

Von Hippel-Lindau protein and respiratory diseases

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Abstract

Von Hippel-Lindau protein (pVHL) was first identified as a tumor suppressor gene as mutations in the *VHL* gene predispose individuals to systemic benign or malignant tumors and cysts in many organs, including renal cell carcinoma of the clear-cell type and hemangioblastoma. Although pVHL is best known to act as a component of ubiquitin protein ligase for the proteasomal degradation of hypoxia inducible factor (HIF)- α , pVHL also interacts with extracellular matrix proteins and cytoskeleton, regulating extracellular matrix assembly, cell signaling, and many other cellular functions. Recent studies suggest that pVHL contributes to many lung diseases, including pulmonary arterial hypertension, lung cancer, pulmonary fibrosis, and acute respiratory distress syndrome. Mutation or loss of function of pVHL activates HIF and induced expression of vascular endothelial growth factor, endothelin-1, and FoxM1, leading to pulmonary arterial hypertension. Loss of pVHL in lung cancer cells promotes epithelial-mesenchymal transition and cancer migration and invasion while decreasing lung cancer cell proliferation and colonization. In patients of idiopathic pulmonary fibrosis, elevated expression of pVHL induces expression of fibronectin/integrin $\alpha 5\beta 1$ /focal adhesion kinase signaling, resulting in fibroproliferation and fi-

brosis. In alveolar epithelial cells, pVHL mediates Na, K-ATPase degradation in an HIF independent pathway, causing decreased edema clearance during hypoxia. These studies suggest that pVHL plays key roles in the pathogenesis of many lung diseases, and further investigations are warranted to elucidate the underlying molecular mechanisms.

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Key words: Von Hippel-Lindau protein; Lung cancer; Pulmonary fibrosis; Pulmonary hypertension

Core tip: Although von Hippel-Lindau protein (pVHL) was first identified as a tumor suppressor and is best known as a component of ubiquitin protein ligase for the proteasomal degradation of hypoxia inducible factor (HIF)- α , recent studies suggest that pVHL contributes to many lung diseases in both HIF-dependent and HIF-independent pathways. Loss of pVHL promotes pulmonary arterial hypertension *via* activation of HIF. In lung cancer, loss of pVHL promotes epithelial-mesenchymal transition and cancer migration and invasion while decreasing cell proliferation and colonization, pVHL also induces fibronectin/integrin $\alpha 5\beta 1$ /focal adhesion kinase signaling to facilitate fibrogenesis. pVHL mediates Na, K-ATPase degradation to cause decreased edema clearance during hypoxia.

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VON HIPPEL-LINDAU PROTEIN

The von Hippel-Lindau (*VHL*) gene was first identified in patients with von Hippel-Lindau syndrome, an autosomal dominant disease with an incidence of one in 36000

births^[1]. Germline mutation of the *VHL* gene, which is located in human chromosome 3p25^[2], predisposes individuals to benign or malignant tumors and cysts in many organ systems and causes clear-cell renal cell carcinoma, hemangioblastoma, pheochromocytoma in adrenal glands, pancreas tumors, endolymphatic sac tumors of middle ear, and epididymal cystadenomas in testes. There are also findings that VHL contributes to liver and pulmonary hemangiomas^[3]. Individuals with VHL disease usually have an inherited mutant copy of the *VHL* gene and an inactivated wild-type allele through somatic mutation or hypermethylation.

pVHL regulates HIF activity

The *VHL* gene, cloned in 1993^[4], consists of three exons and produces two alternatively spliced mRNAs, translating into two proteins pVHL³⁰ and pVHL^{19[5,6]}. The larger protein contains 213 amino acids and the shorter is internally translated and is missing the first 53 amino acids. VHL³⁰ is found in the nuclear, cytosolic and membrane fractions while VHL¹⁹ localizes mostly in the nucleus. The VHL protein contains two domains, α and β domain. The α domain serves as an elongin C binding site while the β domain acts as a substrate-recognition/docking site. VHL is found in a multiple-protein complex with Elongin B, Elongin C, Cullin (Cul) 2, and Rbx 1 (also known as ROC1/Hrt1), forming an ubiquitin protein ligase (E3) complex named VEC^[7,8]. Elongin C brings VHL and Cul2 together. Cul2 is associated with Rbx1, which recognizes a cognate ubiquitin conjugating enzyme (E2).

