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Advances in Brief

Somatic Mutations of the Von Hippel-Lindau Tumor Suppressor Gene in Sporadic Central Nervous System Hemangioblastomas

Hiroshi Kanno, Keiichi Kondo,[1] Susumu Ito, Isao Yamamoto, Satoshi Fujii, Soichiro Torigoe, Naoki Sakai, Masahiko Hosaka, Taro Shuin,[2] and Masahiro Yao[2]

Departments of Neurosurgery [H. K., S. I., I. Y., S. F.], Urology [K. K., N. S., M. H., T. S., M. Y.], and Radiology [S. T.], Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236, Japan

Abstract

Hemangioblastoma is one of the benign tumors in the central nervous system. It is often associated with the von Hippel-Lindau (VHL) disease, a well known hereditary tumor syndrome. It is believed that inactivation of both alleles of *VHL* tumor suppressor gene is essential in the tumorigenic processes in hemangioblastomas associated with VHL disease. The molecular basis for the development of sporadic hemangioblastomas is not known. Here, we analyzed 13 cases of primary sporadic hemangioblastomas for somatic mutations of *VHL* gene with single strand conformational polymorphism analyses of the tumor DNAs. We detected abnormal single strand conformational polymorphism pattern in 7 tumors (54%). Of these 7 possibly mutated tumors, we successfully characterized 3 tumors by direct sequencing. We were unable to sequence 4 tumors because of the poor quality of DNA obtained from paraffin blocks. Somatic mutations in the 3 tumors were 2 missense mutations and 1 microdeletion. These mutations were observed in 1 tumor in exon 1 and 2 tumors in exon 2. Our results suggest that mutations of *VHL* tumor suppressor gene are involved in the development of at least 20% of sporadic central nervous system hemangioblastomas.

Introduction

Hemangioblastomas are rare tumors of the central nervous system and account for only 1–2% of all intracranial tumors (1). Although hemangioblastomas are usually treated successfully with surgical resection, some tumors show a frequent recurrence (2). Familial hemangioblastomas develop in a well known autosomal dominant hereditary tumor syndrome, VHL[3] disease, which is characterized by frequent development of retinal angiomas, pancreas and kidney cysts, renal cell carcinomas, and pheochromocytomas (3). However, the genesis of sporadic hemangioblastomas is not well understood. Recently a tumor suppressor gene for VHL disease was isolated (4). Knudson's theory predicts that the *VHL* gene could be mutated in the tumors that are sporadic counterparts of the tumors found in the von Hippel-Lindau disease. Thus far somatic *VHL* gene mutations have been detected in close to 60% of sporadic clear cell renal carcinomas. However, somatic *VHL* gene mutations were not detected in sporadic pheochromocytomas.[4] No reports have appeared on sporadic hemangioblastomas. Therefore, we examined the alterations of *VHL* tumor suppressor gene in sporadic hemangioblastomas. Here, we showed that *VHL* tumor suppressor gene was mutated in at least 20% of sporadic hemangioblastomas.

Materials and Methods

Materials. A total of 13 primary sporadic hemangioblastomas and matched peripheral blood samples were collected at Yokohama City University Hospital (Table 1). One tumor specimen was frozen tissue and the rest were paraffin-embedded tumors. None of the patients have a family history of the von Hippel-Lindau disease. These patients were diagnosed as having sporadic hemangioblastomas from standard screening tests, including detailed family histories and pedigree analyses, physical neurological and opthalmological examinations, abdominal ultrasound imaging, and radiological examinations. They did not have the tumors that are hallmarks of VHL disease (retinal angiomas, renal cell carcinomas, pancreatic cysts, or pheochromocytomas). All tumors were classified according to WHO pathological classification.

