controversial whether the histological term "hemangioma" vs "hemangioblastoma" should be appropriate in calling retinal vascular tumors of VHL disease. Recent clinical studies have proved efficacy of various therapeutic options depending on the location of retinal tumors between peripheral and optic disc/juxtapapillary hemangioma. Long-term follow-up observation can be achieved in VHL disease patients, showing favorable outcomes of conventional standard treatments. However, there still exists a population suffering from irreversible severe visual disturbance even though the conventional treatments had been performed enough. Further challenging of molecular targeting therapy as well as development of vitreoretinal surgeries and gene/protein transfer technique may contribute to preservation of the patients' vision in the future.

INTRODUCTION

von Hippel-Lindau (VHL) disease is a rare hereditary (1/36,000 live births) autosomal dominant syndrome [1]. VHL disease is manifested by a range of different benign and malignant tumors, including hemangioma/hemangioblastoma of the retina and central nervous system, renal cell carcinoma, pheochromocytomas, pancreatic carcinoma, and cysts in the kidneys, liver, and pancreas [2]. There are two different clinical patterns for diagnosis of the disease: 1) patients with a positive

history of developing retinal hemangioma as well as systemic tumors such as renal cell carcinoma, pheochromocytoma, pancreatic tumors or cysts, epididymal cystadenoma associated with VHL, and 2) patients without a family history of VHL who present with retinal hemangioma in combination with other various tumors [3]. VHL is an age-dependent and highly penetrant disease with the more common manifestations being retinal hemangiomas. VHL-associated retinal hemangioma occurs in over 60% of the patients [4], and is the first manifestation of the disease in 43% of patients [5]. On the other hand, about half of retinal hemangiomas are related to VHL [6]. However, it remains unclear whether retinal capillary hemangioma that occurs without a family history is most commonly sporadic or most commonly represents an initial manifestation of VHL [7,8].

It is indisputable that biotechnology and molecular science have developed dramatically during a couple of decades. Indeed, long-term follow-up cases have been accumulated according to recent literatures. These backgrounds certainly provide an opportunity to reconsider the previous concept and clinical aspects of the disease. In this review, the current development of molecular pathological studies, clinical characteristics, histopathology, differential diagnosis and managements are included and discussed.

Recent advances in molecular pathology of VHL

VHL disease is caused by a mutation in the *VHL* gene [2], resulting in the functional disorder in VHL-encoded protein (pVHL). Originally identified as a tumor suppressor, the pVHL is now known to repress expression of mRNAs that are normally induced under hypoxic conditions [9]. The hypoxia-inducible factor (HIF) is a key transcription factor responsible for up-regulation of various hypoxia-inducible genes' expression. When mutated, the *VHL* gene produces a protein that is unable to regulate HIF, permitting accumulation of HIF and subsequent activation of vascular endothelial growth factor (VEGF) and other hypoxia-inducible genes [10]. This can result in growth of retinal hemangiomas and other tumors associated with VHL disease. pVHL is a substrate recognition component of an E3-ubiquitin ligase that rapidly destabilizes HIF-alpha under normoxic, but not hypoxic, conditions. Thus, pVHL is known to be regulating angiogenic factors through HIF-alpha depending on tissue oxygen concentration. Chemokines are a group of structurally related secretory and transmembrane proteins whose major tasks are to coordinately recruit various leukocyte populations into target tissue sites via specific receptors. Chemokine receptor CXCR4 is reported to be down-regulated by the pVHL and upregulated by HIF [11].

Apart from HIF dysregulation, further conditions that favor tumorigenesis may be related to both non-functional or absent pVHL in the cell. VHL-deficient cells lose the ability to exit the cell cycle, making it the initial step in VHL tumorigenesis. Ultimately, cells lacking pVHL are deficient in assembly of an extracellular fibronectin matrix or in regulating growth arrest mediated by cell-extracellular matrix signaling [12,13]. To clarify how the HIF-independent mechanisms by pVHL underlie pathogenesis of retinal hemangioma is a future issue.

Recent *in vivo* studies have shown that VHL gene is essential for normal development of the retinal tissue and vitreoretinal vasculature. Kurihara et al. generated retina-specific conditional-knockout mice for VHL (Vhl(alpha)(-CreKO) mice. These mice exhibit arrested transition from the fetal to the adult circulatory system, persistence of hyaloid vessels and poorly formed retinal vessels. These defects are suppressed by intraocular injection of FLT1-Fc protein [a VEGF receptor-1 (FLT1)/Fc chimeric protein that can bind VEGF and inhibit its activity], or by inactivating the HIF-1alpha gene. These suggest that not only macrophages mediating programmed cell death, but also tissue oxygen-sensing mechanisms regulate the transition from the fetal to the adult circulatory system in the retina [14]. VHL also plays a crucial role in the tissue maintenance of the neural retina as well as retinal pigment epithelium (RPE). Lange et al. demonstrated that VHL-dependent regulation of HIF-1alpha in the RPE is essential for normal RPE and iris development, ocular growth and vascular development in the anterior chamber, whereas VHL-dependent regulation of other downstream pathways is crucial for normal development and maintenance of the retinal vasculature [15].

Clinical features of retinal capillary hemangioma

Ocular VHL disease typically occurs as retinal capillary hemangioma found either in the peripheral retina and/or the optic disc/juxtapapillary region (Figure 1). Retinal hemangioma originates from the inner, midperipheral retina and usually grow, causing visual impairment due to leakage leading to various secondary changes in the eye [16]. Approximately one-third of patients have multiple retinal capillary hemangiomas, while two-third have isolated tumor [4]. The most common clinical finding of these retinal hemangiomas is a highly vascularized tumor in the superotemporal region of the retina [17]. The coloration of the vascularized tumors is commonly found as reddish, while orange-yellow tumors can be seen [18]. These tumors are often endophytic and peripheral spherical masses classically associated with a dilated tortuous feeding artery and a draining vein. Usually, two or three tortuous feeding vessels with dilatation are accompanied with the peripheral tumors. Even in case of peripheral retinal hemangiomas, coloration of the optic disc can be reddish, and the optic disc margin may be unclear (Figure 1) in the same eye. Alternatively, retinal hemangioma may

