れている[15]。

上記のサーベイランス（図1）により治療適用ありとされる症例には以下の原則に従い治療を行う（図2)。すなわち、遠隔転移の有無にかかわらず、切除可能な症例は出術を行う。手術は腫瘍核出術を基本とし、可能な限り膵機能を温存する術式を考慮する。また、膵臓の手術に際し、他の腹部臓器の合併疾患に対する手術時期も考慮する。手術不可能、非根治手術または術後に再発した症例では組織型検索が必要である。組織学的分化度に加えKi-67/MIB-1指数(%)の検索も重要である。低分化型の膵神経内分泌癌であればCDDPおよびVP-16併用の全身化学療法が適応となる（保険適応なし）[16]。高分化型の膵神経内分泌癌では現在まで確立した全身化学療法のコンセンサスはないが、NCCNガイドライン(2009年)では分子標的薬（mTOR阻害剤）などの臨床試験、サンドスタチン投与（非機能性腫瘍には保険適応なし)、または経過観察の選択枝が提示されている[17]。最近、切除不能の高分化型非機能性の中腸神経内分泌腫瘍の患者を対象にOctreotide（サンドスタチンLAR®）の効果をみたRCT（PROMID試験）により、Octreotideが無増悪期間を含めた予後を明らかに改善したとの報告があり[18]、非機能性の膵臓神経内分泌性腫瘍に対してもその効果が期待されている。低悪性度から中等度悪性度の進行性神経移行内分泌腫瘍に対するmTOR阻害薬RAD001とOctreotide（サンドスタチンLAR®）の併用臨床第II相試験の結果では、部分寛解と不変が計90%程度であり腫瘍制御効果が期待されている[19]。また、いずれの組織型でも肝転移が存在する場合は、塞栓術、抗癌剤肝動注、ラジオ波焼灼などの治療も考慮すべきである[20]。

2. 膵嚢胞病変（漿液性嚢胞線腫）

2-1. 背景（一般的臨床事項）

VHL病の7-71%の症例において膵嚢胞性病変が見られ、組織型の判明した症例ではほとんどが漿液性嚢胞線腫（Serous cystadenoma）である[3, 6, 21, 22]。膵臓漿液性嚢胞線腫の悪性化はごく稀であり、嚢胞径が大きくなり他臓器の圧迫症状などの臨床症状が出現するまで、経過観察あるいは治療の必要はない[1]。ただし、成人のVHL症例では、悪性化する可能性のある他の膵嚢胞性病変（膵管内粘液性乳頭腫瘍および粘液性嚢胞腫瘍）との鑑別に注意が必要である。

2-2. 経過観察（サーベイランス）方法

臨床症状（多臓器の圧迫症状）のない場合、サーベイランスの必要はない。上記P-NETに対するサーベイランスに際し、膵嚢胞性病変についても評価を行う。

2-3. 治療の指針

臨床症状（多臓器の圧迫症状）の出現時に切除術を考慮する。

E．結論

欧米からのこれまでの報告をもとに、本邦の臨床と研究の実態を考慮し、VHL病における膵臓神経内分泌性腫瘍と膵嚢胞性病変に対する経過観察と治療についての指針（案）を策定した。本指針は日本において同病態についての初めての臨床指針（案）であり、今後、公聴会などによる意見の集約を重ね、広くコンセンサスを得ていくこ

とが必要である。

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G．研究発表

1.論文発表

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2. 学会発表　　該当なし

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得　　該当なし
2. 実用新案登録　該当なし
3. その他　　該当なし