DNA Extraction. The high molecular weight DNA from one frozen tumor and those from peripheral blood samples were extracted by standard procedures. DNAs of 12 tumors were extracted from paraffin-embedded sections according to the method of Sarkar (5). Paraffin-embedded tissues were cut into 5-μm sections and then paraffin was removed with xylen and tissues were washed with ethanol. One hundred μl of cell lysis buffer (10 mM NaCl-10 mM EDTA-0.45% Tween 20) and 10 mg of proteinase K were added to the tissues and they were slowly shaken at 50°C for 48 h. Finally they were boiled for 15 min and supernatant centrifuged at 10,000 × g that contains tumor-derived DNA as used for the analysis.

SSCP Analysis and DNA Sequencing. PCR and SSCP analyses were performed as described previously (Fig. 1), except that [P^{32}]-deoxy ATP (Amersham, England) was included for PCR and subsequent SSCP analysis in the DNAs of paraffin-embedded specimen (6). PCR products were sequenced directly according to the manufacturer's protocol (United States Biochemical Corp.) using Dynabeads (Dynal, Inc., Oslo, Norway) (Table 2).

Results and Discussion

We detected abnormal electrophoresis patterns in 7 of 13 tumor DNAs by DNA-SSCP analyses (Table 1). These SSCP abnormalities were not detected in the peripheral blood DNAs in the same patients. We then performed sequence analyses in these 7 PCR products. We were able to confirmed the presence of mutations in 3 of the 7 PCR products by direct sequencing. Direct sequencing was not successful in 4 tumors with SSCP changes because of the poor quality of DNA. Sequence analyses revealed 2 cases of missense mutation and 1 case of deletion (Table 1). These mutations were confirmed as somatic mutation by comparing the tumor DNAs with those in peripheral blood DNAs of the same patients. These mutations occurred at nucleotide 476 (G to C) in exon 1 and nucleotide 618 (A to C) in exon 2; a deletion of nucleotide 663 (T) occurred in exon 2 (Table 1). Typical results are shown in Figs. 2 and 3. Case 12 shows abnormal bands in SSCP analysis (Fig. 2). Sequence analysis reveals that case 12 has a microdeletion at nucleotide 663 (T) (Fig. 3). Interestingly, 3 of 7 cases (42%) with an abnormal electrophoresis pattern in SSCP analyses showed a recurrence of tumor (Table 1); in contrast, none of the 6 cases with normal SSCP patterns showed tumor recurrence.

Central nervous system neuroaxial hemangioblastomas occur in more than 50% of patients with VHL disease. Previous studies on

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[1] H. K. and K. K. contributed equally to this work.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: VHL, von Hippel-Lindau; SSCP, single strand conformational polymorphism; PCR, polymerase chain reaction.

[4] J. Gnarra, submitted for publication.

Table 1 *Pathological and clinical features and mutations of VHL* gene in hemangioblastomas

Case No.	Pathological feature	First date of surgery	Region of tumor	Recurrence of tumor	Site of SSCP change	*VHL* gene mutation
1	HB[a]	1983	Cerebellum			
2	HB	1984	Cerebellum		Exon 1	NT 476 G to C (trp to ser)
3	HB	1987	Cerebellum	+	Exon 3	ND
4	HB	1987	Cerebellum	++	Exon 2	NT 618 A to C (leu to phe)
5	HB	1986	Cervical spinal cord			
6	HB	1987	Cerebellum			
7	HB	1986	Cerebellum			
8	HB	1982	Cerebellum			
9	HB	1983	Cerebellum		Exon 1	ND
10	HB	1989	Cerebellum			
11	HB	1985	Cerebellum		Exon 2	ND
12	HB	1993	Cervical spinal cord	+	Exon 2	Deletion NT 663, T
13	HB	1985	Cerebellum		Exon 3	ND

[a] HB, capillary hemangioblastoma; ND, unable to perform sequence analysis due to the poor quality of DNA; ++, tumor recurrence observed more than two times; +, tumor recurrence observed one time; NT, nucleotide number in VHL complementary DNA; trp, tryptophan; ser, serine; leu, leucine; phe, phenylalanine.

