20%	非常に重症、入院が必要で精力的な治療が必要
10%	死期が切迫している
0%	死

VHL 症例検討会は 3 回出席し、泌尿器科医の立場から、主に腎腫瘍、副腎腫瘍の診断、治療に関して意見を述べた。また遺伝子診断や当院での遺伝カウンセリングの現況についても紹介した。

D．考察

重症度基準については、これを用いて複数の自験例について判定を行ったところ、概ね適用できるとの印象をもった。今後、一般医にも使用してもらい使いやすさや再現性などを検証していくことが重要と考えられた。

また VHL 病は稀な全身性の難治疾患であり、患者ごとに合併疾患や全身状態が大きく異なることから、各領域専門医参加型の症例検討を継続し、またこれらの診療経験を集積していくことが重要と考えられた。

E．結論

VHL 病患者の重症度を判定するための分類を新規に作成し、その中で特に VHL 病に発症した褐色細胞腫に対する重症度基準を作成した。また各領域専門医が参加した VHL 病症例検討会に定期的に参加し、症例検討を積み重ねた。

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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

厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

フォン・ヒッペル・リンドウ病の診療指針に基づく診断治療体制確立の研究

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福島　敦樹　高知大学教育研究部医療学系眼科学
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【研究要旨】
VHLにおける網膜血管腫の重症度分類を作成した。程度分類は０～４とした。具体的には網膜血管腫の有無、網膜滲出性病変の有無、治療への反応性の有無により分類することとした。治療後で網膜血管腫を認めない場合は、視力で重症度を判断することとした。両眼性、片眼性により区別せず、視力低下の判定は障害程度等級表を参考とすることとした。

A．研究目的

１）VHL 病における網膜血管腫の重症度分類を作成する。

B．研究方法

１）網膜血管腫の有無、網膜滲出性病変の有無、治療への反応性の有無、視力、両眼性、片眼性などの観点から、VHL 病における網膜血管腫の程度分類を試みた。視機能障害の指標として障害程度等級表を参考とした。

C．研究結果

１．網膜血管腫の重症度分類の作成。以下の通りに分類。
０：網膜血管腫を認めない
１：網膜血管腫を認めるが、（網膜滲出性病変がないため）治療の必要がなく、日常・社会生活に問題なし（視力低下なし）
２：網膜血管腫を認め、（網膜滲出性病変に対する）治療によく反応して、日常・社会生活に問題なし（視力低下なし）
３：網膜血管腫を認め、（網膜滲出性病変への）治療に対する反応が不充分で、日常・社会生活に軽度の問題あり（視力低下あり）
４：網膜血管腫を認め、（網膜滲出性病変に対する）治療が困難で、日常・社会生活に支障が大きい（視力低下が著しい）

D．考察

VHL 病全体の重症度を判定するには、各臓器の重症度を判定し、総合的に評価する必要がある。網膜滲出性病変の有無、治療への反応性の有無を基本に網膜血管腫の重症度を分類した。視機能障害の評価は身体障害者程度等級表に準拠することにより社会通念上相当とした。本研究成果をもとに VHL 病網膜血管腫の重症を把握する疫学調査が必要になると考えられた。

E．結論

VHL 病における網膜血管腫の重症度分類

を作成した。

F．参考文献

1)　身体障害者程度等級表

G．研究発表

1. 論文発表　　該当なし

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

4. 厚生労働科学研究費補助金（難治性疾患克服研究事業）
分担研究報告書

フォン・ヒッペル・リンドウ病の診療指針に基づく診断治療体制確立の研究（膵神経内分泌腫瘍、膵のう胞）

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【研究要旨】
フォン・ヒッペル・リンドウ（VHL）病は常染色体優性遺伝性で各種の腫瘍が多発する難治性疾患で、主に中枢神経系と網膜血管芽腫、腎細胞癌、副腎褐色細胞腫、膵腫瘍、内耳リンパ嚢腫、精巣上体嚢腫が発症する。前年度までに膵病変について「フォン・ヒッペル・リンドウ（VHL）病診療ガイドライン」を作成、更に患者向けにVHL病の膵病変について解説したガイドブックを作成した。今回本研究班で、治療の必要性と日常・社会生活への影響度を主体とした重症度判定基準の作成に取り組み、我々は1）膵神経内分泌腫瘍、および、2）膵嚢胞について、それぞれ重症度判定基準を作成した。今後、これらの重症度分類について本邦VHL病患者に協力を依頼し、有用性の検証と問題点について再検討する予定である。昨年度、国内で初めてインターネットを用いた各診療科の専門医による症例検討会が行われ、本年度も継続した。これらの取り組みによりVHL病の病態解明はもとより、国内全体のVHL病診療の向上に寄与できると考える。