図1. VHL病の膵神経内分泌腫瘍に対するサーベイランス
（文献2より改変引用）

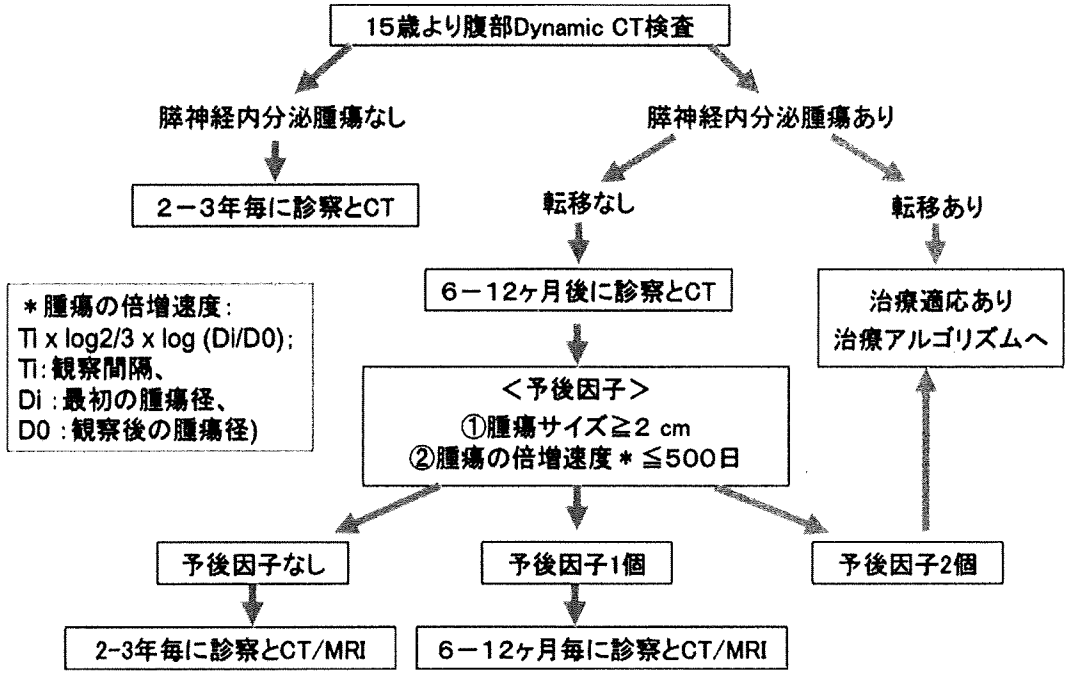

図2. VHL病に伴う膵神経内分泌腫瘍の治療アルゴリズム

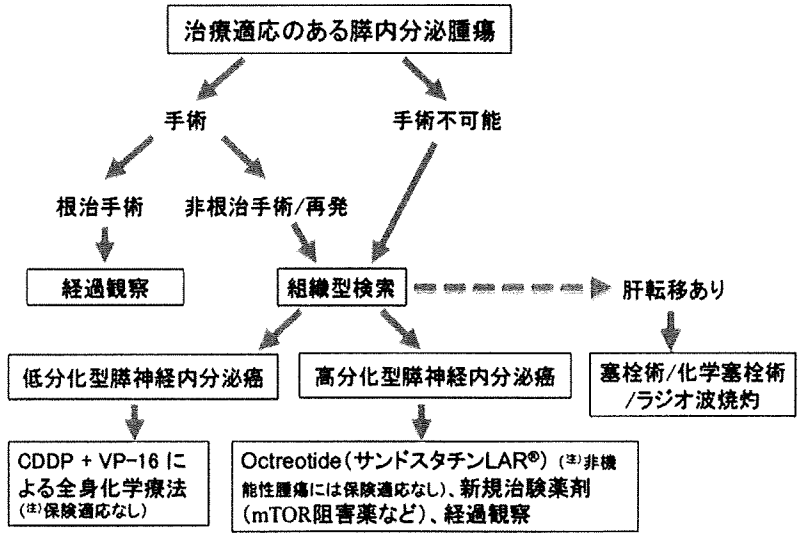

表1. P-NETのWHO分類

WHO分類	高分化型 膵神経内分泌腫瘍	高分化型 膵神経内分泌癌	低分化型 膵神経内分泌癌
生物学的活性	良性/低悪性度	低悪性度	高悪性度
転移	-	+/-	+
Ki-67/MIB-1指数(%)	<2	2～20	>20
病理組織学的分化度	高分化	高分化	低分化
血管浸潤	-	+	+

（文献23より改変引用）

〔Ⅳ〕

平成21年度研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

和文書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
菅野　洋	フォン・ヒッペル・リンドー病	日本脳腫瘍病理学会	脳腫瘍臨床病理カラーアトラス第3版	医学書院	東京	2009	172-173

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kanno H, Yamamoto I, Nishikawa R, et al.	Spinal cord hemangioblastomas in von Hippel-Lindau disease.	Spinal Cord.	47(6)	447-452	2009
Kanno H, Nakano S, Kubo A, et al.	Neuronal differentiation of neural progenitor cells by intracellular delivery of synthetic oligopeptide derived from Von Hippel-Lindau protein.	Protein Pept Lett.	16(11)	1291-6	2009
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Ito S, Natsume A, Wakabayashi T, et al.	Human neural stem cells transduced with IFN-beta and cytosine deaminase genes intensify bystander effect in experimental glioma.	Cancer Gene Ther	17(5)	299-306	2010
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雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ohno M, Natsume A, Wakabayashi T, et al.	Interferon-beta, MCNU, and conventional radiotherapy for pediatric patients with brainstem glioma.	Pediatr Blood Cancer	53(1)	37-41	2009
Wakabayashi T, Natsume A, Hatano H, et al.	p16 promoter methylation in the serum as a basis for the molecular diagnosis of gliomas.	Neurosurgery	64(3)	455-61	2009
Ito M, Natsume A, Wakabayashi T, et al.	Type I interferon inhibits astrocytic gliosis and promotes functional recovery after spinal cord injury by deactivation of the MEK/ERK pathway.	J Neurotrauma	26(1)	41-53	2009
Oi S, Natsume A, Wakabayashi T, et al.	Synergistic induction of NY-ESO-1 antigen expression by a novel histone deacetylase inhibitor, valproic acid, with 5-aza-2'-deoxycytidine in glioma cells.	J Neurooncol	92(1)	15-22	2009
Maeda H, Nishimori I, Okabayashi T, et al.	Total pancreatectomy for multiple newuroendocrine tumors of the pancreas in a patient with von Hippel-Lindaw disease.	Clin J Gastroenterol	2	222-225	2009

〔V〕

研究成果の刊行物・別刷

65 von Hippel-Lindau disease

フォン・ヒッペル・リンドー病

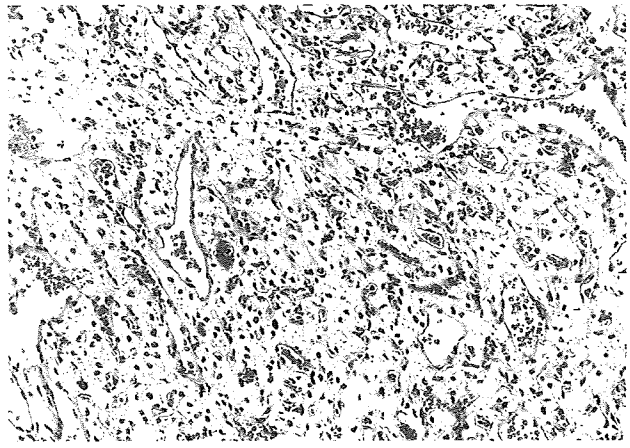


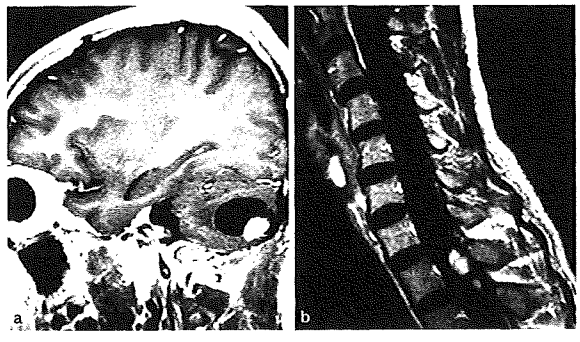
図1 VHL病患者に発生したhemangioblastoma 多角形あるいは類円形の明るく淡い好酸性の胞体を有する間質細胞がシート状に並び，その間に豊富な毛細管網が存在している．(HE染色，弱拡)

