Third International Meeting on von Hippel-Lindau Disease

Berton Zbar, William Kaelin, Eamonn Maher, and Stephane Richard

Laboratory of Immunobiology, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland 21702 [B. Z.]; Dana Farber Cancer Institute, Boston, Massachusetts 02115 [W. K.]; Division of Medical and Molecular Genetics, University of Birmingham, Birmingham B15 2TT, England [E. M.]; and Neuro-Oncologie EPHE, Department of Nephrology, Necker Hospital, Paris 75743, France [S. R.]

Abstract

Five years after the identification of the von Hippel-Lindau (VHL) gene, physicians, scientists, and concerned VHL family members met to review the current state of knowledge on the diagnosis and treatment of VHL and to summarize the latest information on the biochemistry of the VHL protein (pVHL). The NIH and University of Pennsylvania groups reported the detection of germ-line mutations in 100% (93 of 93) of VHL families studied. Several studies determined the frequency of VHL germ-line mutations in individuals with a single manifestation of VHL without a family history of VHL. National groups to improve the diagnosis and treatment of individuals with VHL disease have been established in Great Britain, Denmark, France, Holland, Italy, Japan, Poland, and the United States. Evidence for the existence of genes that modify the expression of VHL was presented. The VHL protein appears to have several distinct functions: (a) down-regulation of hypoxia-inducible mRNAs; (b) proper assembly of the extracellular fibronectin matrix; (c) regulation of exit from the cell cycle; and (d) regulation of expression of carbonic anhydrases 9 and 12.

Introduction

Five years after the identification of the VHL gene, physicians, scientists, and concerned VHL family members met to review the current state of knowledge on the diagnosis and treatment of VHL and to summarize the latest information on the biochemistry of the VHL protein (pVHL).

Genetic Diagnosis of VHL

The NIH and University of Pennsylvania groups (NCI, Bethesda, MD; and the DNA Diagnostic Laboratory, University of Pennsylvania, Philadelphia, PA) reported the detection of germ-line mutations in 100% (93 of 93) of VHL families studied (1). The improvement in detection of germ-line VHL mutations was attributed to the use of quantitative Southern blotting, and fluorescence in situ hybridization to detect deletions of the entire VHL gene (1). Deletions of the entire VHL gene were not detected by procedures ordinarily used for VHL mutation detection. The improved ability to detect germ-line VHL mutations should enable clinicians to distinguish disorders that simulate VHL from VHL and to facilitate identification of germ-line mutations in VHL families whose mutations were not detected previously, making genetic testing available in these families. The use of long-range PCR, a nonradioactive method to detect germ-line deletions in the VHL gene, was reported by Dr. Cybulski (Hereditary Cancer Center, Szczecin, Poland). Overlapping sets of primers were used in long-range PCR to detect germ-line deletions. This method for mutation detection may be helpful when limited amounts of DNA are available. However, long-range PCR will not replace quantitative Southern blotting, or FISH for detection of large germ-line deletions. The Polish group is sequencing the breakpoints to determine whether there are hot spots for germ-line deletions in the VHL gene. The Polish group also identified VHL disease caused by germ-line deletions restricted to exon 2 of the VHL gene.

Detection of Germ-Line VHL Mutations in Patients without a Family History of VHL

The diagnosis of VHL disease is established when an individual with a family history of VHL develops a single manifestation of the disorder, for example, a clear cell renal carcinoma, retinal angioma, or pheochromocytoma. Several studies determined the frequency of VHL germ-line mutations in individuals with a single manifestation of VHL without a family history of VHL. These studies provide valuable information for physicians seeing patients with these disorders.

H. Neumann (University of Freiburg, Freiburg, Germany) reported that 1.6% of 189 German patients with sporadic renal carcinoma had a germ-line VHL mutation. F. J. Hes (Utrecht University, Utrecht, the Netherlands) found that 5% (1 of 22) of patients with isolated CNS hemangioblastoma had a germ-line VHL mutation. S. A. McKee (West Midlands Regional Genetics Service, Birmingham, United Kingdom) reported that 1 of 42 patients with an isolated CNS hemangioblastoma studied to date had a germ-line VHL mutation. From a national study of retinal angiomas in the United Kingdom (Addenbrookes Hospital, Cambridge, United Kingdom; and University of Birmingham, Birmingham, United Kingdom), it was estimated that individuals with an isolated retinal angioma had a 30% probability of having VHL.