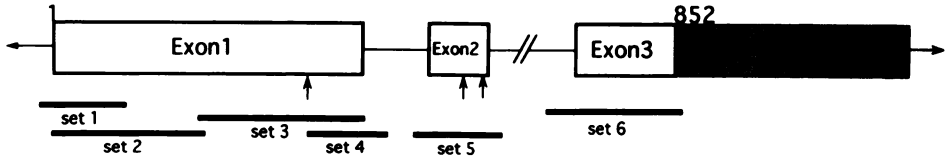

Fig. 1. Sites of *VHL* gene mutation and ranges of PCR with 6 sets of primers. *Arrows*, 3 sites of VHL gene mutations detected in sporadic hemangioblastomas; □, 3 exons; ▨, untranslated region of exon 3.

tumor suppressor genes revealed that various sporadic tumors are characterized by somatic inactivation of genes responsible for hereditary tumor syndromes. We and others recently showed frequent somatic mutations and loss of heterozygosity of the *VHL* disease gene in sporadic clear cell renal carcinomas, also one of the major manifestations of the VHL disease (6, 7). Although hemangioblastoma is one of the major manifestations of the VHL disease, the molecular basis for the development of sporadic hemangioblastoma is not known. Our results show that mutations of the *VHL* gene are involved in at least 20% of sporadic hemangioblastoma. Since the quality in some of the tumor DNAs is poor because they were derived from paraffin-embedded and old specimens, we were unable to analyze the loss of heterozygosity by a polymorphic base change at nucleotide 19 in VHL complementary DNA sequence.

Interestingly, hemangioblastoma apparently has similar pathological features to metastatic clear cell renal carcinoma in the central nervous system. Therefore, it is sometimes difficult to pathologically differentiate metastatic renal cell carcinomas from sporadic hemangioblastomas (8). This fact suggests that alterations of the same gene may contribute to the appearance of similar morphological features in these 2 types of tumors with different origins.

Central nervous system hemangioblastomas include 2 cell types from the pathological viewpoint (endothelial cells and lipid-filled interstitial cells). It is not known which of these 2 cell types is a neoplastic cell. Microdissection of sporadic tumors combined with mutation detection may provide an answer.

Previous clinical studies showed that hemangioblastomas of multicentric origin and those developed from the VHL disease are major risk factors for tumor recurrence. Since our result showed that 3 of 7 cases with abnormal SSCP pattern showed tumor recurrence, mutations of the *VHL* gene might be another risk factor for tumor recurrence.

Former histopathological studies suggested that human hemangioblastomas are classified into several subgroups by the pathological features and immunohistochemical staining. Interestingly, recurring hemangioblastomas have lower frequencies of cyst formations and a lower proportion of lipid-laden stromal cells (2). Further detailed studies with molecular biological analysis on the *VHL* gene, together

Table 2 *PCR primer sets for SSCP analysis of VHL* gene

Set	Exon		Sequence of primers
1	1	Upstream	5′-AAATACAGTAACGAGTTGGCCTAGC-3′
		Downstream	5′-GTCGACCTCCGTAGTCTTCG-3′
2	1	Upstream	5′-CCTAGCCTCGCCTCCGTTACAAC-3′
		Downstream	5′-GTACTCTTCGACGCCTGCCTACC-3′
3	1	Upstream	5′-TGGTCTGGATCGCGGAGGGAAT-3′
		Downstream	5′-CCCAGCTGGGTCGGGCCTAAGCGCCGGGCCCGT-3′
4	1	Upstream	5′-CTGCCCGTATGGCTCAACTTCGA-3′
		Downstream	5′-TGCTATCGTCCCTGCTGGGT-3′
5	2	Upstream	5′-GTGGCTCTTTAACAACCTTTGC-3′
		Downstream	5′-CCTGTACTTACCACAACAACCTTATC-3′
6	3	Upstream	5′-TTCCTTGTACTGAGACCCTAGT-3′
		Downstream	5′-AGCTGAGATGAAACAGTGTAAGT-3′