臨床症状

小脳および脊髄のhemangioblastomaによる神経症状で発症する場合が最も多く，小脳失調，中脳水道閉塞による頭蓋内圧亢進を認めることが多い．多血症をしばしば伴う．次にretinal angiomaによる眼症状を呈することが多い．

画像所見

小脳および脊髄のhemangioblastomaは，境界鮮明でcystを高率に伴い，CT，MRIにて著明な造影効果を示し(図I)，血管撮影では腫瘍の顕著なstainingを呈する．



図I MRI像
a：嚢胞を伴った小脳hemangioblastoma．(T1強調Gd造影，矢状断)
b：脊髄空洞症を伴った脊髄hemangioblastoma．(T1強調Gd造影，矢状断)

定義

von Hippel-Lindau (VHL) 病は，hemangioblastoma (→132頁)，retinal angioma，renal cell carcinoma，pheochromocytoma，腎，精巣上体のcystなどを発生する常染色体優性の遺伝性多発腫瘍性疾患であり，母斑症(神経皮膚症候群)の一種である．本疾患の病名の由来は，1904年に家族性のretinal angiomaを報告したEugene von Hippelと1926年に網膜のhemangiomaと小脳のhemangioblastomaの合併例を報告したArvid Lindauによる．

VHL病の診断基準[1]は，家族歴の有無によって異なる(表1)．

発生頻度・部位

VHL病で発生する腫瘍性あるいは嚢胞性病変には，小脳，脳幹，脊髄のhemangioblastoma (80%)，retinal hemangioma (70%)，renal cell carcinoma (40 %)，pheochromocytoma (20%)，膵臓，腎臓，脾臓，精巣上体の嚢胞(50%)，膵のislet cell tumor，側頭骨内のendolymphatic sac tumorなどがある．これらの腫瘍性，嚢胞性病変は，両側性，多発性に発生し，散発例に比べると，若年に発生するのが特徴である．発生率は，約36,000人に1人であり，VHL病のうち家族歴を欠く散発例は約10%に認められる．

中枢神経系のhemangioblastomaはVHL病患者の約8割に認められ，その好発部位は小脳(約70%)，脊髄(約40%)，脳幹部(約10%)である(図1)．小脳hemangioblastomaは，多くは小脳半球，虫部内，または小脳表面に認められ，約70%が嚢胞形成し，この場合は，大きな嚢胞壁に赤みを帯びた壁在結節として認められ，嚢胞液は淡黄色を呈する．脊髄hemangioblastomaは，VHL患者の約5割に認められ，多くは髄内に発生するが髄外にみられることもある．

分子生物学的知見

VHL病は，染色体3pに存在する*VHL*遺伝子の変異により生じる．*VHL*遺伝子は，1993年にZbarらのグループにより，3p25領域からpositional cloning法により単離同

表1　VHL病の診断基準

VHL病の家族歴のある場合	VHL病の家族歴のない場合
以下の1病変以上を発症 •中枢神経の血管芽腫 •腎癌 •褐色細胞腫 •網膜血管腫 •膵ラ氏島腫瘍 •膵癌 •膵嚢胞 •精巣上体嚢胞	A) 中枢神経の血管芽腫と網膜血管腫の合併 B) Aの病変のどちらか1つおよび以下に示す1病変以上を合併 •腎癌 •褐色細胞腫 •精巣上体嚢胞 •膵病変（嚢胞，腫瘍）

定された[2]．*VHL* 遺伝子の翻訳領域は639塩基対，3個のエクソン exon より成る．この遺伝子を用いた本疾患患者の遺伝子診断を行うと，結合蛋白の結合部位に集中して遺伝子変異を認める．約60%がアミノ酸置換を来す missense mutation で，約40%が frameshift あるいは nonsense mutation である[3]（図2，3）．

VHL蛋白は，elongin B，C と Clu-2 との複合体 VBC を作成し，hypoxia-inducible factor（HIF）の α subunit を正常酸素濃度下でユビキチン化してプロテアソームにより分解することにより HIF の発現を制御する[2]．しかし，相同染色体の両側の *VHL* 遺伝子の不活性化あるいは低酸素濃度下では *VHL* 遺伝子が機能しないため，HIF が分解されずその下流の vascular endothelial growth factor（VEGF）などの発現を誘導し，これらが腫瘍発生に関与すると考えられている．生まれつき相同染色体の片方の *VHL* 遺伝子に変異（germline mutation）がある場合は，対立した相同染色体上の正常な *VHL* 遺伝子がヘテロ接合性の消失 loss of heterozygosity（LOH）やメチル化 methylation などによって不活性化されると *VHL* 遺伝子の機能が喪失し，上記のメカニズムにより hemangioblastoma や腎細胞癌など VHL 病でみられる様々な腫瘍の発生が誘導されると考えられている[4]．また，最近 hamangioblastoma の起源に関して，造血幹細胞のマーカーである stem cell leukemia（Scl），CD133，CD34，c-kit，erythropoietin が陽性であり，その陽性細胞の分布が hemagioblastoma の発生部位と一致することから，間葉系の embryonic hemangioblast がその由来であると報告されている[5]．

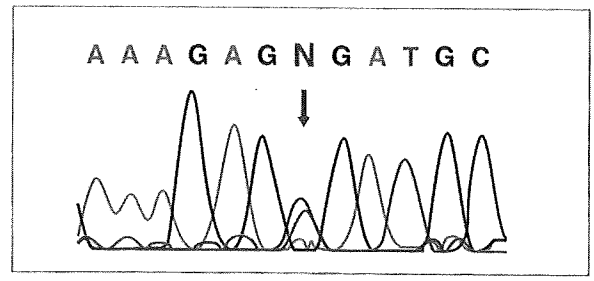


図2　VHL病患者にみられた germline mutation
481番目の塩基が cytosine から thymine へ変わり（矢印），ストップコドンとなっている（nonsense mutation）．