F. J. Hes studied 17 patients with pheochromocytoma without a family history of VHL. (7 with solitary, 4 with multiple or recurrent, 3 with familial, and 3 with bilateral pheochromocytomas). No germ-line VHL mutations were detected in this group of patients with isolated pheochromocytoma. S. Giraud (Hôpital Édouard Herriot, Lyon, France) studied 6 kindreds with familial pheochromocytoma, 5 patients with isolated bilateral pheochromocytoma, and 11 patients with isolated unilateral pheochromocytoma. Germ-line VHL mutations were detected in two of six kindreds with familial pheochromocytoma and in four of five patients with isolated bilateral pheochromocytomas.

Formation of National Groups to Study VHL

National groups to improve the diagnosis and treatment of individuals VHL disease have been established in Great Britain, Denmark, France, Holland, Italy, Japan, Poland, and the United States. T. Shuin (Kochi Medical School, Kochi, Japan) reported that 28 Japanese VHL families were identified recently, bringing the total number of Japanese VHL families to 62. Dr. Shuin surveyed university hospitals in Japan to identify the treatments for VHL renal cancer in use in these hospitals. Dr. Shuin prepared a VHL web site in Japanese. C. J. M. Lips (Utrecht University) reported that 24 VHL families have been...
identified in the Netherlands (~50% of the estimated number of Dutch VHL families; Ref. 2). A national network has been set up to promote early detection and improve clinical management.

Drs. G. Opocher and A. Murgia (University of Padua, Padua, Italy) have organized a coordinated working group for the diagnosis and treatment of VHL patients. This service sees patients from northern Italy. Facilitated access to different clinical specialists is provided along with a coordinated follow-up. Thus far, 10 Italian VHL families have been identified. Dr. K. Krzystolik (Hereditary Cancer Center, Szczecin, Poland) has established a Polish VHL Registry. Seventeen unrelated Polish VHL families have been identified. S. Richard (Hôpital Necker, Paris, France) described the French cooperative VHL working group, which was organized in 1990. Data are available on 650 VHL affected individuals in 165 distinct VHL families. A reference program for managing VHL families is being developed in Denmark (E. Kjeldsen; Clinical Genetics, KKA, Odense, Denmark).

Genotype-Phenotype Correlations

No specific type of germ-line VHL mutation was associated with endolymphatic sac tumors, or retinal angiomas. H. Neumann identified several new, missense germ-line VHL mutations associated with VHL type 2A (pheochromocytoma without renal carcinomas). C. Beroud (INSERM, Hôpital Necker, Paris) described a VHL mutation database (3).5

Pancreatic Tumors in VHL

P. Choyke (NIH, Bethesda, MD) found solid pancreatic tumors in 12% of the NCI VHL patients. Metastases from VHL pancreatic tumors were observed in 2% of NCI VHL patients. Computerized axial tomography with contrast enhancement was the best method to screen for pancreatic tumors in VHL patients. P. Hammel (Hôpital Beaujon, Clichy, France), in a large series of VHL patients studied with abdominal computed tomography scan, demonstrated an incidence of 70% for all pancreatic manifestations.

VHL Disease: Evidence for Modifier Genes

Variation in disease severity among VHL related family members (with the identical germ-line mutation) has been observed frequently. E. Maher, A. Webster, and coworkers looked for evidence of gene(s) modifying the effects of germ-line VHL mutations (4). These workers compared the number of retinal angiomas in the eyes of closely related VHL family members to the number of retinal angiomas in the eyes of distantly related family members. All patients were examined by a single ophthalmologist eliminating bias attributable to differences between observers. The number of retinal angiomas in the eyes of closely related families members was more similar than the number of retinal angiomas in the eyes of distantly related family members. This observation supported the concept that there are gene(s) that modify the number of retinal angiomas in VHL patients. Furthermore, the retinal angioma number predicted the course of VHL later in life. The greater the number of retinal angiomas, the greater the problem with renal cell carcinoma and hemangioblastoma will be in subsequent life (4).