A．研究目的

フォン・ヒッペル・リンドウ（VHL）病は常染色体優性遺伝性で各種の腫瘍が多発する難治性疾患である。主に中枢神経系と網膜血管芽腫、腎細胞癌、副腎褐色細胞腫、膵腫瘍、内耳リンパ嚢腫、精巣上体嚢腫が発症する。発症頻度は欧米では3−4万人に1人とされる。しかし、国内での病態は不明であり、この病気に特化したガイドラインは発刊されていなかった。本研究班では、昨年度までにVHL病に合併する膵神経内分泌腫瘍と膵嚢胞について疫学的調査を行い、日本におけるガイドラインを作成1、2、更には患者用のガイドブックの作成・発刊3を行った。本年度は更に以下の3点を研究の主目的とした。1）作成したVHL病ガイドラインを実践し、その有用性を評価する。2）VHL病における疾患別の重症度基準を作成し、重症度判定と予後調査を行う。3）昨年度も施行したインターネット会議（3eConference）を用いたVHL病症例検討会の継続を行う。

B．研究方法

1）作成したVHL病ガイドラインを実践し、その有用性を評価する。

各施設にてガイドラインに基づいた VHL 病の診療を実践し、問題点や改訂点について引き続き検討していく。

２）VHL 病の重症度基準の作成と重症度判定について

特発性間質性肺炎、網膜色素変性症、再生不良性貧血、潰瘍性大腸炎、強皮症、パーキンソン病、筋委縮性側索硬化症の重症度分類が提示され、VHL 病に関する重症度判定基準に対して協議が行われたところ以下のような意見が出された。

１：各病態を何段階評価にするか統一すべき（4 段階評価が一般的）

２：機能と病変の範囲の両方で分類を作成すべき

３：患者さんの要望も大事なので、要望とすり合わせての分類の作成が重要

４：潰瘍性大腸炎でも入院日数や腫瘍の数を因子として加えたので、これらも加えるほうが良い。

これらの意見を元に各臓器別に重症度分類に対する検討が行われた。

３）インターネット会議（3eConference）を用いた VHL 病症例検討会

第 3 回は 9 月 27 日（木）、第 4 回は 12 月 26 日（木）に開催された。

C．研究結果

今年度は上記の２）、３）につき報告する。

２）VHL 病の重症度基準の作成と重症度判定について：膵病変

VHL 病に合併する膵病変には１）膵神経内分泌腫瘍、および２）膵嚢胞があり、下記のようにそれぞれ独立させた重症度判定基準を作成した。

膵神経内分泌腫瘍

PNET 0：膵神経内分泌腫瘍を認めない。

PNET1：膵神経内分泌腫瘍を認めるが経過観察で良く、日常・社会生活に支障なし。

PNET2：膵神経内分泌腫瘍を認め、治療が必要である。日常・社会生活に問題ないか、軽度の支障あり。

PNET3：膵神経内分泌腫瘍および遠隔転移を認め、治療が必要である。日常・社会生活に問題ないか、軽度の支障あり。

PNET4：膵神経内分泌腫瘍および遠隔転移を認め、治療が必要である。日常・社会生活に支障が大きい。

当初は疾患の進行度を主体とした重症度判定基準を提案したが、治療の必要性と日常・社会生活への影響度を主体とした上記の形に修正した。

膵嚢胞

PC0：膵嚢胞を認めない

PC1：膵嚢胞を認めるも症状なし。日常・社会生活に支障なし。

PC2：膵嚢胞により症状を認めるが、治療の必要がなく、日常・社会生活に支障は軽度である。

PC3：膵嚢胞により腹痛などの症状や膵内外分泌機能低下を認め、治療が必要である。日常・社会生活に支障は軽度である。

PC4：膵嚢胞により腹痛などの症状や膵内外分泌機能低下を認め、治療が必要である。日常・社会生活に支障が大きい。

３）インターネット会議（3eConference）を用いた VHL 病症例検討会

第 3 回：症例 1：37 歳、女性。多発性膵嚢

胞に対する方針と左腎腫瘍の治療方針と時期について討議された。

症例2：15歳、女性。これまでに2回小脳血管芽手術の既往あり。頚髄C3/4，頭蓋頚髄移行部の腫瘍に対する手術時期と、他臓器のスクリーニング時期について討議が行われた。

第4回：症例1：36歳、女性。両側腎癌に対し、右腎部分切除術＋腫瘍核出術、焼灼術、左腎腫瘍核出術、焼灼術施行。右腎背側に認められる緩徐に増大する腫瘍性病変の治療方針について討議された。

症例2：33歳、男性。小脳血管芽腫摘出術、左腎癌手術、腎嚢胞に対する手術の既往あり。腎癌・腎嚢胞に対する方針と脊髄と頭蓋頚椎移行部血管芽腫に対する治療方針が討議された。

症例3：23歳、女性。母親がVHL病と診断された際に遺伝相談を受け、VHL病と診断。右腎癌、右副腎領域の腫瘍、腎動脈下の傍大動脈領域の腫瘍についての治療方針が討議された。

D．考察

VHL病は難治性疾患であり、種々の腫瘍性疾患を併発する。同時に複数の腫瘍に罹患することも稀ではなく、治療に難渋し日常・社会生活に支障が出る患者も認められる。本研究では、VHL病の病態を疾患ごとではなく包括的に捉えるために重症度判定基準が提唱された。

