von Hippel-Lindau: A Tumor Suppressor Links Microtubules to Ciliogenesis and Cancer Development

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Abstract

Loss of von Hippel-Lindau (VHL) tumor suppressor gene function occurs in familial and most sporadic renal cell carcinoma. The tumor suppressor role of the protein pVHL is based on its ability to target transcription factors of the hypoxia-inducible factor family for degradation, but other functions of pVHL are less clearly defined. New findings show that pVHL is necessary for cilia formation. pVHL interacts with PAR proteins, a complex that specifies the membrane domains of polarized epithelial cells, and directs the orientation of growing microtubules. Loss of pVHL results in aberrant orientation of newly formed microtubules and prevents ciliogenesis. These results add to a growing body of evidence linking cilia and the cell cycle and suggest that the tumor suppressor role of pVHL may involve previously unrecognized pathways. [Cancer Res 2007;67(10):4537–40]

Background: von Hippel-Lindau and Renal Cysts

The von Hippel-Lindau (VHL) syndrome is an inherited disease characterized by the development of tumors in the adrenal glands (pheochromocytomas), blood vessels (hemangioblastomas), and the kidney (clear cell carcinomas; ref. 1). It is caused by an inherited mutation in one of two alleles of the tumor suppressor gene VHL. Somatic mutations in the unaffected second gene copy result in malignant transformation of individual cells and tumor formation (2). The VHL protein (pVHL) is part of an E3 ubiquitin ligase complex that targets proteins for proteasomal degradation. This function is best established for the degradation of members of the hypoxia-inducible factor protein family (2). Hypoxia-inducible factor transcription factors are tightly controlled under normoxic conditions. Low oxygen tension prevents the posttranslational modifications of hypoxia-inducible factors, necessary for their ubiquitylation by the pVHL containing E3 ubiquitin ligase complex, and results in activation of several target genes to guide the adaptation to hypoxia (3). Hypoxia-inducible factors are up-regulated in renal cancers in VHL disease and in a large proportion of sporadic clear cell carcinomas as well. Despite substantial evidence from animal models, that the lost control of hypoxia-inducible factors by pVHL is a key step in tumor formation, the contribution of other pVHL functions to tumorigenesis is less clear (4).

In the kidneys of patients with VHL disease, renal tumors are preceded by the development of premalignant cysts that may resemble classic autosomal-dominant polycystic kidney disease (5). Polycystic kidney disease occurs in all age groups and encompasses a spectrum of clinically and genetically heterogeneous disorders (6). Cystic kidney diseases in general are caused by mutations in proteins that are localized to primary cilia or centrosomes (7, 8). Cilia are hair-like structures containing longitudinal strands of microtubules that originate from the surface of polarized renal epithelial cells (9). In polarized epithelial cells, the centrosome is located beneath the apical cell surface and is anchored to the plasma membrane, where it forms the basal body, from which the cilium originates (Fig. 1). Primary cilia in the kidney are nonmotile organelles, which have been shown to act as flow sensors (10). Bending of cilia by flow, leading to intracellular calcium transients, has been linked to activation of signaling cascades, such as the noncanonical Wnt signaling pathway involved in regulating epithelial cell polarity (11, 12). Most importantly, ciliary signaling also seems to inhibit canonical Wnt signaling, preventing the stabilization of β-catenin and the downstream events that have been implicated in tumorigenesis of various cancer types.

pVHL Is Required for Cilia Formation and Regulates Microtubule Orientation

The fact that pVHL disease causes kidney cysts and the link between kidney cysts and dysfunction of cilia and centrosomes strongly suggest that pVHL plays a role in the function of these organelles. Indeed, recent evidence from three independent groups indicates that pVHL is localized to primary cilia and required for ciliary assembly (13–15). Renal cancer cells lacking pVHL are deficient of cilia, whereas ectopic expression of pVHL in these cells reestablished cilia growth. Conversely, reduction of pVHL by RNA interference–mediated knockdown in renal epithelial cells resulted in the loss of primary cilia, strongly suggesting that pVHL function is required for ciliogenesis (15). The fact that loss of pVHL results in the absence of cilia may explain why VHL disease is associated with kidney cysts and provides further proof for the ciliary hypothesis of cyst formation. However, the question remains how pVHL functions in the process of ciliogenesis. Hergovich et al. (16) found that pVHL binds to and stabilizes microtubules. This finding is potentially pertinent to cilia because the ciliary axoneme contains stable microtubules, as indicated by a high content of acetylated tubulin. A recent report, examining microtubular polymerization by live cell imaging of fluorescence-tagged end binding protein-1, suggests that pVHL controls the orientation of microtubule formation (15). End binding protein-1 is present in a large cytoplasmic pool and caps the plus ends of growing microtubules. When tagged with GFP, end binding protein-1
marks the plus end of growing microtubules and can be used to dynamically monitor microtubule growth (Fig. 1). Schermer et al. showed that microtubule growth is oriented toward the periphery of the cells in cells expressing pVHL. In pVHL-deficient cells, however, the orientation of microtubule growth is disturbed, resulting in disordered directions of microtubule assembly (Fig. 1).