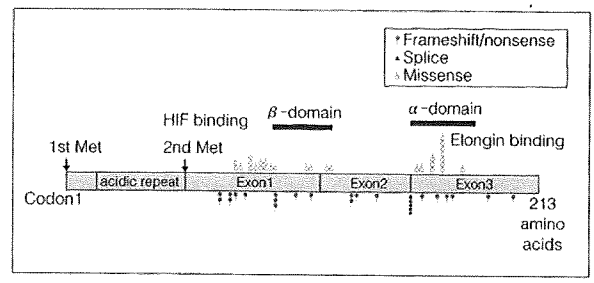


図3　わが国の VHL 病家系の *VHL* 遺伝子変異の分布

治療・経過・予後

VHL病の発症年齢は18～30歳くらいで，腫瘍別には retinal hemangioma（平均25歳），CNS hemangioblastoma（平均30歳），renal carcinoma（平均37歳），pheochromocytoma（平均27歳）である．VHL病の患者の平均寿命は50歳未満とされてきたが，現在では通常よりもやや短い程度で，renal cell carcinoma（32%）と CNS hemangioblastoma（53%）が死亡の原因になることが多い．VHL病のキャリアであれば，65歳くらいまでにはほとんどがなんらかの病変が認められる．治療は，原則として手術による摘出術であるが，定位放射線治療による治療は腫瘍を消失させる効果はないものの腫瘍の増大を制御することは可能であり，特に多発性の場合は選択肢の1つである．　（菅野　洋）

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ORIGINAL ARTICLE

Spinal cord hemangioblastomas in von Hippel–Lindau disease

H Kanno[1], I Yamamoto[1], R Nishikawa[2], M Matsutani[2], T Wakabayashi[3], J Yoshida[3], N Shitara[4], I Yamasaki[5], T Shuin[5], and Clinical VHL Research Group in Japan

[1]Department of Neurosurgery, Yokohama City University School of Medicine, Yokohama, Japan; [2]Department of Neurosurgery, Saitama Medical University, Hidaka, Japan; [3]Department of Neurosurgery, Nagoya University School of Medicine, Nagoya, Japan; [4]Tokyo Metropolitan Hiroo General Hospital, Tokyo, Japan and [5]Department of Urology, Kochi University School of Medicine, Nangoku, Japan

Study Design: Retrospective data analysis.
Objective: To clarify the clinical features and surgical management of spinal cord hemangioblastomas in patients with von Hippel–Lindau disease (VHL).
Setting: Clinical VHL Research Group in Japan, Japan.
Methods: Forty-eight out of 66 patients with associated spinal cord hemangioblastoma among 142 VHL patients were retrospectively examined with respect to clinical features, accompanying lesions and outcome of surgical treatment.
Results: Among these 48 patients, 46 of them (95.8%) also had a central nervous system (CNS) hemangioblastoma at another site: 42 (87.5%) with cerebellar hemangioblastoma and 11 (22.9%) with brain stem hemangioblastoma. Twenty-three patients (47.9%) had more than one spinal cord hemangioblastoma. The 48 patients with spinal cord hemangioblastomas collectively had a total of 74 tumors. The tumor was accompanied with a syrinx in 64 and without it in 10 patients. Forty of the 48 patients underwent surgical treatment for their spinal cord hemangioblastomas, and 7 of these 40 underwent surgical treatment twice. When functional changes in the patients after these 47 operations were examined by postoperative evaluation by McCormick's classification, 39 of these operations (83.0%) resulted in improvement/no change and 8 (17.0%) in aggravation of symptoms.
Conclusion: Von Hippel–Lindau disease patients bearing spinal cord hemangioblastomas mostly had a CNS hemangioblastoma at another site. These tumors can be removed in the majority of VHL patients without aggravation. In these patients, when the timing of treatment for spinal cord hemangioblastoma is determined, the probability of occurrence and treatment of other lesions should be considered.
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Keywords: spinal cord hemangioblastoma; von Hippel–Lindau disease; clinical features; surgical outcomes

Introduction

Von Hippel–Lindau disease (VHL) is an autosomal dominant hereditary disease in which central nervous system (CNS) and retinal hemangioblastomas; renal cell carcinomas; pheochromocytomas; abdominal cystic lesions such as those in the kidneys, liver, and pancreas; and epididymal cysts develop. VHL manifests itself by approximately 65 years of age and shows an annual incidence of one per 36 000 live births. Manifestations in the CNS include cerebellar, brain stem, spinal cord, and suprasellar hemangioblastomas and endolymphatic sac tumors.[1–6] The causative gene, located in the chromosome 3p25–26 region,[7] is also related to the occurrence of sporadic CNS hemangioblastoma[8] and renal cell carcinoma.[9]

Sixty to 80% of VHL patients manifest CNS hemangioblastomas in the cerebellum, brain stem and spinal cord.[1,10–13] Spinal cord hemangioblastoma is the second most common among CNS hemangioblastomas, accounting for more than 40% of VHL-associated CNS lesions.[14] Spinal cord hemangioblastomas have morphological features similar to CNS hemangioblastomas elsewhere. Histologically, CNS hemangioblastomas are highly vascular tumors composed of neoplastic stromal cells, pericytes and endothelial cells. The neoplastic cell of the origin for CNS hemangioblastomas is the mesoderm-derived, embryologically arrested hemangioblast.[15] Although a small spinal cord hemangioblastoma is mostly asymptomatic, it becomes symptomatic after the syrinx associated with the tumor has enlarged. Among manifestations in patients with VHL, the earliest features