問題点としては、1）重症度判定は一定期間もしくは、治療後に見直しを行うか、2）膵嚢胞や網膜血管腫などの生命予後に直接かかわらない疾患の重症度分類と、腎癌や褐色細胞腫などとを同等に扱ってもよいのか、などが挙げられた。今後、これらの重症度分類について本邦VHL病患者に協力を依頼、有用性の検討を行い問題点について再検討する予定である。

一方、インターネットによる症例カンファレンスは、今までに4回施行された。いずれも複数の臓器にわたって疾患を持ち単一診療科で治療方針を決定することが難しい症例を討議した。複数の異なった領域の専門家によるインターネットカンファレンスは、VHL病患者の診療において非常に有用であり、複数の診療科を患者が受診する必要がなかった。VHL病患者にとって理想的な診療体系の一つとみなされ、今後も継続すべきと考えられる。

E．結論

本研究班で平成24年度に行ってきた研究内容につき、膵病変について報告した。重症度判定基準については更なる検証と必要に応じた改訂が必要である。

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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

〔Ⅳ〕

平成24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
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研究成果の刊行に関する一覧表

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執印太郎、篠原信雄、矢尾正祐、他	von Hippel-Lindau 病全国疫学調査における腎癌の臨床的解析	日本泌尿器科学会雑誌	103	552-556	2012
執印太郎、矢尾正祐、篠原信雄、他	von Hippel-Lindau 病に伴う褐色細胞腫の特徴：全国疫学調査とその解析結果	日本泌尿器科学会雑誌	103	557-561	2012
菅野 洋	家族性脳腫瘍の基礎と臨床	BRAIN and NERVE－神経研究の進歩	64	557-564	2012

〔V〕

研究成果の刊行物・別刷

Acta Neurochir (2013) 155:1–7
DOI 10.1007/s00701-012-1514-y

CLINICAL ARTICLE

Clinical features of patients bearing central nervous system hemangioblastoma in von Hippel-Lindau disease

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Received: 10 July 2012 / Accepted: 25 September 2012 / Published online: 19 October 2012

Abstract
Background Central nervous system (CNS) hemangioblastoma (HB) is one of the most common manifestations in von Hippel-Lindau disease (VHL), but large-scale studies on clinical features of CNS HB in VHL are scarce.
Methods On the basis of the results of a questionnaire, we collected data of VHL patients with CNS HB.
Results The total number of CNS HBs in 111 VHL patients (male 59, female 52) was 264 with the following distributions: cerebellar, 65.4 %; brainstem, 9.9 %; spinal cord, 23.9 %; and pituitary, 1. 1 %. The follow-up period was 0.6 to 39.2 years, with the mean 12.5 years. Patients bearing brainstem or spinal cord HB also had another HB significantly more frequently than those bearing cerebellar HBs ($P<0.05$). The mean onset age of CNS HB was 29.1 years, and that of patients bearing a single HB (mean 34.4 years) was significantly greater than that of multiple HBs (mean 25.7 years). Patients with multiple HBs under 40 years are more dominant than those with a single HB. The distribution rate of brainstem HB is significantly smaller in patients below 30 years than patients above 29 years. Although ECOG PS score increased along with number of operations, the onset age decreased with increasing number of operations. The mean ECOG PS score of patients below 20 years is significantly smaller than patients above 19 years.
Conclusions When the onset age of CNS HB is under 40 years, and CNS HB is located at the brainstem or spinal cord HB, the probability of multiple occurrence can be predicted. Since patients with an onset age under 20 years old preserve a high performance status, early detection of CNS HB would be important. In addition, since a multiple operations aggravate performance status, number of operations should be reduced.

Keywords Central nervous system hemangioblastoma · Von Hippel-Lindau disease · Clinical features · Quality of life

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Introduction

Von Hippel-Lindau disease(VHL) is a familial neoplasia disorder with an autosomal dominant pattern of inheritance that results from a germline mutation in the *VHL* gene, and which is characterized by a predisposition to develop