VHL Animal Models

J. Gnarra (Louisiana State University, New Orleans, LA) described his attempts to produce an animal model for VHL disease. Gnarra replaced the wild-type VHL gene with a mutant allele lacking exons 2 and 3 and part of exon 1 (5). Mice that were homozygous for the mutant allele died during embryonic life.

The cause of death appeared to be defective placenta formation. The trophoblasts failed to differentiate; the placentas in VHL –/+ mice were avascular. VHL +/- mice, which have been observed for 2 years, have shown no increased tumor formation. A number of possible explanations were raised for the failure of renal tumors to form in the VHL +/- mice, including: (a) insufficient number of precursor cells and (b) the possible role of other tumor suppressor genes/oncogenes in murine renal carcinoma. The phenotype of the VHL –/– mice was similar to the phenotype of VEGF –/– mice. Gnarra also presented evidence for interaction of the VEGF and the MET proto-oncogenes.

Functions of the VHL Protein

W. Kaelin (Dana-Farber Cancer Institute, Boston, MA) and A. Pusic (Max-Planck Institute for Biochemistry, Martinsreid, Germany) presented studies on the function of the VHL protein (pVHL). The VHL protein appears to have several distinct functions (6–8). The function that has been studied most intensively is the regulation by VHL of hypoxia-inducible mRNAs including the VEGF mRNA. The VHL protein down-regulates VEGF. An understanding of how pVHL regulates VEGF has come from studies of proteins that bind to pVHL. pVHL has been found to form a tetramer with elongins B and C and Cul2. This tetramer bears structural similarities to tetrinamers in yeast that participate in degradation of cellular proteins. VHL may participate in the degradation of proteins that regulate expression of VHL target genes.

Cells with mutant VHL proteins failed to make a fibronectin extracelluar matrix (9). This defect could be corrected by transfection of the wild-type VHL gene. VHL binds, at least indirectly, to fibronectin associated with endoplasmic reticulum.

Pause reported that wild-type VHL was required to exit the cell cycle (10). When renal carcinoma cells with wild-type VHL were exposed in culture to low serum concentration, the cells exited the cell cycle and entered G0; renal carcinoma cells with mutant VHL were unable to exit the cell cycle and continued proliferating. Pause reported that the VHL-elongin B/C complex-bound NADD8.

B. Zbar (NCI, Frederick, MD) reported studies by S. Ivanov and M. I. Lerman (NCI, Frederick) that identified carbonic anhydrases 9 and 12 as target proteins of the VHL gene (11). VHL down-regulates carbonic anhydrases 9 and 12. Mutant VHL proteins that lacked the elongin binding domains had no ability to down-regulate CA9 and CA12. Mutant VHL proteins with intact elongin binding domains retained some ability to down-regulate CA9 and CA12. Of particular interest, other workers independently described the presence of a unique antigen on the surfaces of clear cell renal carcinoma cells. This antigen (MN), the target of antibody (G250), is identical to CA9. In other words, a cell surface antigen that is characteristic of clear cell renal carcinoma cells is regulated by the gene, that, when mutated, leads to clear cell renal carcinoma.

Experimental Treatment of VHL

D. Gross (The Hebrew University Hadassah Medical Center, Jerusalem, Israel) treated irradiated nude mice grafted with a paraganglioma from a VHL type 2A patient. Gross showed inhibition of tumor growth by treatment with lonidamine (quinoline-3-carboxyamide), an antiangiogenic drug. In both the prevention and intervention experiments, tumor size was decreased in the drug-treated mice. Histological examination of grafted tumors showed a decrease in blood vessels in the tumors of treated mice. Kaelin reported plans for clinical trials of drugs that inhibit binding of VEGF with receptor.
Treatment of VHL Renal Carcinoma