pVHL is best known as a component of E3 for the proteasomal degradation of hypoxia inducible factor (HIF)-1/2 α ^[9,10]. In normoxic conditions, HIF- α is hydroxylated at the conserved proline residues within the oxygen-dependent degradation domain by the prolyl-hydroxylase domain proteins^[9,11]. The prolyl-hydroxylated HIF- α is recognized by pVHL, leading to poly-ubiquitination and degradation^[9,10]. During hypoxia, the prolyl-hydroxylases are inhibited, HIF- α is not hydroxylated and is unable to bind pVHL, leading to its stabilization. Stabilized HIF- α forms a heterodimer with a HIF- β family member and acts as a transcriptional factor to activate expression of downstream genes that contain the HIF-responsive elements in their promoters^[12]. Many of these genes are involved in promoting adaption to hypoxic conditions, including the angiogenic genes vascular endothelial growth factor (VEGF) and erythropoietin (EPO), glycolysis-involving gene phosphoglycerate kinase, and glucose transporter Glut-1, *etc.*^[12,13].

pVHL was identified as a tumor suppressor because the *VHL* gene mutation was shown to be associated with tumors in the kidney^[3,14]. Iliopoulos *et al.*^[14] and Kondo *et al.*^[15] demonstrated that reintroduction of the wild-type VHL into the VHL-mutated renal carcinoma cell line 768-O inhibited the tumor formation in nude mice after injection of the renal carcinoma cells. HIF activation is a crucial for carcinogenesis in absence of pVHL since HIF induces expression of angiogenic factors VEGF, EPO

and platelet-derived growth factor^[3,12,16]. pVHL also regulates a proliferative response to hypoxia since the loss of pVHL leads to constitutively elevated Cyclin D1 and abnormal proliferation rate of the renal carcinoma cells^[17,18]. Additionally, the pVHL mutant is known to increase Akt-mTOR signaling^[19], induce fibroblast growth factor receptor signaling^[20,21], disrupt cilia formation^[22], and downregulate p53^[23] to promote cancer initiation and progression.

pVHL regulates extracellular matrix assembly

In the other characterized pVHL pathway, fibronectin interacts with pVHL in cells and co-localizes with a fraction of pVHL on the endoplasmic reticulum (ER)^[24]. Moreover, renal carcinoma cells with loss of pVHL have a defective assembly of extracellular fibronectin matrix^[24]. These data support a direct role of pVHL in fibronectin matrix assembly^[24]. Although pVHL null cells have intact extracellular matrix (ECM) expression and secretion levels^[25,26], pVHL deficient 786-O renal cancer cells are unable to organize an adequate matrix even in the presence of an excess of exogenous fibronectin, suggesting that proper assembly of fibronectin matrix requires adequate interaction between fibronectin and its receptor^[27]. Further studies demonstrated that association of fibronectin with α v β 1 integrin is crucial for the fibronectin matrix assembly. In cells with pVHL expression, α v integrin forms “patch-like” focal contacts and relocates to the intercellular junctions where α v and β 1 integrin form large fibrillar adhesions and anchor firmly to the fibronectin substrate. In the absence of pVHL, α v integrin focal contacts remain unchanged; however, they are unable to assemble β 1 fibrillar adhesions^[27]. Activation of β 1 integrin by exogenous divalent cations or activating antibodies partially restores the capability of VHL null cells to assemble β 1 fibrillar adhesions and fibronectin fibers^[27]. These studies suggest that pVHL is an important regulator of α v β 1 integrins and is essential for the formation of β 1 fibrillar adhesions and the organization of extracellular fibronectin.