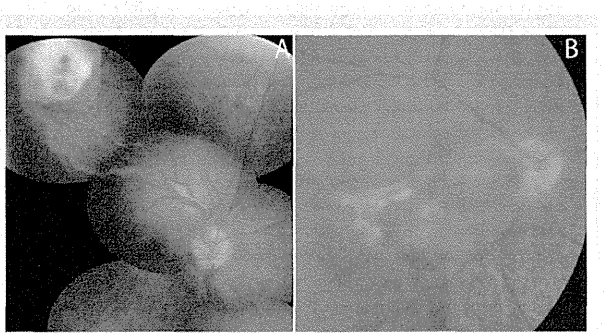

Figure 1 Fundus photograph of two von Hippel Lindau Disease patients with retinal capillary hemangioma in the temporal periphery (A), and juxtapapillary hemangioma (B) of the right eye. Two dilated vessels emanate from the tumor (A). In contrast, no dilated feeder vessels are noted in juxtapapillary hemangioma (B). (B: Case courtesy of Dr Hiroshi Yoshikawa).

be exophytic, and arise from the outer retinal layers. Exophytic tumors are not usually associated with arteriovenous shunting. They tend to develop in the juxtapapillary region (Figure 1), and are frequently misdiagnosed as papilledema, choroidal neovascularization or tumors of RPE or choroid.

Approximately one quarter (22%) of eyes demonstrated de novo ocular involvement, with 18% demonstrating new retinal hemangiomas in a peripheral retinal location only, 2% demonstrating new tumors in a juxtapapillary location only, and 2% demonstrating new tumors in both juxtapapillary and peripheral locations [19]. These anatomical features between peripheral and optic disc/juxtapapillary tumors may reflect on different clinical course and therapeutic approach. In a recent cross sectional study, vision loss in 335 patients with VHL-associated retinal hemangiomas more likely occurred when the lesions were in the juxtapapillary region [20]. Severe vision loss in the affected eyes was also related to the patients' age, the number and size of tumors located in the periphery [20]. The study also showed that although bilateral involvements are common, the rate of bilateral visual impairment is less common due to the asymmetric disease burden. However, the tumor can still lead to blindness and the rate of significant morbidity in one eye remains high. Among eyes with ocular VHL disease at baseline, 88% did not demonstrate retinal hemangiomas in a new retinal location, 70% remained stable in the number of retinal hemangioma, and 79% remained stable in the extent of the tumor involvement. Mean visual acuity for all study eyes decreased by 5.1 ± 0.6 letters across follow-up, with 16.1% of the eyes decreasing by more than 10 letters in visual acuity. Among eyes affected at baseline, greater vision loss was associated with the presence of juxtapapillary hemangioma, development of retinal hemangioma in a new location, and increase in peripheral hemangioma number and extent [19].

Secondary changes of the disease usually entail exudative or tractional effects surrounding the tumor [21]. Exudation occurs in 25% of cases, of which 10% is intraretinal exudation and a further 16% can cause retina detachment. Fibrovascular proliferation secondary to peritumoral ischemia in the 9% of cases produces tractional retinal detachment, macular ectopia, epimacular proliferation and traction leading to rhegmatogenous retinal detachment [22].

Of all the ancillary tests available to detect retinal capillary hemangioma, fluorescein angiography (FA) is the most informative diagnostic tool because of the vascular nature of the tumor. The retinal tumor has fine capillary filling, which rapidly becomes homogeneous. The draining vein becomes prominent in the venous phase, while the tumor demonstrates progressive hyperfluorescence with late leakage of dye into the surrounding structures [4]. Optical coherence tomography (OCT) is useful in diagnosis of the exophytic retinal hemangioma [23] despite the presence of the juxtapapillary or peripheral region [24], in detecting subretinal fluid, and in monitoring the response to treatments [25].

Histopathology of surgically excised retinal tissues

Whether we should use terms "hemangioma" or "hemangioblastoma" in calling retinal vascular tumors has yet to be determined. Hemangioblasts are the multipotent precursor cells that can differentiate into both hemopoietic and endothelial cells [26]. Principally, the term "hemangioblastoma" should be used in tumorigenesis of the hemangioblasts. Recently, isolated erythropoietin-positive cells, indicative of developmentally arrested hemangioblasts, were detected in retinal vascular tumor associated with VHL [27,28], suggesting that the retinal tumor may be closely associated with the term "hemangioblastoma". On the other hand, when the term "hemangioblastoma" is used, the tumor cells morphologically should have nuclear atypia and dense cell proliferation with some undifferentiated changes, malignancy and/or malignant potential rather than "hemangioma". For example, these tendencies are clearly seen in histology of retinoblastoma, which is totally different from that of retinoma [29]. As described below, the tumor cells do not present with nuclear atypia, mitotic figure, or high cellularity in case of retinal vascular tumor in VHL disease. These findings are marginally different from cerebellar hemangioblastoma in VHL. In order to prove this, comparative studies on histopathology will be needed between the retinal and cerebellar vascular tumors of VHL disease. Although traditionally the vascular tumor in VHL disease has been recognized as hemangioblastoma, various authors have recommended that capillary hemangioma rather than hemangioblastoma be used to describe vascular tumors in VHL disease [4], based on histological findings. Therefore, in this review, the term "hemangioma" has been used in retinal vascular tumor observed in VHL disease.

Histologically, the basic lesion is a capillary hemangioma, but not cavernous hemangioma, in vascular tumors of the retina in patients with VHL. The tumor, a capillary hemangioma, is composed of endothelial cells, pericytes, and stromal cells. It is likely that the hemangioma replaces full-thickness of the retina. Retinal hemangioma reveals a variety of vacuolated "foamy" stromal cells reside among the thin capillary-like channels [30]. Morphologically, the stromal cells can present with characteristic pale, polygonal cells (Figure 2). The stromal cells appear foamy due to the presence of phagocytosed lipids produced by leaking capillary endothelium. The lipid is plasma-derived cholesterol stearate [31]. In addition to these typical histological findings, Chan and associates have identified sporadic tumorlet-like cells characterized by small cellular clusters, which form angiomesenchymal islands in optic disc hemangioma of VHL disease [28,32]. Immunohistochemical studies show that the