multiple neoplastic lesions, including central nervous system (CNS) hemangioblastomas (HBs), retinal HBs, renal cell carcinomas (RCCs), pheochromocytomas (PhCs), pancreatic tumors (PTs), endolymphatic sac tumors (ELSTs), and epididymal cystadenomas (ECs) [9, 11–13]. VHL gene, located at chromosome 3p25 region, was isolated by a positional cloning approach [7] and shown to be mutated in the germline of VHL patients, as well as in sporadic tumors including CNS HBs [5] and RCCs [16]. Common CNS lesions in affected individuals include cerebellar, spinal cord, brainstem HBs, as well as retinal HBs, pituitary stalk HBs, and ELSTs [3, 4, 8–10, 13]. The annual incidence of VHL is approximately one in 36,000 live births and has over 90 % penetrance by 65 years of age [9, 11–13]. The earliest feature among manifested lesions in VHL patients is usually a retinal or cerebellar HB. At present, metastases from RCC and neurological complications from CNS HBs are the most common causes of death. Although the median survival of VHL patients previously did not reach 50 years, the prognosis of VHL patients has been improved and the complications related to VHL-associated lesions were reduced by comprehensive serial screening and routine follow-up, earlier diagnosis of lesions in VHL patients by computed imaging and laboratory studies including DNA tests, progress in treatment such as micorosurgery or radiotherapy for CNS HB and tumor enucleation for RCC, and increased knowledge of VHL [9]. At present, even small asymptomatic HBs in VHL patients are detected on an MRI without difficulty [11]. Since most VHL patients with a CNS HB also have other CNS HBs and/or visceral tumors, these patients might have opportunities to undergo treatment for other CNS HBs or visceral tumors in the future. Large-scale epidemiological studies involving over 100 patients have rarely been done, and the clinical features of CNS HBs in VHL disease are not yet fully defined. In addition, the previous studies mostly have treated a smaller number of only cerebellar or spinal cases; and therefore the clinical aspects and the quality of life of CNS HBs in VHL patients have not yet been fully elucidated [1, 17]. Here, focusing particularly on onset age of CNS HB, we describe the clinical features and quality of life of HBs in the CNS of VHL patients.

Materials and methods

VHL patients were gathered by the Japanese VHL Study Group in the Japanese Health & Labor Ministry during 2009–2010 via the results of a questionnaire (Table 1) answered by Japanese neurosurgeons at 1020 hospitals approved as training facilities for neurological surgery in Japan. The clinical diagnosis for VHL was made on the basis of the following criteria [9]. In the presence of a positive family history, VHL can be diagnosed clinically in a patient with at least one typical VHL tumor, retinal or CNS HB; RCC; PhC; and PT. ELSTs 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 they exhibit two or more CNS HB or a single HB in association with a visceral tumor such as RCC, PhC, and PT. All CNS HBs or visceral tumors were diagnosed on the basis of clinical histories, symptoms, histories, laboratory studies, and radiological findings made by CT or MR. Among the collected VHL patients, those bearing CNS HBs were investigated with respect to the following: patients' characteristics, location of CNS HB, onset age of CNS HB, follow-up period, number of operations for CNS HB, and radiation therapy. In addition, performance status (PS) at the point based on the results of the questionnaire was assessed according to the following Eastern Cooperative Oncology Group (ECOG) PS [14]. The relationship between the number of operations and the PS or onset age of CNS HB was also examined. Exclusion criteria were as follow: incomplete description about location of tumor, onset age or performance status. For statistical analysis, we applied the non-parametric Mann-Whitney's U test or Spearman's correlation coefficient rank test. Statistical significance was set at $P<0.05$. This study was conducted with the approval of the ethics committee of Kochi University School of Medici,ne which is the center of this study.

Results

According to the criteria described in the Methods section, 294 patients were defined as VHL according to results of the questionnaire. Among them, 200 (68.1 %) bore CNS HBs. Among these 200 patients, their tumor locations, onset age, ECOG PS,and follow-up period were clarified in 111 patients. Summary of data on CNS HBs in VHL patients is shown in Table 2. Among 111 patient, 2 died due to CNS HB at 44 and 47 years, and remaining 109 patients were living.

Among the 111 patients bearing CNS HBs, those bearing cerebellar ones were 92 (82.9 %); brainstem, 22 (20.7 %); spinal cord, 43(38.7 %); pituitary, 3 (2.7 %). Fifty-three patients had only cerebellar HBs (30, single tumor; 23, multiple ones). Ten patients had only spinal cord HB (seven, single tumor; three, multiple ones); and three patients had only brainstem HBs (two, single tumor; one, multiple ones). In addition, the result of the present study revealed that 83.7 % of the patients with spinal cord HB had another CNS HB at

Table 1 Questionnaire on central nervous system hemangioblstoma (CNS HB) in VHL

Onset age of CNS HB ( ) years old or the Year ( )				
Times of therapy	Age ( )years old or the Year ( ) Location □cerebellum □brainstem □spinal cord	○C(cervical)	○ T(thoracic)	□ tumor removal □ stereotactic radiotherapy □ external irradiation □ other therapy ○ L/S(lumbosacral)
The second time	( ) years old or the Year ( ) Location □cerebellum □brainstem □spinal cord	○C(cervical)	○T(thoracic)	□ tumor removal □ stereotactic radiotherapy □ external irradiation □ other therapy ○ L/S(lumbosacral)
The third time and following	( ) years old or the Year ( ) Location □cerebellum □brainstem □spinal cord	○C(cervical)	○T(thoracic)	□tumor removal □stereotactic radiotherapy □external irradiation □other therapy ○ L/S(lumbosacral)

