Somatic von Hippel-Lindau Gene Mutations Detected in Sporadic Endolymphatic Sac Tumors

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Abstract

Endolymphatic sac tumors (ELSTs) occur sporadically or in association with an autosomal dominantly inherited tumor syndrome, von Hippel-Lindau (VHL) disease. In VHL disease, a germline mutation of the VHL tumor suppressor gene is inherited, and loss of function of the wild-type allele occurs through genetic deletion with subsequent development of neoplastic growth. Genetic alterations associated with sporadic ELSTs are less well understood. In this study, we used tissue microdissection to selectively analyze neoplastic cells from four sporadic ELSTs. In two cases, we detected somatic mutations involving VHL gene exons 1 and 2, respectively. Additionally, one of these cases revealed deletion of the VHL gene locus. Two cases did not reveal VHL gene mutation; one of these two cases showed VHL gene deletion. These results suggest that mutations and allelic deletions of the VHL tumor suppressor gene play a role in the tumorigenesis of sporadic ELSTs.

Introduction

VHL disease has been characterized by a variety of neoplasms including hemangioblastomas of the central nervous system, renal cell carcinomas, pheochromocytomas, and cysts involving the pancreas, kidney, and epididymis (1, 2). The VHL tumor suppressor gene responsible for VHL disease has been mapped to chromosome 3p25 (3) and subsequently identified (4). Previous studies on renal cell carcinomas (5), pheochromocytomas (6), hemangioblastomas (7), and pancreatic cystadenomas (7) from patients with VHL disease support Knudson’s hypothesis that both an inherited germline mutation and loss of function of the wild-type allele of the VHL gene are essential for the development of these neoplasms.

Only recently ELSTs have been observed in association with VHL disease (8, 9). Evidence for the definite association of ELSTs with VHL disease first has been provided by the demonstration of the frequent occurrence of ELSTs in the VHL patient population (10). Subsequently, identification of both germline mutation and VHL gene wild-type deletion strongly suggested a causative association between VHL disease and ELSTs (11–13).

In sporadic tumors, tumorigenesis is thought to be initiated by somatic alteration of both alleles of a tumor suppressor gene. Accordingly, allelic deletions at 3p25 and mutations of the VHL gene have been documented in sporadic renal cell carcinomas (14), hemangioblastomas (15) and cystic lesions of the pancreas (7) and epididymis (16). Genetic changes of the VHL gene, however, have not yet been investigated in sporadic ELSTs. In this study, we analyzed four ELSTs from patients without evidence of VHL disease for the presence of allelic deletions and somatic mutations at the VHL gene.

Materials and Methods

Tumors. Anonymized samples were obtained from four tumors. Cases 1–3 were female; case 4 was male. Patients were 26, 37, 43, and 49 years old at the time of tumor removal (cases 1–4, respectively). None of the patients had a previous history of neoplastic disease.

Microdissection. Six-micron sections, obtained from paraffin-embedded material from four ELSTs (Fig. 1) after formalin fixation, were deparaffinized in xylene, rinsed in ethanol from 100% to 70%, and briefly stained with H&E. A modified microdissection procedure was performed under direct-light microscopic visualization using a 30-gauge needle as described previously (5). For comparison, samples of nontumor control tissue were obtained from the same slides in all cases.

DNA Extraction. Procured cells were immediately resuspended in 25 μl of buffer containing Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 1% Tween 20, and 0.1 mg/ml proteinase K and were incubated at 37°C for two days. The mixture was boiled for 10 min to inactivate the proteinase K and 1.5 μl of this solution was used for PCR-amplification of DNA.

Mutation Analysis and DNA Sequencing. PCR-based SSCP analyses were performed using primers covering the open reading frame of the VHL gene in the presence of [α-32P]-dCTP. After detection of mutation alleles, DNA was extracted from excised bands of the SSCP gel by overnight incubation in 100 μl of distilled water at room temperature, and subsequently PCR-amplified. The amplified PCR products were used for DNA sequencing (Perkin-Elmer, Cyclin Sequencing Kit).

LOH Analysis. LOH analysis was performed using polymorphic primers D3S1038 and D3S1110 mapped to the VHL gene locus 3p25/26 in the presence of [α-32P]-dCTP. Amplified products were separated on a 6% polyacrylamide gel.

Results and Discussion

DNA was successfully procured from four archival sporadic ELSTs and normal control tissue by tissue microdissection. Extracted DNA was PCR-amplified with primers covering the open reading frame of the VHL gene. VHL gene mutation analysis was performed using SSCP gel electrophoresis and sequencing analysis (Fig. 2). Aberrant bands in the SSCP gel were detected in two cases (cases 1 and 4). VHL gene deletion analysis revealed LOH of the VHL gene locus in two cases (cases 2 and 4).