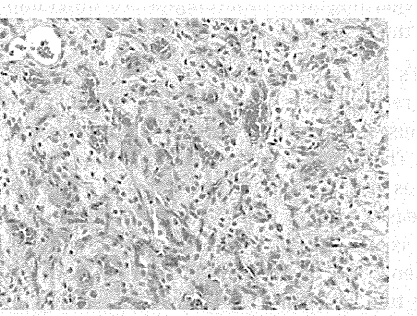

Figure 2 Histological finding of retinal hemangioma in a patient with von Hippel Lindau Disease (Case courtesy of Professor Akito Hirakata). Note collection of marked microvessels without nuclear atypia admixed with foamy stromal cells.

foamy stromal cells reveal positive reaction for glial fibrillary acidic protein, which appear to be of glial origin. However, the stromal cells also showed positive for neuron-specific enolase (NSE), indicating that the stromal cells are not originated from glial cells only. Some of the stromal cells stained positively for inhibin alpha [27]. These results suggest that stromal cells in retinal hemangiomas are neuroectodermal in origin with immunohistochemical features. Miyazawa et al. recently demonstrated that the NSE-positive stromal cells expressed VEGF protein in retinal hemangioma. Chan CC et al. showed expression of VEGF, and HIF, as well as several stem cell markers including erythropoietin, erythropoietin receptor, and CD133, in human retinal and juxtapapillary hemangioma in VHL disease [28,33,34]. In contrast, CD117, the stem cell factor receptor, was not expressed in retinal tumor cells of VHL disease, suggesting that the tumor may not have myeloid/neural crest lineage [28]. Recently, Liang et al. demonstrated CXCR4 immunoreactivity in the cytoplasm and nuclei of the stromal and vascular cells in retinal hemangiomas of VHL cases, whilst CXCL12 was negative in the retina [35]. They also showed that gene expression of VEGF and CXCR4 was highly detected in retinal hemangioma tissues. The VHL gene deletion may be restricted to the stromal cells, suggesting that the stromal cells are the neoplastic component in retinal hemangiomas, and induce the associated neovascularization [36]. Further pathological examinations will definitely characterize the morphology and biological features in hemangioma/hemangioblastoma.

Differential diagnosis

Primitive retinal vascular abnormalities are benign conditions of the retinal circulation that comprise vascular tumors and telangiectasias. Retinal vascular tumors include not only retinal capillary hemangioma, but also vasoproliferative retinal tumors (VPRTs), cavernous hemangioma of the retina, and racemose hemangiomatosis of the retina or Wyburn-Mason syndrome [25]. Of particular importance, many of the vascular tumors of the retina have significant associations with systemic disease. As ocular symptoms are often the most common presenting disease manifestation, the ophthalmologist plays an important role in accurate and early diagnosis [37]. Especially, recent several researchers have conducted pathological and immunohistological analyses in patients with VPRT. Therefore, clinical and molecular differences in VPRT from retinal hemangioma are discussed in this review; otherwise, please refer to recent and previous review articles regarding differential diagnosis of other important retinal vascular tumors [4,25].

VPRTs are benign glial and vascularized tumors of the sensory retina (Figure 3), located at the temporal periphery. VPRT causes retinal neovascularization or exudative retinal changes. The tumors are associated with no large retinal feeder vessels, as observed in retinal hemangiomas associated with VHL disease. Approximately 75% of cases are idiopathic and the rest is secondary to retinitis pigmentosa, chronic retinal detachment, and Coats disease. Patients with VPRTs have no systemic tumors such as VHL disease Laser photocoagulation or cryotherapy is commonly performed for these tumors [38]. Plaque radiotherapy, photodynamic therapy (PDT), or pals plana vitrectomy might be performed when the tumors do not respond to these procedures [39]. The histology of the tumor

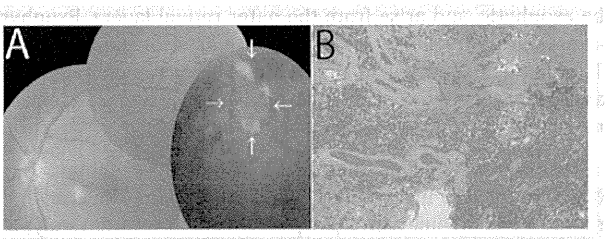

Figure 3 Fundus photographs (A), and histological findings (B) of the surgically excised vasoproliferative retinal tumor tissue.

Fundus photograph shows elevated reddish retinal tumor at the temporal mid-periphery (arrow), involving neovascularization on the surface (A). Light micrograph demonstrates a correction of small vessels with thickened vessel walls and marked hyalinization. Spindle-shaped stromal cells are intermingled in the tumor. (hematoxylin-eosin, original magnification x200).

demonstrated a correlation between small vessels with a thickened vessel wall and marked hyalinization, where glial cells are intermingled (Figure 3). The pathological findings in the abnormal vessels are different from those in retinal hemangioma of VHL disease. Liang et al. demonstrated that there was no immunoreactivity against VEGF or CXCR4 in VPRT [35], whereas we have shown immunoreactivity for VEGF in tumor tissues of VPRT [40]. Although the immunohistochemical results are still controversial, anti-VEGF antibody therapy is basically effective for patients with VPRT [40]. Loss of heterozygosity of VHL genes is detected with microsatellite marker D3S1110 in retinal tumor tissues coming from VHL disease but not in VRPT [35].

Managements of peripheral retinal hemangioma

The universal goal in the treatment of retinal hemangioma is preservation of visual acuity and the visual field without destruction of the function of the retina around the tumor. In order to achieve the therapeutic goal, it may be a significant process to reduce tumor volume via sclerosis of dilated feeder vessels. A study showed that patients with smaller lesions (less than 1.5 mm) were more likely to remain stable. Those that progressed in this group were well controlled with standard therapies including cryotherapy and/or photocoagulation [41]. Hence, retinal hemangiomas are generally treated with cryotherapy or laser photocoagulation, and patients receive a 72% and 74% success rate, respectively [41]. Laser treatment is sufficient in small peripheral tumors and cryotherapy could be carried out in patients with large retinal tumors. We herein demonstrate a VHL case of peripheral retinal hemangioma treated with laser photocoagulation (Figure 4). A twenty-year-old female complained of blurred vision in both eyes. She had a medical history of cerebellar and spinal cord hemangioblastomas. Her father had been diagnosed with VHL disease. At the age of 23, visual acuity became no light perception in her right eye due to retinal detachment although she received several times of laser photocoagulation. In contrast, she also received laser photocoagulation to the small retinal tumors for 21 times in total, which has been keeping favorable vision over 20 years (Figure 4). In addition to those therapies, Kreusel et al. conducted retrospective study including 25 retinal capillary hemangiomas of 24 patients treated with brachytherapy using 106-ruthenium/106-rhodium plaques. Of 25 tumors, 23