ECOG Performance Status		
Check	Score	definition
○	0	Full active, able to carry on all pre-disease performance without restriction.
○	1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature.
○	2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of working hours.
○	3	Capable of only limited self-care, confined to bed or chair more than 50% of working hours.
○	4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
○	5	dead

the same and/or another site; and, similarly, 91.3 % of the patients with brainstem HB also had another CNS HB at the same and/or another site, and 68.4 % of the patients with cerebellar HBs had CNS HB at the same and/or another site (Fig. 1). The distribution of 63 spinal cord HBs is as follows: cervical, 50.6 %; thoracic, 37.0 %; lumbar, 12.3 %. The number of patients having undergone radiotherapy was 10, and all radiotherapies were performed with stereotaxic radiosurgery . In VHL patients below 40 years old, patients bearing multiple HBs are more dominant than those bearing single HB, while in VHL patients above 39 years old, those bearing single HB are more dominant than those bearing multiple HBs. The distribution rate of brainstem HB is

Table 2 Summary of data on CNS hemangioblastomas (HBs) in VHL patients

Characteristics	
Male/female	59/52
VHL patients with treated CNS HBs	108
VHL patients with untreated CNS HBs	3
Onset age of CNS HB (mean years±sd.)	7 to 73 (29.1±12.6)
VHL patients with a single HB	34.4±15.8
VHL patients with multiple HBs	25.7±9.8
Period of follow-up (mean years±s.d.)	0.6 to 39.2 (12.5±9.3)
ECOG Performance status (PS) (mean score±s.d.)	0.77±1.16
ECOG PS 0	63 (56.8 %)
ECOG PS 1	29 (26.1 %)
ECOG PS 2	8 (7.2 %)
ECOG PS 3	6 (5.4 %)
ECOG PS 4	3 (2.7 %)
ECOG PS 5	2 (1.8 %)
Distribution of all CNS HBs	264
Cerebellum	172 (65.2 %)
Spinal cord	63 (23.9 %)
Brainstem	26 (9.8 %)
Pituitary	3 (1.1 %)
Distribution of onset CNS HBs in VHL patients	111
Cerebellum	79 (71.2 %)
Spinal cord	21 (18.9 %)
Brainstem	10 (9.0 %)
Pituitary	1(0.9 %)
Total number of operation	251
Times of operation per patient (mean tumes±s.d) 1 to 9	1 to 9 (2.2±1.8)

significantly higher in patients below 30 years old than patients above 29 years old ($P<0.01$) (Table 3).

The onset age of VHL patients with a single HB (34.4± 15.4 years) was significantly higher than the patients with multiple HBs (25.7±12.6 years) ($P<0.001$). Similarly, the mean onset age of patients bearing a single cerebellar HB (33.8 ±15.0 years) was significantly higher than that of patients bearing multiple cerebellar HBs and/or HBs at another site (26.2 ±8.9 years). Likewise, in the case of patients bearing a single spinal cord HB, this mean age (35.9 ±17.5 years) was significantly higher than that of patients bearing multiple spinal cord HBs and/or HBs at another site (24.4 ±12.1 years old) ($P<0.01$). Patients bearing multiple HBs are more dominant than those bearing single HB in patients below 40 years old, while those bearing single HB are significantly more dominant than those bearing multiple HBs in patients above 39 years old ($P<0.01$) (Table 3).

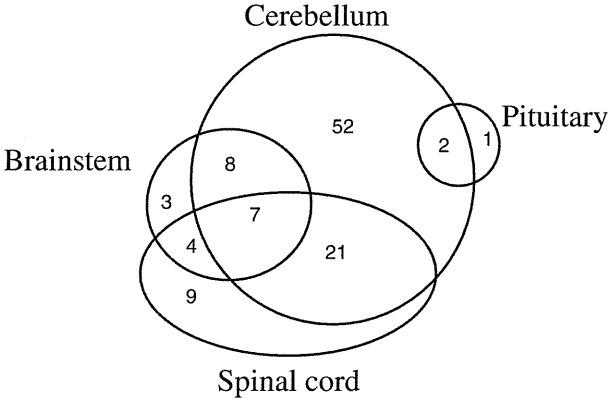

Fig. 1 Distribution of CNS HBs in 111 VHL patients

ECOG PS was assessed based on the results of the questionnaire. Those patients having low ECOG PS scores ($PS=0, 1$) were 82.9 %, while higher ECOG PS scores ($PS\geq 2$) were 17.1 % of the total 111 patients. The relationship between ECOG PS score and the onset age of CNS HB showed a tendency that patients having a lower PS score were at a lower onset age, but there was no significant correlation ($P=0.06$). The mean ECOG PS score of patients below 20 years old was significantly smaller than patients above 19 years old ($P<0.01$). The mean ECOG PS of patients with a single CNS HB (0.34±0.76) was significantly lower than that of patients with multiple CNS HBs (0.87± 1.14) ($P<0.005$). In addition, the mean ECOG PS of patients bearing cerebellar HB was 0.67±1.04, and the mean ECOG PS of patients bearing a single cerebellar HB (0.24± 0.69) was significantly lower than that of patients bearing multiple cerebellar HBs and/or HBs at another site or single cerebellar HB with HB at another site (0.87±1.12, $P<0.005$). The mean ECOG PS of patients bearing a spinal cord HB was 0.88±1.1. The mean ECOG-PS of patients bearing spinal cord HB was 0.88±1.1, and that of those bearing a single spinal cord HB (0.69±0.95) was not significantly lower than that of patients bearing multiple spinal cord HBs and/or HBs at another site HB or a single spinal cord HB with an HB at another site (0.93±1.20, $P=0.46$). ECOG PS score of VHL patients bearing a single CNS HB is significantly smaller than that of those bearing multiple CNS HBs in all onset age groups ($P<0.05$).