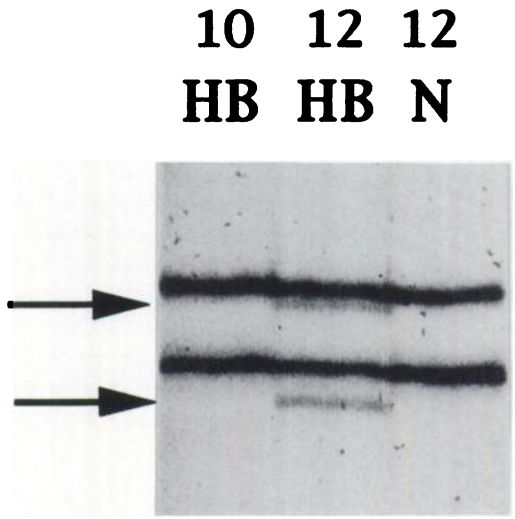

Fig. 2. SSCP analyses of DNAs from tumor samples and normal blood. A typical result of SSCP analysis is shown. *Top*, case numbers; *N*, normal DNA from the patient's blood; *HB*, hemangioblastoma. PCR and subsequent gel electrophoresis were performed using primer set 5 that covers exon 2. Case 10 and normal blood DNA from patient's blood show normal electrophoresis pattern, whereas case 12 showns abnormal bands in SSCP analysis *(arrows)*.

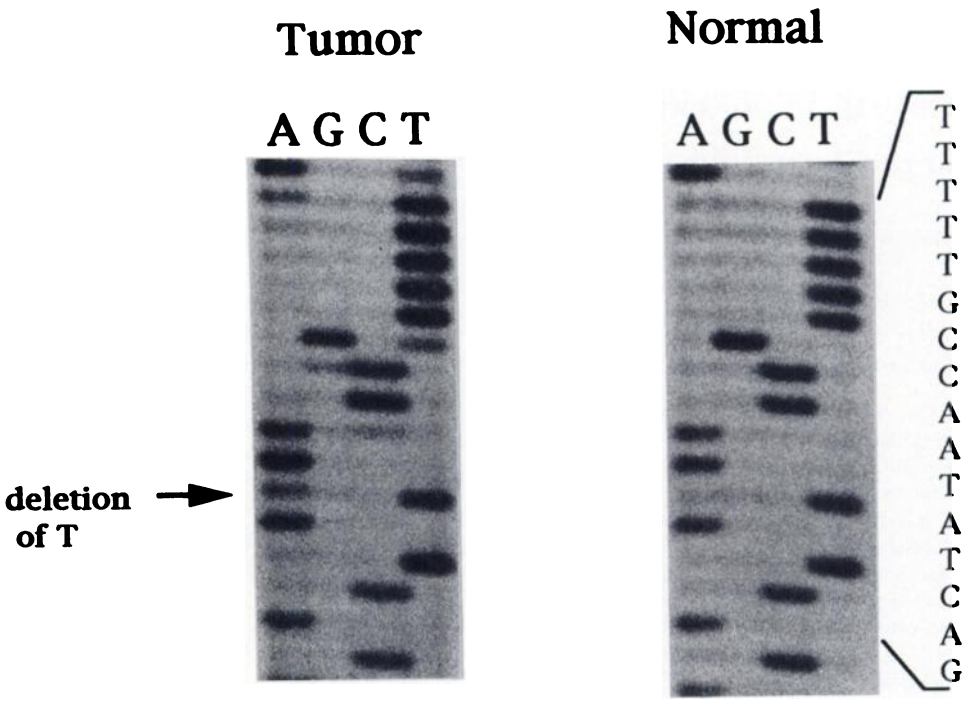

Fig. 3. Sequence analysis of PCR products in a sporadic hemangioblastoma. Tumor (case 12) and normal sequences are shown. *Right ordinate*, normal sequences. Tumor (case 12) has a deletion of nucleotide 663 (*T*).

with immunohistochemical technique, should be performed to distinguish a special type of hemangioblastoma from the previously defined classical hemangioblastomas.

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