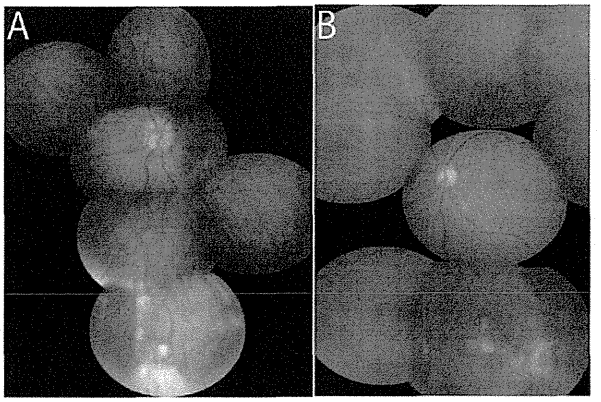

Figure 4 Fundus photograph before and after laser photocoagulation in a patient with VHL disease.

A 21-year-old female shows retinal capillary hemangioma at the inferior periphery (A) before treatments. She eventually has received laser photocoagulation 21 times at outpatient ward. At the age of 44, fundus delimits the tumor lesion with regression of the feeding vessels (B).

hemangiomas could be destroyed by single brachytherapy. They also concluded that a favorable outcome could be expected if a hemangioma's diameter is 5.0 mm or smaller and if there is no preoperative exudative retinal detachment [42]. External beam radiotherapy has been also shown to be useful when standard therapy has not prevented progression [43]. Palmer et al. have found proton-beam irradiation to be an efficacious and safe treatment for large retinal hemangiomas, measuring more than 3 mm, and for cases complicated by exudative retinal detachments or for tumors involving the optic nerve [44]. However, there may exist a problem if the tumor recurs. In such cases, it may be hard to conduct additional radiotherapy to the eye. Combination therapies including ruthenium plaque radiotherapy, cryotherapy, and PDT can be required to induce complete tumor regression and sclerosis of the dilated vessels [18] in selected cases.

Anti-VEGF agents are also candidates for retinal hemangiomas in VHL patients. There are two ways for administration of anti-VEGF agents to the human body: systemically and intravitreously. Intravitreal injections of anti-VEGF therapy (pegaptanib) may decrease retinal thickening minimally and reduce retinal hard exudates in some patients with advanced hemangiomas in patients with VHL [45]. However, the efficacy of agents in this class such as VEGF receptor inhibitor SU5416, and anti-VEGF agents including bevacizumab, ranibizumab and pegaptanib are uncertain [46-50].

Interferon (IFN)-α has an established role in cancer therapy in some cancer types such as hairy cell leukemia and melanoma. Niemela et al. reported that recombinant human IFN-α-2a (Roceron-A; Roche) was injected subcutaneously into VHL patients at a dose of 3×10^6 IU, 3 times/week for 12 months. There was a transient decrease in size and fluorescein leakage from the retinal hemangioma during the therapy. They concluded that IFN-α-2a might decrease blood flow in hemangiomas as suggested by shrinkage and diminished leakage of retinal hemangiomas [51].

On the other hand, larger tumors may have been shown to be non-responsive to medical treatments. A retrospective study of patients showed that for lesions between 7–9 mm, surgical resection of retinal tumors improved visual acuity or kept it the same [35,52]. Therefore, surgical resection of the tumor should be considered for patients with large retinal hemangiomas. The surgery should consist of pars plana vitrectomy, argon endolasing of the feeder vessels, endodiathermy of the vascular lesion, artificial posterior vitreous detachment formation, and filling of the vitreous cavity with silicone oil [35]. Expected intraoperative or postoperative complications include cataract, hemorrhage during resection, epiretinal membrane, and intraoperative retinal breaks, and recurrent retinal detachments [52]. Bimanual technique is useful to reduce intraoperative bleeding and to resect tumor tissues safely during vitrectomy [27].

Managements of optic disc/juxtapapillary hemangioma

Ophthalmologists may choose observation unless the associated visual impairments happen in patients with optic disc/juxtapapillary hemangioma, because overtreatments may lead to an irreversible optic nerve disorder. Instead, the treatments should be considered if the optic disc tumors complicate serous retinal detachment and retinal exudation formation in the macula, and subsequent visual disturbance. Laser photocoagulation may be applied if the optic disc is completely covered with the tumor, which should be confirmed using FA. Even though the tumor partially involves the optic disc, laser may be possible in case of cooperative patients to the treatments, and favorable vision fixation during laser irradiation. Otherwise, the laser photocoagulation should be avoided.

PDT can be effective in reducing macular edema associated with retinal hemangioma; however, this does not always correspond with an improvement in visual acuities especially for juxtapapillary tumors, which is more characteristic for VHL-positive patients [53]. Reynolds et al. reported that VHL patients with juxtapapillary hemangioma could experience treatment complications, including a vitreous hemorrhage and rhegmatogeneous retinal detachment. At that time, scleral buckling procedure, vitreoretinal surgery, and endo-laser photocoagulation may be required [54].

Anti-VEGF agents are also candidate treatments for optic disc hemangioma. von Below et al. demonstrated that bevacizumab, a humanized anti-VEGF antibody, was also used systemically (6mg/kg body weight); treatment decreased tumor exudation transiently, but did not improve eventual visual outcome [50]. Aiello et al. reported the treatment involving the systemic administration of a VEGF receptor inhibitor SU5416. The juxtapapillary hemangioma did not result in a decrease in tumor size but effected an improvement in visual acuity and visual field [46]. As mentioned above in the peripheral retinal hemangioma, effects of anti-VEGF treatments on suppression of tumor growth vary in each case [55]. The reasons may be related to a reduction in vasopermeability, because there was no apparent effect of treatment on the size of the primary retinal hemangiomas [45].