Among 111 VHL patients, 108 patients underwent a total of 251 operations, while the remaining three patients did not undergo treatment for CNS HB. All three patients with untreated CNS HB were above 39 years old, with a mean of 42.7 years old. Among these three patients with untreated CNS HB, two have one cerebellar HB and one has two CNS HBs (cerebellum, 1 and spinal cord, 1). ECOG PS scores of patients with untreated HBs were as follows: two patients, PS 0; and one, PS 4. The follow-up period of patients with untreated HB ranged from 1 to 7 years (mean 5 years). On the other hand, the number of

Table 3 Onset age of CNS HB and other clinical features

Onset age of CNS HB (years)	−19 (*N*=26)	20–29 (*N*=41)	30–39 (*N*=24)	40- (*N*=20)
Male/Female	11/15	26/15	14/10	9/11
Single/Multiple	7/19	17/24	8/16	15/5
Follow-up period	13.54±9.14	13.61±8.92	13.54±10.87	7.5±6.81
Total number of CNS HB	75	103	62	24
Mean number of CNS HB	2.88±1.97	2.51±1.80	2.58±1.86	1.2±0.52
Distribution of all CNS HB	C47/B12/S15/P1	C58/B12/S33/P1	C52/B1/S9	C15/B1/S6/P1
Distribution of onset CNS HB	C19/B2/S4/P1	C28/B6/S7	C18/B1/S5	C14/B1/S5
Total number of operations	67	101	63	20
Mean number of operations	2.58±1.94	2.46±1.83	2.63±1.95	1±0.65
Mean ECOG PS score	0.29±0.46	0.73±1.11	0.83±1.34	0.89±1.18
ECOG PS score single	0	0.5±1.03	0.13±0.35	0.77±1.17
ECOG PS score multiple	0.41±0.51	0.88±1.15	1.19±1.51	1.2±1.3

operations per patient with treated CNS HBs ranged from 1 to 9, with a mean of 2.2±1.8. In the first operation, cerebellar HB was dominant; but in the second operation, the rate of the spinal cord HB operation increased. In the spinal cord operations for HB, the first and third operations were dominantly performed at the cervical level, while the second operation was dominantly at the thoracic one (Fig. 2). As to the relationship between number of operations and ECOG PS, the ECOG PS score significantly increased together with the operation number ($P<0.001$). In contrast, in the relationship between operation number and onset age of CNS HB (Fig. 3), the latter significantly decreased with increasing number of operations ($P<0.005$); with a mean of 32. 9 years for one operation; a mean of 26.2 years for two operations; and a mean of 23.9 years for three and more operations. As to the relationship between number of operations and onset age of CNS HBs, the mean numbers of CNS HB and operation for CNS HB in onset age of VHL patients above 39 years old are significantly smaller than other onset age groups ($P<0.05$) (Table 3, Fig. 3).

Discussion

Approximately two-thirds of CNS HBs are sporadic in origin, while the remaining third are associated with VHL. HBs in the CNS are a common feature in VHL patients, and are often also accompanied by such lesions at other sites, although sporadic CNS HBs are almost always universally solitary. Approximately two-thirds of VHL patients bear

