THE MOLECULAR BASIS OF VON HIPPEL-LINDAU DISEASE

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THE VON HIPPEL-LINDAU SYNDROME

von Hippel-Lindau (VHL) disease is an autosomal dominant familial cancer syndrome characterized by the development of retinal angiomata, central nervous system hemangioblastomata, renal cell carcinomas, pheochromocytomata, and endolymphatic sac tumors (1). This disease affects 1 in 36,000 individuals and has a variable but high degree of penetrance (2). The development of renal carcinomas and cerebellar hemangioblastomata are the major causes of morbidity and mortality for VHL patients.

THE VON HIPPEL-LINDAU GENE

Clinical and molecular epidemiological data have suggested that VHL disease, like hereditary retinoblastoma, is due to inactivation of a tumor suppressor gene (3). In keeping with this view, the VHL gene was mapped to chromosome 3p25-26, a region that is frequently deleted or altered in sporadic renal cell carcinomas. Based on this positional information, a group headed by Drs. Michael Lerman and Bert Zbar cloned the VHL susceptibility gene in 1993 (4). The VHL gene consists of three exons (5), encodes a 4.5-kB mRNA (6,7) and is highly conserved across species (4,8,9). VHL gene mutations have now been identified in ~75% of VHL families (10–12). Tumor development in VHL patients is associated with somatic loss or inactivation of the remaining wild-type VHL allele, in keeping with the notion that the VHL gene functions as a tumor suppressor.

The risk of developing the various tumors that comprise the VHL syndrome differs among VHL kindreds. One classification scheme distinguishes families at low risk (VHL type I) or high risk (VHL type II) of developing pheochromocytomata (13). The latter category is further subdivided into type IIA (low risk of renal cell carcinoma) and type IIB (high risk of renal carcinoma). Certain genotype–phenotype correlations are emerging. For example, missense mutations are often associated with the development of type II disease, whereas mutations predicted to lead to truncated versions of the VHL protein are primarily associated with type I disease (10,11,13–16). It is now also apparent that certain VHL germ-line mutations give rise to familial pheochromocytomata without the other classical manifestations of VHL disease (14,17–19).

A prediction of Knudson's tumor suppressor model is that loss of VHL gene function should also play a role in the sporadic counterparts of the tumors observed in VHL disease. Indeed, inactivation of both VHL alleles, either as a result of mutation, hypermethylation, or loss, has been documented in ~75% of sporadic renal cell carcinomas of the clear cell type (12,20–23) and ~50% of sporadic cerebellar hemangioblastomata (24). Mutation of the VHL gene, with loss of the remaining VHL allele (manifest as loss of heterozygosity at the VHL gene locus), has been documented in early premalignant lesions of the kidney, such as atypical cysts and in microscopic renal cell carcinoma in situ (25,26). Thus, inactivation of VHL function appears to be an early, and possibly requisite, step in the pathogenesis of hereditary and sporadic renal carcinomas of the clear cell type. In contrast to renal carcinomas and cerebellar hemangioblastomata, however,

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inactivation of the VHL gene appears to be a relatively rare event in sporadic pheochromocytomata (20,27).

THE VON HIPPEL-LINDAU PROTEIN

The VHL protein (pVHL) contains 213 amino acid residues and migrates with an apparent molecular weight of ~30 kD (pVHL-L) (6,8). A second VHL isoform (pVHL-S), with an apparent molecular weight of ~19 kD, is generated by translation initiation at an internal ATG (O. I. and W. G. K., unpublished observation). In situ RNA analysis and immunostaining of adult tissues have documented high levels of expression in organs such as the kidney and cerebellum, which are sites of tumor formation in VHL disease (28–30). Immunostaining, as well as biochemical fractionation experiments, suggest that pVHL is largely, but not exclusively, cytoplasmic (6,29,30). Under certain experimental conditions, pVHL appears to "shuttle" between the nucleus and cytoplasm (31). The physiological relevance of this latter observation is currently being investigated.

Reintroduction of wild-type, but not mutant, pVHL into VHL (-/-) renal carcinoma cell lines inhibits their ability to form tumors in nude mice (6,32). With one exception (33), restoration of pVHL function in VHL (-/-) renal carcinoma cells has not led to significant alterations in cell growth in vitro. In particular, pVHL, although capable of suppressing tumor growth in vivo, does not dramatically alter the ability of most renal carcinoma cell lines to grow in low serum or to form colonies in soft agar (6).