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are mostly retinal and cerebellar hemangioblastomas, while it is relatively rare that spinal cord hemangioblastoma is the earliest manifestation. Although most of these hemangioblastomas are located in the intramedullary region accompanied with a syrinx, postoperative deterioration is not often encountered.[14,16] Since the advent of the era of magnetic resonance imaging (MRI), small asymptomatic spinal cord hemangioblastomas can be easily detected on an MRI.[1] In VHL patients, spinal cord hemangioblastomas are frequently found. VHL patients harboring spinal cord hemangioblastomas face various treatment problems that are not encountered by patients harboring sporadic spinal cord hemangioblastomas, because VHL patients with spinal cord hemangioblastomas may also require treatment for other CNS hemangioblastomas or visceral tumors simultaneously or in the future. However, most of the previous studies have dealt with isolated sporadic spinal cord hemangioblastomas, and the clinical features and the management of spinal cord hemangioblastomas in VHL patients have not yet been fully clarified.[14] Here, we describe clinical features and management for VHL patients with associated spinal cord hemangioblastomas.

Methods

Patients with VHL were collected by the Clinical VHL Research Group in Japan in 2000–2002. The members of the Clinical VHL Research Group in Japan consisted of neurosurgeons who belonged to 336 hospitals approved as training facilities for neurosurgery in Japan and joined this study. Diagnosis for VHL was based on the following the criteria[3]: (i) patients with a family history of developing hemangioblastoma in the CNS and retina, renal cell carcinoma, pheochromocytoma or pancreatic tumors or cysts, epididymal cystadenoma; (ii) patients without a family history of VHL disease, but who develop hemangioblastoma in combination with other tumors, such as renal cell carcinoma, pheochromocytoma, pancreatic tumors or cysts, or epididymal cystadenoma. All symptomatic and asymptomatic spinal cord hemangioblastomas were diagnosed based on clinical symptoms and neuroradiological findings by MRI (Figure 1), and all surgically removed spinal cord hemangioblastomas were histologically verified. Sixty-six patients having spinal cord hemangioblastomas among 142 VHL patients in 81 VHL families were retrospectively examined with respect to the following: gender, age, type of VHL, spinal tumor location, onset age of spinal cord hemangioblastoma and other lesions (cerebellar hemangioblastoma, brain stem hemangioblastoma, retinal hemangioblastoma or renal cell carcinoma), presence of a syrinx, number of spinal tumors, other CNS hemangioblastomas, other complicating lesions (endolymphatic tumor, renal cell carcinoma, pheochromocytoma, epididymal cyst, renal cyst, pancreas cyst and liver cyst), presence of VHL family history, manifestations in the family, follow-up period and outcome of surgical treatment. In addition, the age of the patient at the onset of spinal cord hemangioblastoma, the period between the onset of the earliest manifestation in VHL and the operation for spinal cord hemangioblastoma and times of surgical treatment for spinal cord hemangioblastoma were examined. Data from the clinical charts and radiological records were used to obtain the above information. Evaluation of surgical treatment was assessed in terms of McCormick's functional grade (Table 1)[17] before and 6 months after the operation. The change in the status of the patient after the operation was classified into three types: improved, no change and aggravated. The relationships between the postoperative McCormick's functional grade and age of patient at the time of the operation, number of simultaneously resected spinal cord hemangioblastomas during the operation, volume of the tumor preoperatively, spine level of the tumor, McCormick's functional grade before the operation and presence of a syrinx were assessed on MRI. Exclusion criteria were as follows: incomplete description in clinical charts and neuroradiological records about site of tumor, accompanying lesions, family history of VHL and surgical outcome evaluation. Then, among 66 patients bearing spinal cord hemangioblastomas associated with VHL, 48 of them, representing 45 VHL families, were examined. For statistical analysis, non-parametric test was applied by Mann–Whitney's *U*-test or Spearman's correlation coefficient by rank test. Statistical significance was set at $P<0.05$.

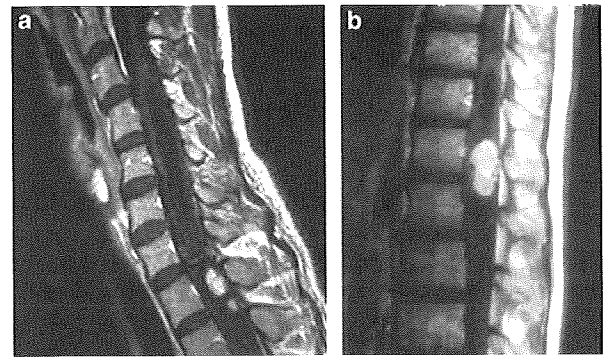

Figure 1 T1-weighted magnetic resonance imaging (MRI) of spinal cord hemangioblastomas in von Hippel–Lindau (VHL)-diseased patients. Spinal cord hemangioblastoma usually shows intramedullary homogeneously enhanced mass lesion frequently associated with syrinx on MRI. (**a**) Cervical cord hemangioblastoma in a VHL patient. (**b**) Lumbar cord hemangioblastoma in a VHL patient.