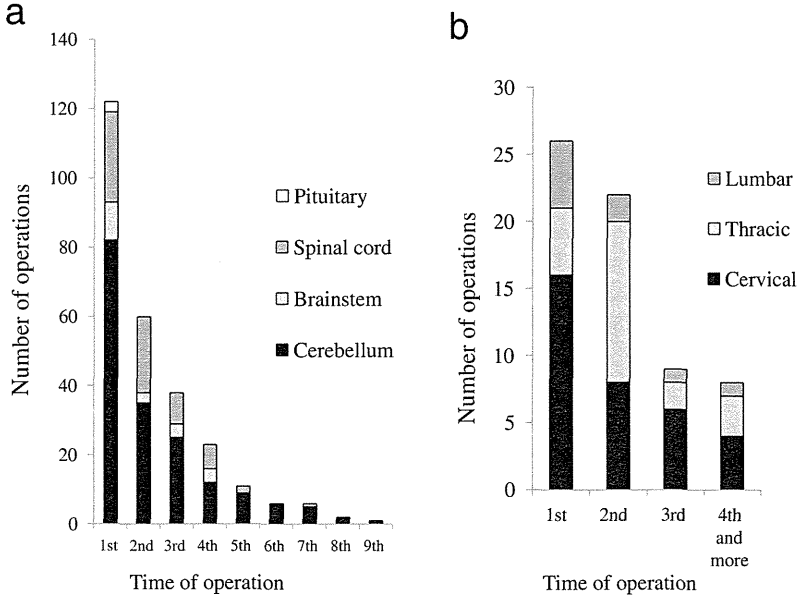

Fig. 2 Sites of CNS HBs with respect to operation number are shown. **a** Cerebellar HBs were always the most dominant, while spinal cord and brainstem HBs were always the second and the third, respectively. The proportion of spinal cord HBs increased with operation number, particularly in the second operation. **b** Number of spinal cord HBs with respect to spinal cord level and operation number. In the first operation, the cervical level was the most dominant location; whereas in the second, the thoracic was the most dominant one. In the third and fourth operations, the cervical level was the most dominant

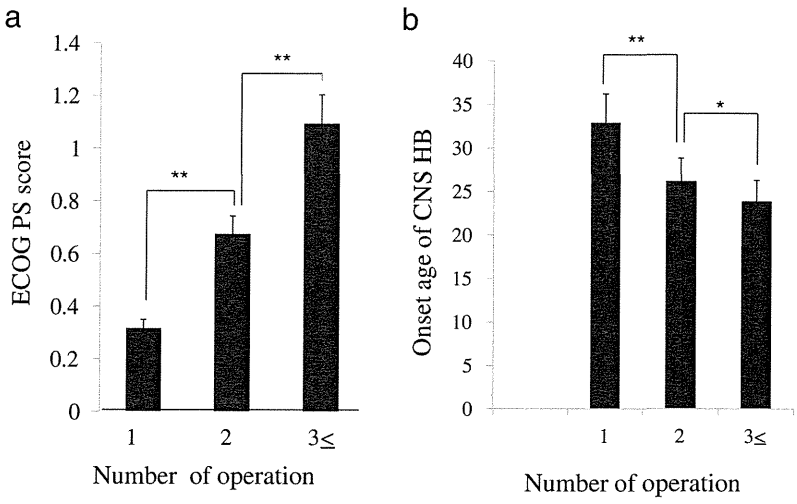

Fig. 3 Relationship between number of operation and ECOG performance status (PS) score or onset age of CNS HB is shown. **a** The ECOG PS score was significantly positively correlated with the number of operations. **b** Relationship between number of operation and onset age (years) of CNS HB is inversely correlated with number of operation. $^{**}P<0.01$, $^{*}P<0.05$

CNS HBs; and among these HBs, cerebellar are the most dominant, and spinal cord ones are next, with brainstem HBs being third [12, 13, 15]. Another frequently found site is the pituitary [16]. In contrast, such lesions in the cerebral intraparenchymal region or intraventricular region are rare. These frequently found regions match those in which hematopoietic embryonic stem cells reside [17]. The HB distribution in this present study is similar to that found in previous studies [1–3, 12, 13].

Spinal cord HB or brainstem HB is frequently also associated with an HB at some other site, mostly cerebellar HB; whereas cerebellar HB is less frequently associated with HBs at other locations [14]. These findings suggest that spinal cord or brainstem HB is usually an accompanied manifestation of cerebellar HB and that cerebellar HB is often an independent pathology. In other words, when a spinal cord HB or a brainstem HB is found in a patient, such a patient should be predicted to have another manifested lesion associated with VHL, particularly a CNS HB; and this possibility should be explored.

Our present study showed that the ECOG performance status score was positively correlated with the number of operations and with the onset age of CNS HB. VHL patients frequently undergo multiple operations for CNS HBs, but multiple operations aggravate performance status. If possible, the number of operation for CNS HBs should be reduced. The present study showed that the first operation was mostly for cerebellar HB and that there was an increase in the proportion of spinal cord HB, particularly thoracic, at the second operation. This change in proportion from the first operation to the second one would be expected to affect performance status. When a CNS HB is identified, and the patient is diagnosed as VHL at an age under 40 years, we can thus predict that another HB will appear in the CNS in the future, particularly in brainstem or spinal cord HB. In contrast, when a CNS HB is identified in the cerebellum and the patient is diagnosed as VHL over 39 years of age, we can predict that another CNS HB will not appear later. Our present study also showed that a single HB was different from multiple HBs in terms of onset age of CNS HB and performance status. The onset age of CNS HB in VHL patients bearing a single HB was significantly older than that for VHL patients bearing multiple HBs. This present study showed that VHL patients will probably have only a single HB when the onset age of CNS HB is over 39 years, but that they will have multiple HBs under the onset age of 40 years. This result indicates that scheduled follow-up is necessary if onset age is under 40 years but that it is not always necessary if onset age is above 39 years. In addition, the present study also showed that the ECOG PS score of patients below 20 years old was significantly smaller than that of patients above 19 years old. This result suggests that early detection of CNS HB in VHL patients and annually follow-up is important, and that thereby the annual follow-up may provide patients opportunities to undergo adequate treatment and may contribute to preserve a high performance status of patients. However, further studies are required to confirm the findings of this study, because the number of VHL patients bearing CNS HB above 39 years or below 20 years are small in this study.

