Somatic Mutations of the von Hippel-Lindau Tumor Suppressor Gene and Loss of Heterozygosity on Chromosome 3p in Human Glial Tumors

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Abstract

Molecular genetic analysis of von Hippel-Lindau tumor suppressor gene (VHL gene) was performed on 38 tissues of human glial tumors (ependymoma, 1; astrocytoma, 6; oligodendroglioma, 1; oligoastrocytoma, 2; anaplastic oligoastrocytoma, 3; anaplastic astrocytoma, 14; glioblastoma multiforme, 11). Somatic DNAs extracted from frozen tumor specimens were examined by single-strand conformational polymorphism analysis and direct sequencing. In addition, loss of heterozygosity (LOH) on chromosome 3p in 15 glial tumor cases, lymphocyte DNAs of which were available, was examined by use of 10 microsatellite probes and two polymorphism markers for the VHL gene. Two cases of low-grade gliomas showed somatic sense mutations in exon 3 of the VHL gene, and 6 of 15 cases (40.0%) showed LOH of chromosome 3p. The VHL gene-mutated cases also showed LOH. The retention of heterozygosity and high pathological grade of glial tumors were correlated significantly. In addition, Kaplan-Meier survival analysis for patients with glial tumors showed that patients with LOH had a significantly longer survival time than those without LOH. These results suggest that somatic mutations on 3p, including the VHL gene, may be involved in tumorigenesis of some low-grade glial tumors.

Introduction

The von Hippel-Lindau (VHL) disease tumor suppressor gene was isolated by positional cloning at chromosome 3p25-26 (1). The human VHL gene encodes a protein of 213 amino acids. The predicted protein contains an acidic pentameric repeat. The VHL gene has the characteristics of a classic tumor suppressor gene; i.e., loss of the wild-type allele has been demonstrated in renal cell carcinoma patients with VHL disease, and somatic mutations of the gene have been detected in sporadic renal cell carcinomas and central nervous system hemangioblastomas with a loss of heterozygosity (2-4).

Somatic mutations of other tumor suppressor genes, such as p53 (5), Rb-1 (6), and p16 (7) have been demonstrated in malignant gliomas. Glioma tumors do not commonly occur as a manifestation of VHL disease. However, recently, a VHL family that manifested low-grade gliomas was reported (8). Both gliomas and hemangioblastomas are vascular-rich central neuroaxial neoplasms, and glioblastomas, and somatic mutations of the gene have been detected in sporadic renal cell carcinomas and central nervous system hemangioblastomas with a loss of heterozygosity (2-4).

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Received 11/26/96; accepted 1/27/97.

The abbreviations used are: VHL, von Hippel Lindau; LOH, loss of heterozygosity; SSCP, single-strand conformation polymorphism.
and subsequent gel electrophoresis were performed with a primer set, which covers exon 3. Case 25 shows an abnormal band in SSCP analysis (additional band).

![Fig. 1. SSCP analyses of DNAs from tumor samples. A typical result of SSCP analysis is shown. G25, glial tumor case 25 (oligoastrocytoma); Normal, normal blood DNA. PCR and subsequent gel electrophoresis were performed with a primer set, which covers exon 3. Case 25 shows an abnormal band in SSCP analysis (additional band).](image)

**Table 1 Data of glial tumors with VHL gene mutations**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age/Sex</th>
<th>Pathological feature</th>
<th>Tumor grade</th>
<th>Region of tumor</th>
<th>VHL gene mutation</th>
<th>Site of mutation</th>
<th>Consequence</th>
<th>Site of amino acid</th>
<th>LOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>73/F</td>
<td>Astrocytoma</td>
<td>II</td>
<td>Right temporal lobe</td>
<td>GCA → GTA</td>
<td>Nucleotide 833</td>
<td>Alanine → Valine</td>
<td>Codon 207</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>44/M</td>
<td>Oligoastrocytoma</td>
<td>II</td>
<td>Left frontal lobe</td>
<td>CGG → TGG</td>
<td>Nucleotide 841</td>
<td>Arginine → Tryptophan</td>
<td>Coden 210</td>
<td>+</td>
</tr>
</tbody>
</table>

![Fig. 2. Direct sequencing analysis reveals a point mutation (transversion from cytosine to thymine) at nucleotide number 841, indicating a missense mutation (from arginine to tryptophan). NT, nucleotide; G25, glioma case 25.](image)