Table 1 Clinical/functional classification scheme[a]

Grade	*Definition*
I	Neurologically normal; mild focal deficit not significantly affecting function of involved limb; mild spasticity or reflex abnormality; normal gait
II	Presence of sensorimotor deficit affecting function of involved limb; mild-to-moderate gait difficulty; severe pain or dysesthetic syndrome impairing patient's quality of life; still functions and ambulates independently
III	More severe neurological deficit; requires cane/brace for ambulation or significant bilateral upper extremity impairment; may or may not function independently
IV	Severe deficit; requires wheelchair or cane/brace with bilateral upper extremity impairment; usually not independent

[a]Cited from McCormick *et al.*[17]

Results

Forty-eight patients bearing spinal cord hemangioblastomas in 45 VHL families were collected from 27 hospitals including all districts in Japan, and consisted of 21 males and 27 females. The age of the patients ranged from 13 to 52 years, and the mean age was 33.5 ± 9.6 years. The follow-up period ranged from 1 to 20 years, with a mean of 6.5 ± 5.4 years. The 48 patients collectively had a total of 74 spinal cord hemangioblastomas. Among these 48 patients, 46 (95.8%) had more than one CNS hemangioblastoma at a site in addition to the spinal cord, and 12 of 46 had the spinal hemangioblastoma as the first manifestation associated with simultaneously recognized lesions. The remaining two patients without another CNS hemangioblastoma developed spinal cord hemangioblastomas at the ages of 12 and 16 years as the first manifestation of VHL. Twenty-three patients (47.9%) had more than one spinal cord hemangioblastoma, and the mean number of tumors in patients harboring spinal cord hemangioblastoma was 1.54. Forty-two patients (87.5%) also had cerebellar hemangioblastoma; and 11 (22.9%), a brain stem hemangioblastoma. Sixteen patients (33.3%) also presented with retinal hemangioblastoma, 16 (33.3%) with renal cell carcinoma, 11 (22.9%) with pancreas cyst, 5 (10.4%) with a renal cyst and 1 (2.1%) with a pheochromocytoma (Figure 2). The onset ages of spinal cord hemangioblastoma, cerebellar hemangioblastoma and brain stem hemangioblastoma ranged from 5 to 49 years, from 10 to 47 years and from 10 to 49 years (mean 27.5 ± 10.5, 24.9 ± 8.2 and 27.5 ± 10.4 years), respectively. For other manifestations, the onset ages of retinal hemangioblastoma and renal cell carcinoma ranged from 11 to 49 years and from 19 to 49 years (mean 23.2 ± 11.5 and 32.9 ± 9.4 years), respectively (Figure 3). Spinal cord hemangioblastoma was the earliest manifestation in 23 patients, the second in 13, and the third in 12 patients. The mean period between the first manifestation of VHL except for spinal cord hemangioblastoma and the operation for spinal cord hemangioblastoma was 4.4 years (Figure 4).

Thirty spinal cord hemangioblastomas (40.5%) were located at the cervical level, 40 (54.1%) at the thoracic and 4 (5.4%) at the lumbar. Although the thoracic site of spinal cord hemangioblastomas was the most dominant, tumors at the cervical level were relatively the most common on a per spine level (cervical, 4.3; thoracic, 3.3; lumbar, 0.8). This occurrence ratio of cervical commonness revealed that spinal cord hemangioblastoma in VHL usually occurred more rostrally than caudally. Sixty-four tumors (86.5%) were associated with a syrinx; and 10 (13.5%) without one. Among the 48 VHL patients harboring spinal cord hemangioblastomas, 40 of them (83.3%) underwent 47 surgical resections for spinal cord hemangioblastomas, whereas 8 patients were under observation because of no symptom with the tumor. Seven patients underwent two operations for spinal cord hemangioblastomas. The mean period between the first operation and the second operation for the latter group was 9.5 years. Among the 47 operations, 16 of them were for plural spinal lesions; and 31 for single lesion. Total removal was performed in 44 operations

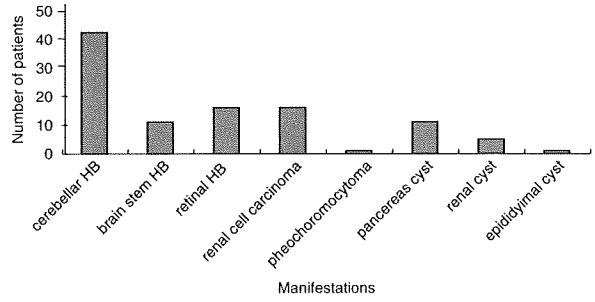

Figure 2 Accompanied manifestations in von Hippel–Lindau-diseased patients harboring spinal cord hemangioblastomas.

Figure 3 The onset ages of central nervous system hemangioblastomas in von Hippel–Lindau (VHL)-diseased patients harboring spinal cord hemangioblastomas. (**a**) Spinal cord hemangioblastomas. (**b**) Cerebellar hemangioblastomas. (**c**) Brain stem hemangioblastomas. The onset ages of spinal cord hemangioblastoma, cerebellar hemangioblastoma and brain stem hemangioblastoma were 27.5 ± 10.5, 24.9 ± 8.2 and 27.7 ± 10.4 years, respectively.

(95.7%), subtotal in 1 (2.1%), and partial in 2 (4.2%) (Table 2). When functional grades before and after the operative stage were evaluated according to McCormick's classification, 6 of the 47 operations resulted 6 (12.8%) in improvement; 33 (70.2%) in no change; and 8 (17.0%) in aggravation. No change of more than 1 grade of McCormick's classification occurred after the operation (Figure 5). In addition, changes in McCormick's functional grade after the operation were not significantly correlated with the number of spinal cord hemangioblastomas surgically treated, presence of a syrinx, preoperative McCormick's

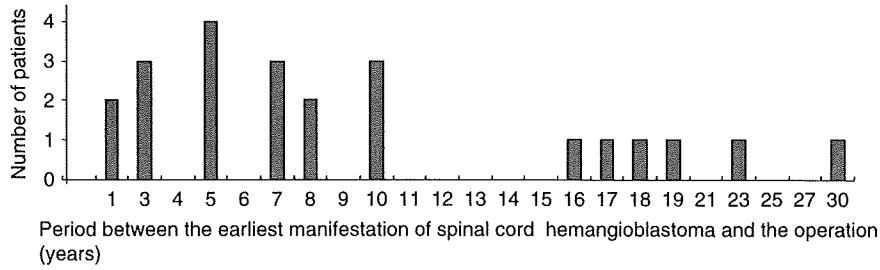

Figure 4 Period between the earliest manifestation of von Hippel–Lindau (VHL) disease preceding spinal cord hemangioblastoma and the operation for spinal cord hemangioblastoma with VHL. The mean period was 4.4 years.