Matsuo et al. reported an 18-year-old woman with optic disc hemangioma in the background of VHL disease [56]. The patient underwent low-dose external beam radiation (20 Gy) to the eye

using a lens-sparing single lateral technique, which led to the inhibition of visual disturbance associated with serous retinal detachment. Therefore, the authors recommended low-dose external beam radiation as the initial treatment option for optic disc hemangioma [56]. In contrast to such destructive therapies, infrared diode laser transpupillary thermotherapy provides a useful modality in the treatment of retinal capillary hemangiomas, and may be particularly favorable for juxtapapillary lesions because of its relatively nondestructive characteristics [57] in selected cases.

Corticosteroids have a significant anti-angiostatic capacity. The primary mechanism of action of angiostatic steroids appears to be in aiding breakdown and blockage of the formation of capillary endothelial basement membranes [58]. Toyokawa et al. reported a case of juxtapapillary hemangioma successfully treated with intravitreal injection of bevacizumab combined with posterior subtenon injection of triamcinolone acetonide (TA) (1.25 mg bevacizumab and 20 mg TA) [59]. Suh et al. showed that verteporfin PDT combined with intravitreal TA appeared to cause involution of the hemangioma with reduction in macular edema and improvement in visual acuity [60]. Therefore, it is likely that TA should be considered one of therapeutic options for patients with retinal and juxtapapillary hemangiomas.

Future prospects on therapeutic approach

Propanolol is a β-blocker commonly used in cardiology that may induce endothelium vasoconstriction and inhibit endothelial proliferation. It has been shown to be effective in infantile facial hemangiomas, and proved safe and effective for the choroidal hemangioma [61]. However, β-blocker has yet to be challenged for patients with retinal capillary hemangioma. Although β-blocker affects systemic circulation of human body including blood pressure, it may be initially tried especially for patients showing refractory to other treatments, after approving the ethical issues in the future.

Recently, adenovirus-mediated VHL intraocular gene transfer has been attempted, and VHL expression in adenovirus-mediated VHL-transduced cells was confirmed at the transcript and protein levels. Adenovirus expressing VHL led to a significant reduction in VEGF expression *in vitro* under normoxic or hypoxic conditions. Akiyama et al. demonstrated that adenovirus-mediated VHL effectively inhibited pathological angiogenesis in the monkey retina [62]. More recently, Sufan et al. analyzed adenovirus-mediated delivery of the bioengineered VHL protein, which contributed to the dramatic inhibition of angiogenesis and growth regression of human renal cell carcinoma xenografts in a dorsal skin-fold window chamber model [63]. Therefore, targeted VHL gene and protein transfer into the eye may open a novel therapeutic approach for retinal hemangioma of VHL disease in the future.

CONCLUSIONS

Recent advances in basic and clinical studies on VHL gene/protein and VHL disease have provided novel concepts in molecular pathology and clinical managements. pVHL plays a critical role in the regulation of HIF-dependent as well as HIF-independent signaling pathways. These mechanisms should underlie the pathogenesis of VHL-related retinal vascular tumors. It is still controversial whether the histological term "hemangioma" vs "hemangioblastoma" should be appropriate in calling retinal vascular tumors of VHL disease. Further morphological and histochemical analyses will be required to resolve the issue. Recent clinical studies have proved application of various therapeutic options depending on the location of retinal tumors between peripheral and optic disc/juxtapapillary hemangioma during a couple of decades. Indeed, long-term follow-up observation can be achieved in VHL disease patients, showing effectiveness of conventional standard treatments. However, there still exists a population suffering from irreversible severe visual disturbance even though the conventional treatments had been performed enough. Further challenging of molecular targeting therapy as well as development of vitreoretinal surgeries and gene/protein transfer technique may contribute to preservation of the patients' vision in the future.

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Review Article

Pathological and Clinical Features and Management of Central Nervous System Hemangioblastomas in von Hippel-Lindau Disease

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Abstract

Central nervous system (CNS) hemangioblastoma is the most common manifestation of von Hippel-Lindau (VHL) disease. It is found in 70-80% of VHL patients. Hemangioblastoma is a rare form of benign vascular tumor of the CNS, accounting for 2.0% of CNS tumors. It can occur sporadically or as a familial syndrome. CNS hemangioblastomas are typically located in the posterior fossa and the spinal cord. VHL patients usually develop a CNS hemangioblastoma at an early age. Therefore, they require a special routine for diagnosis, treatment and follow-up. The surgical management of symptomatic tumors depend on many factors such as symptom, location, multiplicity, and progression of the tumor. The management of asymptomatic tumors in VHL patients are controversial since CNS hemangioblastomas grow with intermittent quiescent and rapid-growth phases. Preoperative embolization of large solid hemangioblastomas prevents perioperative hemorrhage but is not necessary in every case. Radiotherapy should be reserved for inoperable tumors. Because of complexities of VHL, a better understanding of the pathological and clinical features of hemangioblastoma in VHL is essential for its proper management.

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Introduction

Von Hippel-Lindau (VHL) disease is inherited in an autosomal dominant pattern and characterized by the development of hemangioblastomas (HBs) of the central nervous system (CNS) and retina, renal cell carcinoma, pheochromocytoma, pancreatic and endolymphatic sac tumors. CNS HB is the most common VHL-associated lesion, and it is found in 70-80% of VHL patients. HB is a WHO grade 1 tumor, composed of stromal cells and abundant capillaries. Its cytogenesis remains uncertain, but recently it was suggested that HB originates from embryonic hemangioblast. The neurologic morbidity and mortality depend on HB's location and multiplicity. Because of complexities of VHL, a deep understanding of clinical and pathological features of HB in VHL is essential (1).

Histopathology and molecular markers

HBs are characterized histologically by two main components, large vacuolated stromal cells, and a rich capillary network

composed of vascular endothelia and pericytes. The stromal cells represent the neoplastic component of the tumor, but the histogenesis remains uncertain. It has been suggested that the stromal cells are derived from hemangioblast progenitor cells (2, 3) and that the vascular cells represent reactive angiogenesis (4). The nuclei of the stromal cells vary in size with occasional atypical and hyperchromatic nuclei. Their most striking morphological feature is numerous lipid-containing vacuoles, characterizing the typical clear cell morphology. HB histologically mimics the clear cell type of renal cell carcinoma, but differential diagnosis can be made. Renal cell carcinoma commonly stains for markers including cytokeratin, EMA and pan-epithelial antigen, whereas HB does not stain for these markers.

