Commentary

The Tumor Suppressor von Hippel-Lindau Gene Product and Metastasis

New Thoughts on an Old Molecule

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In this issue of *The American Journal of Pathology*, long-time von Hippel-Lindau (VHL) pioneer William G. Kaelin with Nakamura and colleagues¹ demonstrates that cells lacking wild-type *VHL* are defective in the expression and secretion of the glycoprotein clusterin. This phenomenon was observed in cells capable of ubiquitylating hypoxia-inducible factor (HIF) and possessing Type 2C VHL mutants. The authors also found that pVHL-defective renal carcinoma cells overexpress insulin-like growth factor binding protein-3 (IGBP3) and plasminogen activator inhibitor type-1 in a HIF-dependent manner. Previous reports have described pVHL modulating other genes in a HIF-independent manner to promote its tumor suppressor function; however, the overexpression of clusterin by pVHL raises several questions and requires further discussion.

VHL disease is a rare autosomal, dominant syndrome that genetically predisposes affected individuals to tumor development. VHL diseases exhibit diverse genotypic and phenotypic correlations. The most common attributes of this disease are connected with maximum morbidity and mortality and include brain, spinal, and medullary hemangioblastomas, retinal angiomas, renal cell carcinomas (RCCs), pancreatic cancers, and pheochromocytomas (PHEs). Initially, Latif and colleagues isolated the *VHL* gene using a positional cloning strategy.² Since then, naturally occurring complete and partial *VHL* gene deletions, frameshifts, and missense mutations have been implicated in promoting the development of VHL disease.³

VHL disease is classified into distinct clinical subtypes based on both the presence and absence of pheochromocytomas or renal carcinomas.³ Phenotypic differences have been subclassified on the basis of disease type.⁴ Type 1 mutations (more commonly deletions and truncating mutations) predispose to hemangioblastomas and

RCCs but usually not PHEs. Type 2 mutations (more commonly missense mutations) have been identified in PHEs. Type 2 mutations are further subdivided into three groups: type 2A, detected in hemangioblastomas or PHEs but rarely in RCCs; type 2B, detected in hemangioblastomas, RCCs, and PHEs; and type 2C, detected in PHEs only. The Type 2C mutation, associated with the PHE-only phenotype, promotes HIF-ubiquitylation *in vitro* and demonstrates wild-type binding patterns with pVHL-interacting proteins, suggesting that loss of other pVHL functions are necessary for PHE susceptibility.⁵ Therefore, it was suggested that the dependence of VHL tumor susceptibility on *VHL* mutations is based on the variation and/or tissue-specific operations of pVHL. The existence of specific mutations in VHL disease associated with differing tumor risks provides tools to dissect the relationships between pVHL functions and tumor susceptibility.⁴ However, the relationship between pVHL function and its different mutations or tissue-specific expression has been unclear until recently. The present work by Nakamura and colleagues¹ describes differences in gene expression and alterations in protein functions that result from differential pVHL mutations.

Previously, several groups have demonstrated the *VHL* gene product, pVHL, to be part of the protein degradation machinery. pVHL forms stable protein complexes with elongin B, elongin C, Cullin-2, and Ring box-1. Interestingly, these stable protein complexes, which are similar to SCF ubiquitin ligase, polyubiquitylate several proteins including members of the HIF family.⁶ In most cases, the HIF- α subunits of the heterodimeric HIF com-

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plexes are highly unstable in normal oxygen concentrations because of the hydroxylation of conserved prolyl residues, a reaction that is catalyzed by the prolylhydroxylases of the EGLN family. The pVHL complex recognizes the hydroxylated HIF- α subunits, designating them for degradation. However, this function is lost in several VHL diseases that are pVHL-nonfunctional or null. Also, when a hypoxic situation leads to no prolylhydroxylation, HIF heterodimers are stable and able to trigger the transcription of several genes, including vascular endothelial growth factor, Glut1, transforming growth factor- α , erythropoietin, and platelet-derived growth factor-B. As a result, HIF is elevated in many human cancers, further emphasizing its universal implication in tumorigenesis.¹

Other than the ability to destabilize HIFs and control downstream targets of HIF, the tumor suppressing molecular mechanism of pVHL remains unknown. Initially, several groups showed that pVHL does not influence cell cycle; however, other groups have since demonstrated that the reintroduction of *VHL* into VHL-deficient renal cancer cells (RCCs) leads to the accumulation of the cyclin-dependent kinase inhibitor (CDKI) p27Kip1 because of an increase in its stability.⁷ In that particular report, the authors propose that the loss of wild-type *VHL* results in a specific cellular defect in controlling serum-dependent growth, which possibly initiates tumor formation. A similar result was obtained by others when renal cancer cell lines were infected with a recombinant adenovirus containing *VHL* cDNA (AdVHL), resulting in G₁ cell cycle arrest and growth inhibition associated with the induction of p27Kip1 and inhibition of CDK2 and cyclinB1-dependent cdc2 activities.⁸ However, neither study defined whether this cell cycle phenotype is HIF-dependent.