Table 2 Summary of data on spinal hemangioblastomas (HBs) in von Hippel–Lindau (VHL)-diseased patients

Characteristics	
VHL families	45
VHL patients with spinal HB	48
Male/female	21/27
Age (mean years ± s.d.)	33.5 ± 9.6
Period of follow-up (mean years ± s.d.)	6.5 ± 5.4
Associated with >1 spinal HB	23
Associated with extra-spinal HB	46
Cerebellum	42
Brain stem	11
Number of VHL patients having undergone surgical treatment for spinal HB	40
Single operation	33
Multiple operations	7
Total number of surgical treatment	47
Extent of removal	
Total	44
Subtotal	1
Partial	2
Total number of spinal HB	74
With syrinx	64
Without syrinx	10
Volume of spinal HB (mm^3)	
>1000	28
500–1000	37
<500	28

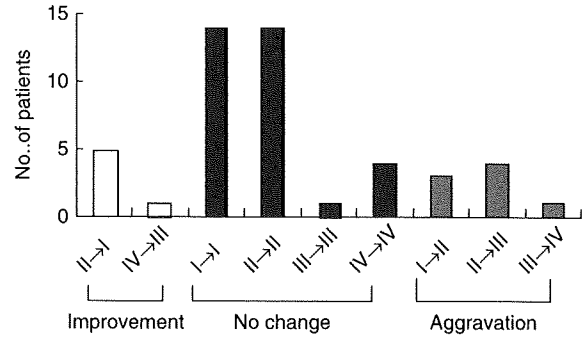

Figure 5 Functional neurological grades before and after the operative stage according to McCormick's classification. Six operations (12.8%) resulted in improvement (white columns); 33 (70.2%), no change (black columns); and 8 (17.0%), aggravation (grey columns). I, II, III and IV means McCormick's functional grades.

functional grade or age of the patient at the time of the operation. However, the preoperative volume of the tumor was significantly correlated with postoperative changes in the grade ($P = 0.02$; Table 3).

Discussion

Spinal cord hemangioblastomas comprise 2–3% of primary intramedullary spinal cord tumors and are the third most common among them.[15,18,19] Spinal cord hemangioblastomas may occur sporadically or in association with VHL, which is a multisystem familial cancer syndrome. Approximately two-thirds are sporadic in origin and the other third are associated with VHL. In VHL patients, spinal cord hemangioblastoma is a common feature, and spinal cord hemangioblastomas associated with VHL are often also accompanied by cerebellar and brain stem lesions; although sporadic spinal cord hemangioblastomas are almost always universally solitary. In addition, VHL patients with spinal cord hemangioblastomas usually also have tumors of other organs such as the eyes, the kidneys, the pancreas, the adrenal glands, paraganglia and the epididymus. This multiplicity accounts for the varied natural history of spinal cord hemangioblastomas associated with VHL, complexities in their management and uncertainties associated with long-term functional outcome.[14,18,19]

Our study revealed that 66 (46.5%) out of 142 VHL patients examined had spinal cord hemangioblastomas. Although sporadic spinal cord hemangioblastomas are found in approximately 10–20% among all CNS hemangioblastomas, spinal cord hemangioblastoma with VHL is a more common manifestation. In addition, 95.8% of the 48 VHL patients with spinal cord hemangioblastoma examined also had CNS hemangioblastomas at another site. In particular, brain stem hemangioblastomas were associated with spinal cord hemangioblastomas. All 23 patients with more than one spinal cord hemangioblastoma also had CNS hemangioblastomas at sites in addition to the spinal region. From these results, when a spinal cord hemangioblastoma is found

Table 3 Change in McCormick's functional grade after the operation in patients with spinal hemangioblastomas (HBs)

Variables	*Number of operation*	*Change of grade*			*P-value*
		Improvement (n = 6)	*No change (n = 33)*	*Aggravation (n = 8)*	
Age (years)					
<21	17	4	10	3	0.54
21–40	23	1	18	4	
>40	7	1	5	1	
Syrinx					
Presence	42	6	29	7	0.51
No presence	5	0	4	1	
Tumor volume (mm^3)					
<500	10	3	7	0	0.02
500–1000	26	3	18	5	
>1000	11	0	8	3	
Operations for spinal HBs					
Single	31	3	25	3	0.43
Multiple	16	3	8	5	
Preoperative McCormick's grade					
I	17	0	14	3	0.26
II	23	5	14	4	
III	2	0	1	1	
IV	5	1	4	0	

in a patient, VHL should be suspected and another manifestation associated with VHL should be explored, particularly a CNS hemangioblastoma. In contrast, when another manifestation of VHL aside from spinal cord hemangioblastoma is the earliest one in a VHL patient, spinal cord hemangioblastoma may be expected later. In our study, the interval between the onset of the initial manifestation and spinal cord hemangioblastoma was 4.4 years as a mean interval. When manifestation associated with VHL is detected and the patient is diagnosed as VHL, we can thus predict that a spinal cord hemangioblastoma will appear within 5 years.

Although previous reports on the spinal cord hemangioblastomas with VHL are limited, Lonser *et al.*[14] examined 44 consecutive cases of patients who underwent 55 operations, and analyzed the relationship between clinical outcome and features. In their report, preoperative neurological function, the presence of a ventral or ventrolateral lesion and tumor size were correlated with postoperative neurological function and were useful as a predictor of outcome, but the presence of a syrinx was not a predictor of outcome. In our study, the preoperative neurological functions, age, number of simultaneously resected tumors and the presence of a syrinx were not correlated with the postoperative functions, but volume of the tumor was correlated with the functional change. The surgical outcome of the tumor volume $<500\,mm^3$ was better than $>500\,mm^3$. Before the tumor volume exceeds $500\,mm^3$ during follow-up by MRI, surgical treatment should be considered.