In HB, the stromal cells and capillary endothelial cells significantly differ in their antigen expressing patterns. Stromal cells are commonly positive for neuron-specific enolase, neural cell-adhesion molecule (CD56), ezrin and vimentin (3). The capillary endothelial cells are commonly positive for CD 34 and CD 31(PECAM) (4). The stromal cells express high levels of epidermal growth factor receptor (EGFR), but the EGFR gene is not amplified (5). A subpopulation of the stromal cells also express transforming growth factor alpha (TGF-α), an EGFR ligand, which may suggest an autocrine TGF-α-EGFR loop (6). Vascular endothelial growth factor (VEGF) is highly expressed in stromal cells corresponding to endothelial expression of its receptors VEGFR-1 and -2 (7) and endothelial receptor Tie-1 (8). In addition to VEGF, erythropoietin and hypoxia inducible factor 2 alpha (HIF2-α) are highly expressed in the stromal cells (9, 10).

Molecular mechanisms

The VHL gene was isolated by positional cloning at chromosome 3p25-26 and encodes a protein of 213 amino acids corresponding to a coding sequence of 639 nucleotides (11). The predicted protein contained an acidic pentameric repeat. The VHL gene has the characteristics of a classic tumor suppressor gene; i.e., loss of the wild type allele in CNS HB patients with VHL, and somatic mutations in sporadic CNS HB with a loss of heterozygosity (12-14). The VHL gene is expressed in a variety of tissues, in particular epithelial cells of the skin, the gastrointestinal, respiratory and urogenital tracts, and the endocrine and exocrine organs (14, 15 16). In the CNS, immunoreactivity for VHL protein is prominent in neurons, including Purkinje cells of the cerebellum (17, 18). Inactivation of the VHL gene in affected VHL family members is responsible for their genetic susceptibility to hemangioblastoma. The mechanism by which VHL protein causes neoplastic transformation has remained unclear.

The VHL protein binds to elongins B and C, which activates transcription elongation by RNA polymerase H, and inhibits elongin (S HI) transcriptional activity (19), suggesting that the VHL protein may play an important role in the transcriptional regulatory network that controls tumorigenesis. The wild-type VHL protein regulates the expression of many hypoxia-induced genes such as vascular endothelial growth factor (Figure 1). The VHL protein inhibits the cellular expression of vascular endothelial growth factor, platelet-derived growth factor, and glucose transporter GLUT1 in hypoxic condition, but not in normoxic condition (19, 20). The VHL protein regulates the mRNA stability of these genes at the posttranscriptional level by interacting with elongins B and C (21).

Germ line or sporadic mutations of the VHL gene are spread all over its three exons. Missense mutations are most common. Non-sense mutations, micro deletions/insertions, splice site mutations and large deletions are also found (22, 23). VHL gene mutations are also common in sporadic hemangioblastomas (13).

Phenotypes of VHL are based on the absence (type 1) or presence (type 2) of pheochromocytoma. VHL type 2 is subdivided into three categories: type 2A, type 2B and type 2C. Type 2A VHL has pheochromocytoma with CNS HB, but not with RCC. Type 2B exhibits pheochromocytoma, renal cell carcinoma and CNS HBs. A recent notion is that type 2C disease has only pheochromocytoma, with no other disease (24, 25) (Table 1).

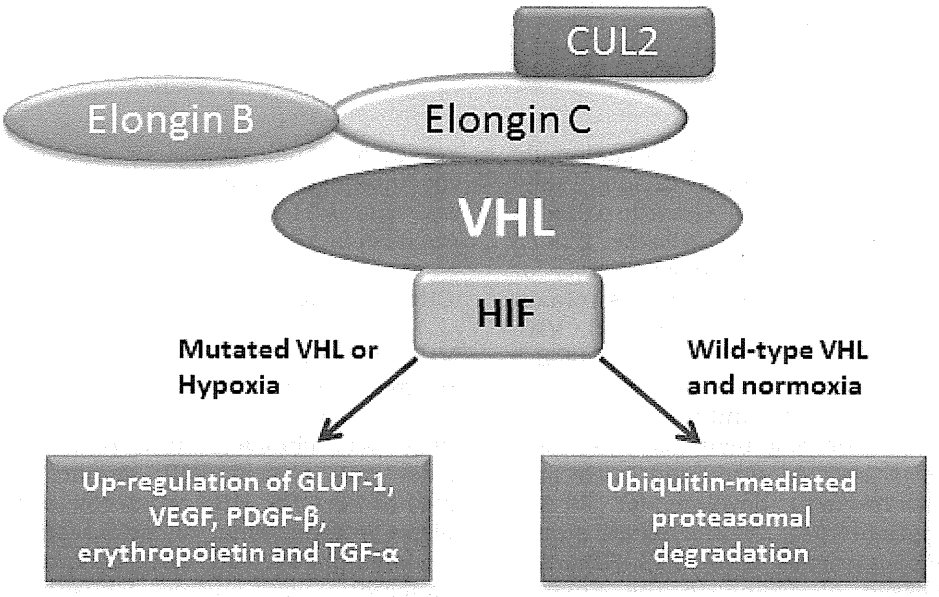

Figure 1. The interaction of VHL protein with HIF and other proteins including elongin B, elongin C and CUL2. A mutated VHL stabilizes HIF and leads to the up-regulation of many pro-angiogenic factors including GLUT-I, VEGF, PDGF-β, erythropoietin and TGF-α. A wild type VHL degrades HIF through ubiquitin-mediated pathway.