In contrast, Mack and colleagues recently showed that decreased growth of VHL-null fibrosarcomas is associated with elevated levels of p21 and p27.⁹ The authors used mouse embryonic fibroblasts (MEFs), a common tool for analyzing cell cycle regulation, to generate VHL-null MEF-derived fibrosarcomas. Unlike RCCs and other VHL-related cancers, growth of both VHL-null MEFs and VHL-null fibrosarcomas was impaired, though tumor vascularity increased. Interestingly, the authors also found that decreased proliferation of VHL-null MEFs correlated with overexpression of p21 and p27, completely contrary to previous findings in RCC. Gene silencing of HIF-1 α by small interfering RNA reduced p21 and p27 protein and mRNA levels in VHL-null MEFs. In the absence of VHL in this study, the induction of p21 and p27 was mediated by a constitutive activation of the HIF pathway in MEFs and fibrosarcomas. The question remains whether differential VHL function is cell- or organ-dependent or whether unknown oncogenes present in RCCs, but not in fibrosarcomas, can modulate VHL.

As mentioned, one function of VHL is to promote the expression of clusterin. In this issue of *The American Journal of Pathology*, Nakamura and colleagues¹ report that every disease-associated pVHL mutant tested was quantitatively defective in promoting the secretion of clusterin. Although clusterin levels in wild-type *VHL*-containing ACHN, a renal carcinoma cell line, and Caki RCC

cells were similar to those in *VHL*-null cells engineered to produce wild-type pVHL, the data suggest that clusterin secretion is a biomarker for a HIF-independent function that is lost in tumor-associated pVHL mutants. To validate their cell culture data, the authors used immunohistochemistry to analyze 63 clear cell renal carcinomas with known *VHL* status. They found that tumors in RCC patients with *VHL* mutations showed a decrease in clusterin relative to wild-type tumors. Additionally, clusterin staining was low in pheochromocytoma samples with *VHL* mutations. However, the data described in this study introduce further complexities in the VHL/clusterin relationship and emphasize the need for understanding VHL function as it relates to its tumor suppressor activity.

Clusterin is a glycoprotein that boosts cell aggregation, and it is overexpressed in several human cancers such as prostate cancer, breast cancer, squamous cell carcinoma, and renal cancer.^{10–15} A recent study suggests that clusterin plays an important role in inhibiting apoptosis in human cancer cells by interfering with Bax activation in the mitochondria.¹⁶ Interestingly, clusterin specifically interacts with conformation-altered Bax in response to chemotherapeutic drugs, and the elevated level of clusterin in human cancers eventually promotes oncogenic transformation and tumor progression by interfering with Bax proapoptotic activities. Furthermore, Kurahashi and colleagues¹⁷ have recently shown that the secreted form of clusterin may be involved in the progression of RCC and that clusterin overexpression could be a useful prognostic marker after radical surgery in RCC patients.

Considering the tumor suppressor function of pVHL, the data presented by Nakamura and colleagues¹ does not corroborate other findings and is, in fact, contradictory. Clusterin has been shown to be up-regulated by most proto-oncogenes and to be activated by membrane-bound receptors.^{18,19} Clusterin accumulates in dying neurons after seizures and hypoxic-ischemic injury, and clusterin-deficient mice have been shown to suffer 50% less brain injury.²⁰ In a different context, clusterin inhibits ischemia-induced death in isolated adult ventricular rat cardiomyocytes in the absence of complement,²¹ suggesting that clusterin protects cardiomyocytes from ischemic cell death via a complement-independent pathway. Taken together, it is clear that clusterin has multiple functions that differ in a context-dependent manner.

In response to the controversial discussion and disparate data on VHL and clusterin in relation to cancer, I would like to finish this commentary with my personal opinion. From the perspective of a tumor biologist, there are several ways to explain the complexity of the data generated by different laboratories. 1) VHL may inhibit primary tumor formation by inhibiting angiogenesis or restricting cells to G₀/senescence but, conversely, may promote metastasis. To examine this theory, studies with metastatic renal tumor models harboring alterations in pVHL will need to be performed. In addition, more survival or prognostic data from patients will need to be collected. 2) Clusterin overexpression in the presence of pVHL may be a feedback response for the tumor to override pVHL function. Several examples exist for such

a response. One of the classic examples is thrombospondin-1, which is well known for its anti-angiogenic activity although highly expressed in several angiogenic tumors. 3) Another explanation involves the possibility that pVHL traffics the clusterin secreted from the tumor. Further studies are required to correlate the serum level of clusterin in response to pVHL overexpression. 4) It also remains unclear whether the overexpression of clusterin induced by pVHL is functionally similar to the anti-apoptotic/prometastatic clusterin secreted by tumors. Thus, Nakamura and colleagues¹ have provided several avenues for future research and discussion into the tumor suppressor function of VHL.

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