The LOH analysis was examined by the comparison of DNAs from the leukocytes and tumor tissues by use of 12 probes (7 microsatellite probes for 3p, 3 microsatellite for 3q, and 2 VHL polymorphism markers). The analyses were performed on 15 astrocytic tumor cases (low-grade glial tumor, 5; high-grade glial tumor, 10) for which blood samples were available. The analysis using those probes revealed LOH on chromosome 3p, including the VHL gene locus in six cases (40.0%) and retention of heterozygosity in eight cases (60.0%). LOH shown in six cases was positioned in 3q11–3p26. The analysis using those probes revealed a significant difference between cases with LOH and those without LOH (Cox-Mantel test; \( P < 0.05 \)). The result showed longer survival time of patients with 3p LOH than of those without LOH (Student’s t test, \( P < 0.05 \)). In addition, Kaplan-Meier analysis for patients analyzed for 3p LOH revealed a significant difference between cases with LOH and those without LOH (Cox-Mantel test; \( P < 0.05 \)). The result showed longer survival time of patients with 3p LOH than of those without LOH.

**Discussion**

Mutations in several tumor suppressor genes are predicted to cause the genesis of glial tumors. Frequent somatic mutation of \( p53 \) has been demonstrated in glial tumors. Fults et al. (11) revealed LOH greater than 10% on 3p, 5q, 7p, 10p, 10q, 11p, 13q, 14q, 15q, 16p, 16q, 17p, 17q, and 18q in malignant glial tumors. The locus of \( p53 \) has been located on 17p. However, the relationship between frequency of LOH on the other chromosomes and mutations of other suppressor genes has not been reported in glial tumors. Using one marker probe of EFD145, an 11% frequency of LOH on 3p was found by Fults et al. (11). His report suggested that a suppressor gene on 3p might be related to the genesis of glial tumors.

Whaley et al. (16) identified VHL gene somatic mutations in 33% of sporadic renal cell carcinomas but did not find any in other carcinomas that were usually not associated with the VHL disease. They proposed that the VHL gene plays an important role in the etiology of sporadic renal cell carcinomas and suggested that functional domains in the 3' end of the reading frame of the VHL gene were critical to the growth-suppressive function of the VHL protein. The predicted protein of the VHL gene contains an acidic pentameric repeat that has homology to the acidic repeat domain in the procysoline surface membrane glycoprotein of *Trypanosoma brucei* (1).

Recently, the VHL protein was shown to bind tightly and specific to Elongins B and C, which activate transcription elongation by RNA polymerase II, and to inhibit Elongin (S III) transcriptional activity (17), suggesting that the VHL protein may play an important role in the transcriptional regulatory network that controls tumorigenesis. Recent results showed that wild-type VHL protein regulated expression of the hypoxia-induced genes such as vascular endothelial growth factor.
factor. The VHL protein inhibits the cellular expression of vascular endothelial growth factor, platelet-derived growth factor B chain, glucose transporter GLUT1 in hypoxic condition, but not in normoxic condition (18). It is supposed that the VHL protein regulates the mRNA stability of these genes at the posttranscriptional level by interacting with Elongins B and C (19). We detected somatic sense mutations in 2 of 33 glial tumors. Both mutations were positioned at the terminal end of the downstream portion of the VHL tumor suppressor gene. In addition, these two cases of glial tumors also showed LOH in 3p. The function of the VHL gene has not been elucidated fully. However, these present mutation in glial tumors may cause the loss of function of the VHL protein, a protein that is related to the transcriptional regulatory network. Thus, this process may lead to the genesis of glial tumors. In addition, the high incidence of LOH on 3p in glial tumors may also suggest the involvement of the VHL tumor suppressor gene. Interestingly, many of LOH-detected cases displayed benign low-grade tumors, whereas retained heterozygosity was found to be dominant in the malignant high-grade tumors. This result suggests that some suppressor genes in 3p, including the VHL gene, may cause tumorigenesis of benign glial tumors. Recently, a VHL family, some members of which had cerebellar benign astrocytomas, was reported (8). This suggests that VHL gene mutations may be related to the development of some astrocytomas.

In addition, VHL gene expression in the central nervous system of adult and fetal human tissues was shown by in situ mRNA hybridization in nerve cells of the cerebral cortex, midbrain, cerebellum, and spinal cord (20). The widespread expression of the VHL gene in the central nervous system suggests that the occurrence of hemangioblastomas and benign glial tumors can be related to a mutation of the VHL gene.

Acknowledgments

We thank Yoko Ibuka for technical assistance.

References


Fig. 5. Kaplan-Meier analysis of patients with glial tumors. Patient with LOH (thin line, ■) and without LOH (thick line, ○) on chromosome 3p, including the VHL gene locus, were compared. Bars on the survival curves indicate patients alive at the indicated time. The Cox-Mantel test showed a significant difference (P = 0.0192).
VHL GENE MUTATION AND 3p LOH IN GLIOMAS


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