Clinical features

CNS HB is a relatively rare brain tumor, accounting for 2.0% of primary brain tumors (26). VHL patients often have multiple HBs at various sites. Twenty-five to 30% of CNS HBs are associated with VHL, with 70-75% of them being sporadic. In VHL, 50 - 60 % of the HBs are located in the cerebellum, 40-50% in the spinal cord, 10 - 20 % in the brain stem, and 2 - 4% in the pituitary stalk, whereas sporadic HBs occur predominantly in the cerebellum. CNS hemangioblastoma is the earliest or the second earliest manifestation, and the onset age ranges from 7 to 73 years, with the mean being 29 years (26, 27, 28). Signs and symptoms vary based on the anatomical tumor location, associated edema and cyst, and tumor size. Tumors that become symptomatic and require resection usually grow faster than asymptomatic ones (1). Patients with cerebellar HBs can present with symptoms owing to cerebellar impairment and increased intracranial pressure. These include: gait ataxia (64%), dysmetria (64%), headaches (12%), diplopia (8%), vertigo (8%), and emesis (8%). Patients with spinal HBs can present with symptoms associated with radiculopathy and myelopathy: hypesthesia (83%), weakness (65%), gait ataxia (65%), hyper-reflexia (52%), pain (17%), and incontinence (14%). Patients with brain stem HBs can display symptoms mainly due to both lower cranial nerve impairment and high intracranial pressure: hypesthesia (55%), gait ataxia (22%), dysphagia (22%), hyper-reflexia (22%), headaches (11%), and dysmetria (11%) (29). In rare cases, CNS HBs present by intra-parenchymal or subarachnoid hemorrhage (29). Approximately 5% of patients develop polyglobulia, which can be cured by removing the solid tumor mass (28, 30). Most symptoms do not arise from the solid tumor itself but from the associated rapidly growing cyst or syrinx

Table 1. Clinical classifications and manifestations of VHL disease

	Clinical manifestations		
	CNS HB	Renal Cell carcinoma	Pheochromocytoma
VHL type 1	+	+	-
VHL type 2A	+	-	+
VHL type 2B	+	+	+
VHL type 2C	-	-	+

(29). Therefore, symptoms can occasionally develop rapidly; however, usually they develop slowly (29, 31-33). Growth patterns vary and are categorized as saltatory (70-75 % of growing tumors), linear (5-7%) or exponential (20-25%). Many tumors remain same in size for several years (32). VHL patients are found to have a mean of 8.5 tumors/patient (range, 1 to 33 tumors/patients) at initial evaluation. Mean tumor development is 0.4 new tumors/year and is correlated with age, with more frequent development in the younger patients (31). Performance status (PS) of VHL patients with CNS HBs has been assessed according to the Eastern Cooperative Oncology Group performance status (EOCG PS; 4). This study results revealed that most patients have a low ECOG PS score (PS=0, 1). The mean ECOG PS of patients with a single CNS HB was significantly lower than that of patients with multiple CNS HBs (27). Patients bearing HBs often show polycythemia owing to erythropoietin secreted from HB cells. In familial cases, genetic testing can detect VHL gene mutations in peripheral blood or tumor tissue (22). In sporadic HB, such mutations can be detected only in tumor tissues (13).

Neuroimaging

HBs are most often visualized by contrast-enhanced T1-weighted MR-imaging (Figure 2). In post-contrast images the tumor tissue appears as a homogenous bright contrast-enhanced mass that clearly stands out from the surrounding tissue. T2-weighted or flair MR-imaging allows excellent quantification of edema and peri-tumoral cysts, which appear as high-signal areas. Cyst walls of HBs are not usually enhanced on MRI. Angiography can be used to highlight the tumor staining, arteriovenous shunting, and early draining veins associated with these tumors prior to resection. Angiography is also performed for intended preoperative embolization in the case of large solid HBs. CT scan has been replaced by MRI (34).

Clinical diagnostic criteria for VHL

VHL is diagnosed according to clinical diagnostic criteria (29). In the presence of a positive family history, VHL can be diagnosed clinically in a patient with at least one typical VHL tumor, such as retinal or CNS HB, renal cell carcinoma, pheochromocytoma or pancreatic tumor. Endolymphatic sac tumors and multiple pancreatic cysts suggest a positive carrier. In contrast, in patients with a negative family history of VHL-associated tumors, diagnosis of VHL can be made when such patients exhibit two or more CNS HBs or a single HB in association with a visceral tumor such as renal cell carcinoma, pheochromocytoma or pancreatic tumor (29).

Management and follow-up

Therapeutic strategy

The therapeutic strategy for each CNS HB in VHL has to be discussed individually with respect to the tumor location, tumor size or associated cysts, as well as symptoms and general condition of the patient, because most VHL patients will develop numerous HBs growing at different rates and at several locations (Figure 3). In addition, a past therapeutic history of each VHL patient should be taken into account. Although the appropriate treatment strategies for CNS HBs are still a matter of debate, there is a general consensus that the symptomatic tumors should be treated (34-38). Since CNS HBs do not grow continuously at the same rate but with intermittent quiescent and rapid-growth phases, therapeutic strategies for

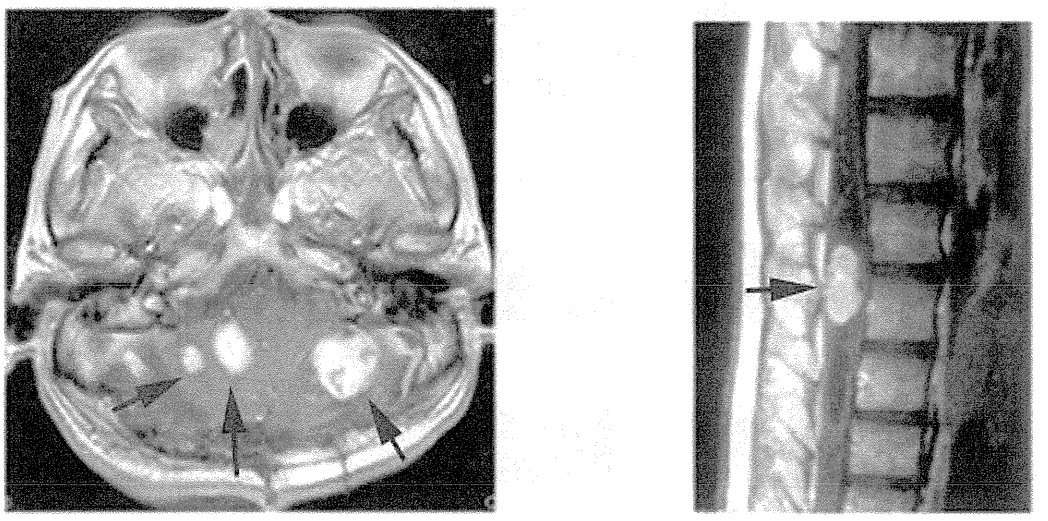

Figure 2. Contrast-enhanced T1 weighted MRI of CNS HBs in VHL. Left, multi-cerebellar HBs; right, lumbar spinal cord HB with syrinx.

asymptomatic tumors in VHL patients are controversial. Asymptomatic tumors, which are stable in MRI screening, are recommended to be followed radiographically. In the case of asymptomatic but progressive tumors, treatment strategies slightly differ in the literature. Some reports recommend early surgery (39) since preoperative neurological symptoms are usually reversible, and surgical resection can be usually performed with low morbidity. For spinal cord HBs, the surgical outcome of the tumor volume less than 500mm^3 was better than that were larger than 500mm^3. If the tumor volume exceeds 500mm^3 during follow-up by MRI, surgical treatment might be considered.