Microtubule stability and polarization play an important role in the differentiation of cells. Rho family GTPases, such as Cdc42, have been shown to modulate microtubule polarity by regulating cortical microtubule capture through Par6 and atypical protein kinase C (aPKC; ref. 17). The Par3-Par6-aPKC complex is also involved in formation and localization of the tight junction, thereby defining the apical and basolateral domains of epithelial cells (18). Additionally Par3, Par6, and aPKC have been shown to localize to primary cilia, and RNA interference–mediated knockdown of the Par3 binding partner 14-3-3σ, a Par5 homologue, resulted in the loss of cilia (19). Thus, the fact that pVHL associates with the Par3-Par6-aPKC complex suggests that pVHL may be involved in polarization of cells (15).

However, the question why microtubular disorientation in pVHL-deficient cells prevents cilia formation remains unanswered. Ciliogenesis is dependent on intraflagellar transport proteins (IFT) proteins (9), but thus far, no link between pVHL and IFT proteins has been found. One prerequisite for cilia formation is the anchoring of the centrosome to the plasma membrane (20). Several studies have analyzed factors involved in the docking of the centrosome (reviewed in ref. 20), but no data exist on the effectors of centrosome positioning during ciliogenesis. In nonpolarized cells, the centrosome functions as the microtubule-organizing center. Interestingly, centrosome positioning in migrating cells is regulated by the Par3-Par6-aPKC complex (17, 21). Thus, it is tempting to speculate that pVHL in concert with Par3, Par6, and aPKC is important in guiding the centrosome and anchoring it to the plasma membrane. The potential inability to target the centrosome correctly to the plasma membrane could explain the defective ciliogenesis in pVHL-deficient cells. In this respect, it is interesting to note that the adenomatous polyposis coli protein, which is another microtubule-interacting tumor suppressor, has been shown to regulate the orientation of the centrosome in migrating astrocytes, probably by influencing

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**Figure 1.** Hypothetical model of pVHL function in ciliogenesis and cell cycle control. In a wild-type cell, pVHL binds to microtubules (MT) and the Par3-Par6-aPKC complex to direct microtubule orientation. This enables the centrosome to migrate to the apical membrane, where it is attached to transition fibers. The membrane-anchored centrosome forms the basal body for the outgrowing cilium, which keeps the cell in G0 as long as a cilium is present. In VHL-deficient cells, microtubule growth is disordered, preventing migration of the centrosome to the plasma membrane. No cilium can be formed and the cells cannot arrest in G0.
microtubule capture at the cortical membrane (21, 22). An alternative hypothesis of how the lack of microtubule orientation in VHL-deficient cells affects ciliogenesis might involve a more general disturbance in the establishment of apicalbasolateral polarity, perhaps through impaired formation of the tight junction or other polarity determinants.

A New Light on Cilia and the Cell Cycle

Can these new findings be linked to the role of pVHL as a tumor suppressor? It has been known for a long time that cilia are in tune with the cell cycle. Differentiated cells in G0 carry cilia, which are resorbed before mitosis (23). The centrosomes duplicate and serve as anchoring points for the mitotic spindle. The cilia reappear after cell division is completed. In the kidney, loss of cilia causes cysts, which are characterized by an increase in proliferation of cyst lining epithelial cells. (The incidence of renal cell carcinoma in most types of cystic kidney disease is not increased, however). Examples of ciliary proteins, which have been linked to the cell cycle, include inversin, a protein mutated in a large autosomal recessive syndrome called the Chlamydomonas reinhardtii, has been reported recently to inhibit cell growth, probably through a defect in cytokinesis (26). Further clues to the role of cilia in cell cycle regulation come from two members of the NIMA-related expressed kinase family in Chlamydomonas, Fa2p and Cnk2p, which both localize to flagella [see also the excellent review by Quarmby and Parker (27)]. NIMA, a kinase first identified in Aspergillus, functionally interacts with cyclin-depen-
dent kinase 1, regulating the entry into the mitotic phase (28). Fa2p similarly plays a role at the G2-M transition but additionally plays a role in an alternative mechanism of ciliary loss called deflagellation, where the flagellum is severed close to the plasma membrane (27). This observation supports a potential link between deciliation and cell cycle regulation and suggests that failure to shed the cilium could negatively affect mitosis. Cnk2p, the second ciliary Nek, is involved in the regulation of cell size and flagellar length, both of which are associated with the cell cycle (27). Interestingly, mutation of two mammalian orthologues of Neks, Nek1 and Nek8, causes cystic kidney disease when mutated in mice, and Nek1 interacts with ciliary proteins, such as kinesin 2. Nek8 is localized in cilia (29) and is up-regulated in human breast cancer, supporting the link between ciliary proteins and cancer (30). Furthermore, components of several signaling pathways, including the Sonic hedgehog and platelet-derived growth factor receptor-α pathways, have been found in cilia (31). These signaling pathways are dysregulated in cancer. Thus, it is tempting to speculate that the cilium restricts the activation of these pathways to prevent uncontrolled proliferation and tumorigenesis.

In summary, recent findings suggest a role for the tumor suppressor protein pVHL in controlling ciliogenesis and microtubule dynamics. These observations have provided new insights into the function of this multifunctional molecule and stimulated the intriguing hypothesis that microtubule dynamics, positioning of centrosomes, and ciliogenesis play important roles in cell cycle control. Further work on the molecular details of pVHL function in this complex relationship will certainly not only provide us with valuable insight into tumorigenesis but also suggest new therapeutic targets for the treatment of cancer.

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