The von Hippel-Lindau Protein Binds to Elongin B and C

The predicted pVHL primary sequence does not closely resemble that of any protein whose function is known. In an attempt to understand the mechanisms underlying tumor suppression by pVHL, several groups sought to identify cellular pVHL-binding proteins (8,34–37). Two such proteins, elongin B and C, bind to a region of pVHL that is frequently mutated in VHL kindreds (34,35,37,38). Elongin B and C, when bound to elongin A, generate a transcriptional elongation complex called elongin or SIII (38). pVHL competes with elongin A for binding to elongin B and C, at least in vitro, thereby inhibiting elongin or SIII activity (35,38). Thus, in the simplest view, tumor suppression by pVHL might be due, at least in part, to its ability to modulate the rate of transcription of certain genes as a result of its ability to inhibit elongin/SIII.

The von Hippel-Lindau Protein Inhibits the Accumulation of Hypoxia-Inducible mRNAs under Normoxic Conditions

VHL-associated neoplasms are typically hypervascular and, on occasion, are associated with paraneoplastic erythrocytosis. The former has been linked to the production of angiogenic peptides such as vascular endothelial growth factor/permeability factor (VEGF/VPF) and the latter to the production of erythropoietin by the tumor cells. VEGF/VPF and erythropoeitin production are normally induced by hypoxia. Several groups have recently shown that VHL (-/-) cells inappropriately produce hypoxia-inducible mRNAs, such as the VEGF mRNA, under both hypoxic and normoxic conditions (32,39,40). Reintroduction of wild-type, but not mutant, pVHL into these VHL (-/-) cells specifically inhibits the production of these mRNAs under normoxic conditions, thus restoring their previously described hypoxia-inducible profile. It seems likely that the overproduction of hypoxia-inducible mRNAs, including those encoding angiogenic peptides such as VEGF/VPF, contributes to the hypervascular nature of VHL-associated tumors. Conversely, inhibition of VEGF has been shown to inhibit tumorigenesis in vivo and thus might contribute to the ability of pVHL to suppress tumor formation (41). If so, this might help to explain the differential effects of pVHL on tumor cell growth in vitro and in vivo.

Surprisingly, the regulation of hypoxia-inducible mRNAs by pVHL appears to be primarily posttranscriptional rather than transcriptional (32,39). Similarly, the regulation of VEGF mRNA abundance by hypoxia occurs primarily at the posttranscriptional level (42). Hypoxia leads to a marked increase in the stability of the VEGF mRNA. This effect depends upon an AUUUA-rich region of the VEGF 3′ UTR. RNA-gel shift analyses have identified cellular proteins that bind to this region under hypoxic, but not normoxic, conditions (43). In the simplest model, these proteins serve to stabilize the VEGF mRNA. In contrast, these RNA binding proteins can be detected in VHL (-/-) cell extracts under both hypoxic and normoxic conditions and, perhaps

as a result, the stability of hypoxia-inducible mRNAs is no longer regulated by changes in oxygen (43).

How might binding to elongin B and C contribute to the above-mentioned regulation of hypoxia-inducible mRNAs? One possibility is that elongin/SIII regulates the transcription of a gene encoding a protein involved in the regulation of mRNA stability. A second possibility is that elongin B and C, when bound to pVHL, directly affect the stability of hypoxia-inducible mRNAs. Such a model might account for the observation that pVHL, elongin B, and elongin C are primarily cytoplasmic (39). Finally, regulation of hypoxia-inducible mRNA stability by pVHL may be unrelated to its ability to bind to elongin B and C. In this regard, it is clear that some VHL-kindred-associated pVHL mutants retain the ability to bind to elongin B and C (8,37). This latter observation, together with the genotype–phenotype correlations described above, might be explained if multiple biochemical activities contributed to tumor suppression by pVHL, one of which is binding to elongins B and C.

CONCLUSIONS

von Hippel-Lindau disease, like many hereditary cancer syndromes, is due to a germ-line mutation affecting a tumor suppressor gene. Inactivation of the VHL gene is also implicated in the pathogenesis of sporadic renal carcinomas and cerebellar hemangioblastomata. A frequently mutated region of the VHL gene product, pVHL, binds to elongin B and C, two components of a tripartite transcriptional elongation factor called elongin or SIII. pVHL negatively regulates the accumulation of hypoxia-inducible mRNAs, such as that encoding the angiogenic peptide VEGF, under normoxic conditions. Whether elongin-binding and regulation of mRNA stability by pVHL are linked and, more importantly, whether they are necessary or sufficient to account for tumor suppression by pVHL, are questions at the center of current studies.

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