Preoperative management

As to preoperative management for CNS HBs, preoperative embolization can be helpful in the case of large solid tumors to prevent intraoperative hemorrhage. There is no general consensus on preoperative embolization since this procedure is occasionally associated with side effects such as swelling, hemorrhage, and infarction. The time span between embolization and an operation should not exceed three days, since peri-focal swelling can cause enhanced unnecessary risks (1, 34).

Surgical treatment

Surgical treatment is usually the first choice therapy for CNS HBs, and its final goal is the complete resection of all tumor components. Since most VHL patients bear multiple CNS HBs and undergo multiple surgeries causing deterioration of performance status (27), at the removal of symptomatic tumors any small asymptomatic tumors in the same anatomical location should be removed simultaneously if they can be found. The cystic wall without contrast enhancement may be left untreated since the cyst wall does not include the tumor cells. The cystic wall usually consists of reactive gliosis without an epithelial lining (38). Occasionally, cysts associated with tumors will refill again in the case of incomplete resection of the solid tumor (40). Since hemangioblastomas are highly vascular tumors, it is not recommended to cut the tumor into pieces since debulking of the tumor can cause extensive bleeding. Without losing sight of the tumor margin, resection must be carried out with careful dissection, and cutting and coagulation of each feeding vessel must be done. It is therefore necessary to consequently dissect

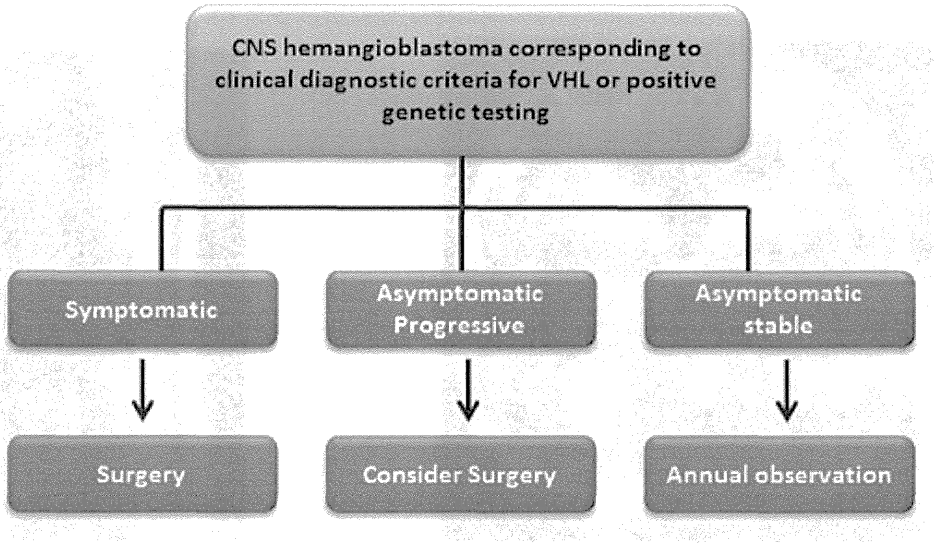

Figure 3. Clinical management of hemangioblastomas.

the plane between the tumor capsule and the surrounding tissue. In many cases the cyst is much bigger than the solid part and is causative of progressive neurological symptoms (37, 38). The solid tumor itself can be distinguished from the surrounding brain tissue due to its reddish or orange color and can usually be removed completely. However, distinction from the surrounding vessels is occasionally difficult. In this case, intraoperative indocyanine green (ICG) video angiography and fluorescent visualization with 5-ALA facilitate to visualize tumors themselves and/or the surrounding vessels (41, 42). Doppler flow sonography with a contrast-enhancing agent can be also useful, since it is a sensitive intraoperative tool to guide the surgical approach and resection (43, 44).

Motor-evoked potentials for spinal cord HBs should be applied in the case of surgery of spinal cord HBs (38). If the spinal cord HB is not visible on the surface of the spinal cord, enlarged arterialized veins can be helpful for finding the tumor. These enlarged arterialized veins except for those penetrating the tumor should be preserved to avoid swelling and hemorrhage from the tumor. Even if a dorsal fascicle is involved in the tumor, it can usually be removed with no neurological deficit or only slight disturbance of deep sensation (38).

Radiotherapy

Stereotactic radiosurgery for HBs results in a high local control rate in CNS HBs with acceptable levels of radiation-induced complications (45). Principally, stereotactic radiosurgery can be used for surgically inaccessible or multiple cranial and spinal tumors (46). More recently, fractionated external beam radiotherapy (EBRT; 47) and infratentorial craniospinal radiation therapy (ICSRT; 48) have been investigated for use against CNS HBs, and favorable outcomes were reported.

Follow-up of CNS HBs in VHL patients

VHL patients with CNS HBs should undergo MRI of the brain and spinal cord at least once a year. VHL patients above 10 years old, who do not display CNS HBs, should undergo MRI screening of their whole neuro-axis every two years. An annual ophthalmoscopy should be performed to screen for retinal HBs. A yearly MRI of the abdomen is recommended to screen for renal cell carcinoma, pancreatic lesions, and pheochromocytoma (29, 34). In addition, a yearly abdominal ultrasound with triennial computed tomography (CT) imaging for renal cell carcinoma, a yearly audiometry for endolymphatic tumor, and pheochromocytoma investigation by urine analysis (metanephrine - VMA) are

recommended. Based upon clinical indication these follow-up modalities should be advanced or extended (49).

Conclusion

The neurologic morbidity and mortality depend on the location and multiplicity of CNS HBs. Because of the complexities of VHL, a deep understanding of pathological and clinical features of HB in VHL is essential, and the management strategies should be tailored to the needs of the individual patients.

Conflict of interest: None

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