In addition, when function before and after the operation was evaluated according to McCormick's classification, 83% of the surgical cases remained unchanged or showed improvement. With respect to the functional outcome, this result indicates that spinal cord hemangioblastomas can be removed in the majority of patients without aggravation.

Central nervous system hemangioblastomas in VHL often have multiple periods of tumor growth separated by periods of arrested growth and exhibit stuttering growth patterns. Therefore, many untreated hemangioblastomas frequently remain asymptomatic, and do not require treatment for long intervals.[5,20] These natural history of CNS hemangioblastomas in VHL should be considered when determining the optimal timing of screening for individual patients and for evaluating the timing and results of treatment.

The VHL patients often have opportunities to undergo plural operations for spinal cord hemangioblastomas. In our study, seven patients underwent operations twice, with a mean interval of 9.5 years. However, the preoperative functional grade at the second operation for spinal cord hemangioblastomas decreased in comparison with the postoperative grade after the first operation. As VHL patients frequently develop multiple spinal cord hemangioblastomas and have opportunities for multiple operations, neurological aggravation should be avoided at the first operation for spinal cord hemangioblastoma with VHL, if possible.

In conclusions, most VHL patients bearing spinal hemangioblastoma also have a hemangioblastoma at some other site. Although most spinal hemangioblastomas are located in the intramedullary region and have a syrinx, the aggravation rate after surgical resection for spinal hemangioblastomas is low. Generally, spinal cord hemangioblastomas can be safely removed in the majority of patients in VHL. The prognosis of spinal cord hemangioblastomas in VHL disease is good as long as consequent yearly surveillance is performed. The probability of the multiple occurrence and treatments of

other VHL-related lesions should be taken into account when the timing of the surgery is determined.

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Neuronal Differentiation of Neural Progenitor Cells by Intracellular Delivery of Synthetic Oligopeptide Derived from Von Hippel-Lindau Protein

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Abstract: Intracellular delivery of synthetic oligopeptides has the potential to promote the occurrence of various cellular events such as cell death, proliferation, growth inhibition, metabolic changes, and morphological changes. However, the regulation of cellular differentiation by intracellular delivery of synthetic oligopeptides has been little studied. Von Hippel-Lindau protein (pVHL) is one of the proteins that functions to induce the differentiation of neural progenitor cells (NPCs). To function in these cells, pVHL forms a complex composed of itself, elongin BC, Clu-2, and Rbx-1. It is suggested that the binding site of elongin BC in pVHL plays a critical role in pVHL function, i.e., ubiquitination, which is related to neuronal differentiation. So, we synthesized an oligopeptide corresponding to the elongin BC binding site, and delivered the oligopeptide into NPCs by using a mixture of trifluoroacetylated lipopolyamine and diloeoyl phosphatidylethanolamine (BioPorter) to form a peptide-lipid complex. After intracellular delivery of the oligopeptide, induction of differentiation of NPCs was shown in terms of neurite outgrowth and by immunocytochemical and electrophysiological means. The intracellular delivery of the synthetic oligopeptide derived from pVHL may provide a safe and valuable approach for the neuronal differentiation of NPCs.

Keywords: Neuronal differentiation, neural progenitor cell, oligopeptide, intracellular delivery, von Hippel-Lindau protein.

INTRODUCTION

Intracellular delivery of synthetic peptides has the potential to promote various cellular events such as cell death [1], proliferation, growth inhibition [2], and metabolic changes [3] and to have transcriptional [4] and morphological [5] effects. Although some functional domains in proteins are responsible for the functions of the full-length proteins, peptides derived from these proteins and delivered intracellularly may have effects similar to those obtained by transfer of the gene encoding the full-length protein [6, 7]. Various proteins are involved in the regulation of neuronal differentiation of NPCs [8-23]. The induction of neuronal differentiation of NPCs by intracelllular delivery has greater advantages in regenerative medicine and clinical use than viral gene transfer, which has the potential for cellular toxicity [24], carcinogenesis [25], and genetic recombination [26]. Although the intracellular delivery of these proteins for such a purpose has been scarcely done, recently generation of neurons by intracellular delivery of PAX6 protein in NPCs has been reported [27]. Von Hippel-Lindau protein (pVHL) is one of the proteins that induce the neural differentiation of NPCs. The crystal structure of the pVHL published previously revealed a ß-sheet structure referred to as the ß-domain in the NH_2 terminus of pVHL and an α-domain consisting of α-helices in the COOH-terminus of pVHL [28]. The amino acid region 156–171 within the α-domain of pVHL was found to be a highly mutated region. This region binds with elongins B and C and CUL-2, a member of the cullin family of proteins [29]. It is thought to form a complex that closely resembles the yeast E3 type ubiquitin ligase complex, and this complex was shown to exhibit E3 ligase activity [30]. Thus, the amino acid 156-171 region of pVHL may play a critical role in the ubiquitination machinery that is involved in the degradation of a broad variety of cellular proteins. This machinery participates in neural differentiation by effecting the degradation of several factors [31-35]. Therefore, this 156-171 region in VHL may be related to neural induction, which is one of the functions of pVHL. Here, we tested our hypothesis that the intracellular delivery of the 156–171 amino acid region of pVHL would cause neural differentiation of NPCs.

MATERIALS AND METHODS

Peptide Design

The amino acid region 156–171 within the α-domain of pVHL binds with elongins C and B and CUL-2, and is thought to form a complex that closely resembles the yeast E3 type ubiquitin ligase complex [36]. It was shown that this complex can indeed exhibit E3 ligase activity. The amino acid residues 156-171 derived from the α-domain of pVHL is competitive with elongin A, which promotes messenger RNA elongation. Therefore, the synthesized peptide was designed as VHL 156-171 (YTLKERCLQVVRSLVK). As control peptides, two peptide mutants, L158V (YTVKERCL QVVRSLVK) and C162F (YTLKERFLQVVRSLVK), were designed.

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