The “3-cm rule” was described by Zbar, speaking for the Urological Oncology Branch, NCI. The policy followed by the Urological Oncology Branch in the management of VHL patients with renal tumors is to delay surgery until one of the renal tumors is ≥3 cm in diameter, as measured by computerized axial tomography. No metastases were detected in 54 patients followed at the NCI with renal tumors <3 cm in diameter (12). Nephron-sparing surgery was the preferred surgical approach in VHL patients with renal carcinoma requiring surgery (12, 13). The average observation period was 5 years. Y. Chrétién (Hôpital Necker) reported on the surgical treatment of 41 patients with VHL renal cell carcinoma. Patients were treated either by nephron-sparing surgery or ex vivo (bench) surgery. Early severe postoperative complications occurred after ex vivo (bench) surgery. In the French series, no metastases were observed with renal carcinomas in VHL patients with tumors <4 cm in diameter. In Neumann’s series of VHL patients, no metastases were observed with renal carcinomas in VHL patients with tumors <7 cm in diameter. D. Goldfarb (The Cleveland Clinic Foundation, Cleveland) described a review of 65 patients treated for VHL renal cell carcinoma (12). Seventy-five % of patients were treated by nephron sparing surgery. The 5- and 10-year survivals were 100 and 81%, respectively. S. Huson (Oxford Radcliffe Hospital, Oxford, United Kingdom) reported a VHL patient with a 2.5–3.0-cm renal carcinoma with metastases to the spinal cord.

Treatment of CNS Hemangioblastomas

S. Richard reported that CNS hemangioblastoma remains the most frequent cause of death in the French series of 215 VHL patients (14, 15). M. Niemala (Department of Neurosurgery, Helsinki) evaluated stereotactic radiosurgery as an alternative to microsurgery for the treatment of hemangioblastomas. Solitary small or medium hemangioblastomas usually shrank or stopped growing after radiosurgery. The recommended margin dose is 10–15 Gy. The adjoining cysts did not respond to radiosurgery but required evacuation, sometimes repeatedly.

Comparative Sequence Analysis of the VHL Tumor Suppressor Gene

E. R. Woodward (Department of Pathology, Cambridge University, Cambridge, United Kingdom) sequenced the VHL gene in a range of primates, Old World monkeys, and New World monkeys. Conservation of homology of the elongin and Sp1 binding domains was found at the amino acid level in all species analyzed, but changes in nucleotide sequence were detected.

Adnexal Papillary Cystadenomas of Probable Mesonephric Origin

Graft (VHL Family Alliance, Boston) described the characteristics of papillary cystadenoma of mesonephric origin (formerly called “broad ligament cysts”). The tumors occur in tissues associated with the mesonephric tubules near the ovaries and uterine tubes and in remnants of the mesonephric duct close to the lateral walls of the uterus and vagina.

Individuals who attended the Third International Meeting on VHL disease felt that considerable progress had been made in germ-line VHL mutation detection and VHL protein biochemistry. Germ-line VHL mutations were found to be an infrequent cause of sporadic renal carcinoma, pheochromocytoma, and hemangioblastoma. The formation of VHL National Working Groups is a worthy development that is likely to improve the care of VHL patients and their families. The report of genes modifying the VHL phenotype, and the predictive effect of retinal angioma number on subsequent development of renal tumors and CNS hemangioblastomas was exciting. It will be important to confirm these findings. Members look forward to the Fourth International Meeting on VHL.

Recommendations for Future Research Initiatives

(a) Development of an animal model to study VHL disease is a high priority. Because VHL mice die in utero, it will be necessary to use techniques such as conditional gene knockout to produce cell populations that lack the VHL gene product. Development of a VHL animal model may produce considerable knowledge about pathogenesis of VHL and provide a model for evaluating experimental treatments.

(b) The high frequency of germ-line VHL mutation detection made by the NCI group must be verified. It will also be important to verify the observations made by the Birmingham/Cambridge groups on retinal angioma number.

(c) It will be important to identify and characterize the proteins that bind to the VBC-CUL2 complex and to demonstrate the VBC-CUL2 complex transports proteins to the proteosome for proteolytic degradation.

References

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