In case 1, sequencing analysis revealed an inactivating G-to-A transversion (Gly to Asp) in exon 2, codon 114 (Fig. 2a). In addition, the mutation was confirmed by BstE II restriction enzyme digestion of amplified DNA (Fig. 2). The mutation was only present in tumor cell samples and was not detected in the normal tissue control samples. VHL gene deletion analysis of case 1 did not reveal LOH (Fig. 3). Retention of heterozygosity in case 1 is further documented by the presence of both wild-type bands and mutation bands after amplification of exon 2.

Sequencing analysis of case 4 showed a one-nucleotide (G) deletion of exon 1, codon 4, resulting in frameshift (Fig. 2b). The mutation was...

[References and further details follow.]
only present in tumor cell samples and was not detected in the normal tissue control samples. No wild-type DNA could be visualized in microdissected tumor tissue of case 4, which is indicative of both genetic mutation and wild-type deletion in this tumor (Fig. 2). As expected, LOH of the VHL gene locus was detected by deletion analysis in this tumor (Fig. 3).

Cases 2 and 3 did not reveal aberrant mutation bands upon SSCP analysis. Analysis with polymorphic markers flanking the VHL gene, however, revealed deletion of the VHL gene locus in case 2. Case 3 showed no evidence of VHL gene deletion or mutation.

ELSTs are rare papillary tumors that may involve the temporal bone and extend into the posterior cranial fossa (17, 18). Although recent studies suggested ELSTs to represent a manifestation of VHL disease (10–13), the occurrence of ELSTs appears not to be confined to patients with VHL disease. Instead, ELSTs may occur sporadically, i.e., in patients unaffected by VHL disease. It has been unknown whether genetic changes of the VHL gene play a role in the tumorigenesis of these sporadic ELSTs. In the present study, we investigated mutations and deletions of the VHL gene in sporadic ELSTs.

Inactivating “somatic” mutations were detected in 2 ELSTs (cases 1 and 4) involving exons 2 and 1, respectively. Furthermore, genetic analysis revealed evidence for both VHL gene mutation and deletion in case 4, whereas the wild-type VHL allele appeared to be preserved in case 1. One additional case revealed VHL gene deletion without VHL gene mutation. In all cases, regular wild-type VHL alleles were detected in normal control tissue confirming the presence of two wild-type alleles and excluding the presence of VHL disease.

The results parallel those of other “sporadic” VHL-associated tumors and indicate that mutations and deletions of the VHL gene are associated with ELST tumorigenesis with or without VHL disease. However, the proposed “two-hit” mechanism (19) for hereditary VHL disease-associated tumors including genetic sequence alterations of both VHL alleles does not consistently occur in sporadic ELSTs. It is, however, possible that additional genetic changes involving the VHL allele contribute to tumor initiation and/or progression. In addition, VHL gene inactivation secondary to CpG island hypermethylation of the VHL gene promoter (20) may represent another mechanism of VHL gene inactivation.

Fig. 1. Histopathology of investigated cases 1–4, characterized by papillary proliferations that are lined by a single row of cuboidal epithelial cells.
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Fig. 2. Mutation analysis of cases 1 and 4. WT, wild type sequence; mut, mutation sequence. A, case 1 with inactivating G to A missense mutation of exon 2 of the VHL gene (top). Presence of mutation was confirmed by BstEI restriction enzyme (G/GTNACC) digestion of amplified DNA. Below: a and b, normal tissue; c and d, tumor tissue; a and c are undigested control tissue, b and d were digested with BstEI. The 208-bp-long PCR product from normal tissue contains a BstEI site and can be cut into a 175-bp and a 33-bp fragment (the 33-bp bands are not shown); the mutation band lost the BstEI site and hence remained undigested. B, case 4 with one nucleotide (G) deletion of exon 1, codon 4 resulting in frameshift (Fig. 2b).

Fig. 3. LOH analysis of cases 2 (left) and 4 (right) with representative marker D3S1038 amplifying the VHL gene locus. Both cases show loss of the upper allele in microdissected tumor tissue (T). Heterozygosity is retained in normal non-neoplastic tissue (N).

In summary, we report genetic alterations of the VHL gene in three of four sporadic ELSTs. Genetic changes of the VHL gene may play a prominent role during the histogenesis of these tumors.

References

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