Conclusion

When the onset age of CNS HB is under 40 years and CNS HB is located in brainstem or spinal cord, the probability of multiple occurrence of CNS HBs can be predicted and close scheduled follow-up is necessary. We would emphasize that since the onset age under 20 years old patients preserve high performance status, early detection of CNS HB in VHL patients and annually follow-up would be important, and that since multiple operations aggravate performance status in VHL patients, number of operations had better be reduced.

Acknowledgments This work was supported by a grant-in-aid for scientific research No. 228 from the Ministry of Health and Labor of Japan.

Conflict of interest None.

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The VHL tumor suppressor protein regulates tumorigenicity of U87-derived glioma stem-like cells by inhibiting the JAK/STAT signaling pathway

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Received November 8, 2012; Accepted December 17, 2012

DOI: 10.3892/ijo.2013.1773

Abstract. The signal transducer and activator of transcription 3 (STAT3) factor plays an important role in the tumorigenicity of cancer stem cells. The purpose of this study was to investigate the inhibitory mechanism of this pathway acting through the tumor suppressor von Hippel-Lindau (VHL) protein in glioma cancer stem cells. We isolated floating neurosphere-forming $CD133^+$ cells as glioma stem-like cells (GSLCs) by the MACS method. Furthermore, we examined these cells for their growth rate, ability to form colonies and neurospheres in soft agar, capacity for implantation into SCID mice and expression of CD133, STAT3, JAK2, Elongin A, PTEN and VHL. Furthermore, we transferred the VHL gene, an inhibitor of STAT3, into GSLCs using an adenovirus vector and compared these transfectants with control vector-transfected GSLCs. GSLCs proved to be implantable and formed a tumor in the subcutaneous tissue of SCID mice, the histology of which was similar to that of human glioblastomas. In addition, GSLCs exhibited a high capacity for soft agar colony and neurosphere formation, nearly all of which were CD133 positive. The majority of GSLCs were immunopositive for STAT3, JAK2 and Elongin A, but immunonegative for PTEN and VHL. When the *VHL* gene was transferred to GSLCs and these cells were transplanted into SCID mice, they did not result in tumor formation. Their capacity for soft agar colony and neurosphere formation was significantly inhibited, although their proliferation was only moderately inhibited. Regarding the expression of various factors, that of CD133 was decreased in the VHL transfectants and those of STAT3, JAK2 and Elongin A were eliminated. However, the expression of PTEN and of VHL was upregulated. These findings suggest that VHL regulated the tumorigenicity and self-renewal ability of glioma cancer stem cells by inhibiting the JAK/STAT signaling pathway.

Introduction

Glioblastoma is one of the most intractable tumors of the central nervous system and is often fatal. Complete remission is rarely achieved. Compared to 30 years ago, the survival time has been prolonged only by a few months (1). Although chemotherapy, usually by oral temozolomide, may prolong survival of glioblastoma patients, it does not significantly affect survival. Bevacizumab, which is an anti-VEGF antibody, also extends survival time by approximately 2 months (2); it is not, however, curative. To date, the majority of glioblastoma patients succumb to the disease within 1 year of initial diagnosis. The therapeutic resistance of glioblastoma possibly results from the presence of cancer stem cells. The cancer stem cell theory does not apply to all types of cancer, but glioblastoma is considered a type of cancer possessing cancer stem/initiating cells (3), which have been isolated from the U87 human glioma cell line (4). The characteristics of glioma cancer stem cells include CD133 positivity, self-reproduction ability, neurosphere formation in non-serum medium containing growth factors, tumorigenicity in SCID mice, chemotherapy and radiotherapy resistance and cellular hierarchy. Signal transducer and activator of transcription 3 (STAT3) was reported to play a critical role in the tumorigenicity of glioma-initiating cells (5,6). Inhibition of translation of STAT3 mRNA by STAT3 siRNA results in inhibition of cell proliferation and self-renewal (7). In addition, the expression of microRNA (miRNA)-21 in glioma-initiating cells is negatively regulated by activated STAT3 (8). It has been suggested that the JAK/STAT pathway is repressed by the von Hippel-Lindau (VHL) protein (9) and that BC-box proteins, including VHL, possibly inhibit the JAK/STAT pathway by binding to Elongin BC (10). Upregulated expression of PTEN coordinately inhibits the PI3K/Akt pathway (11) as well as VHL and PTEN function (12). Glioma stem-like cells (GSLCs) are suggested to be in a hypoxic environment (13). Furthermore, it was recently reported that TRAIL and paclitaxel synergize to kill U87-derived GSLCs *in vitro* (14) and that miRNA-34a suppresses cell proliferation and induces apop-

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Key words: glioma stem-like cells, von Hippel-Lindau protein, tumorigenicity, JAK/STAT pathway