The interaction with pVHL is also necessary for collagen to be assembled into the ECM. Failure of pVHL to interact with collagen correlates with the loss of collagen network and collagen remodeling *in vitro* and *in vivo*^[24,28,29]. Although pVHL can interact with type I, II, IV, and V collagen in denatured conditions, the most specific binding occurs with type IV collagen, specifically the collagen IV α 2 (COL4A2)^[28,29]. The pVHL-COL4A2 interaction occurs on the ER where the N-terminal tail of COL4A2 protrudes from the ER lumen into the cytosol to associate with pVHL. This association requires a collagenous domain of Gly-X-Y triplets in the full-length α -chains of collagen (X and Y can be any amino acid residue but are commonly proline or hydroxyproline). However, hydroxylation of the N-terminal domain leads to disassociation of collagen from pVHL, and collagen folds into the matured triple helical conformation^[29]. Taken together, these findings suggest that pVHL can bind directly to a variety of collagen chains and that this

association is critical for collagen matrix assembly.

pVHL and cytoskeleton dynamics and epithelial cilia maintenance

Accumulating evidence suggests a novel function of pVHL in cytoskeleton dynamics and epithelial cilia maintenance^[30,31]. Hergovich *et al.*^[32,33] reported that pVHL associates with microtubules and protects them from depolymerization. Amino acids 95-123 of pVHL, particularly point mutations such as pVHL (Y98H) and pVHL (Y112H), are critical for the pVHL-microtubule interaction^[32]. The pVHL-microtubule interaction appears to be indirect and is mediated by microtubule motor Kinesin-2, which is responsible for the transport of pVHL to the cell periphery along microtubules, which also stabilizes them^[34,35]. Accordingly, pVHL affects the normal function of primary cilium, a microtubule-based cellular sensory organelle in the kidney. pVHL functions to stabilize microtubules in the axoneme and localizes to primary cilia, but loss of pVHL alone does not affect primary cilia structure in primary cells^[36]. However, inactivation of both the pVHL and GSK3 β leads to loss of cilia, suggesting that mutation of pVHL may sensitize cells to lose their primary cilia and promote the formation of cysts in the kidney^[36]. In absence of functional pVHL, loss of GSK-3 β activates Akt and extracellular signal-regulated kinase, inducing epithelial cell proliferation and kidney cyst formation^[37]. Conversely, mice with the double deletion of VHL and phosphatase and tensin homolog, which mimics Akt activity, display cilia loss and cyst formation in the kidney^[37]. These findings indicate that, although loss of pVHL function alone is insufficient to cause uncontrolled cellular proliferation and cyst formation, additional signaling such as activation of Akt and inactivation GSK-3 β may allow cyst formation.

pVHL also regulates actin cytoskeletal organization and cell motility. Overexpression of pVHL translocates vinculin from the cytoplasm to the cell membrane and induces focal adhesion formation and adhesion^[38]. In contrast, overexpression of pVHL also increases stress fibers which exhibit a spreading morphology, leading to reduced cell motility^[38]. These results suggest that the loss of pVHL may promote cancer progression *via* destabilized actin organization and increased motility in cancer cells. Additional studies suggest that loss of pVHL results in the loss of Brk1 (Brk1), a component of the Wave/Scar pathway that regulates branched nucleation of actin fibers. Consistently, suppression of Brk1 causes abnormal vinculin distribution, loss of Arp2/3 and Wave proteins at the cellular protrusions, and abnormal actin stress fiber formation. Furthermore, suppression of Brk1 decreases proliferation, migration, and invasion in renal carcinoma cells^[39]. A recent genetic study shows that germline and somatic mutations in VHL is associated with loss of *HSPC300* gene, a regulator of actin dynamics and cytoskeleton organization. Depletion of *HSPC300* causes cytoskeleton abnormalities and cytokinesis arrest in tumor cells^[40]. The underlying mechanism of how pVHL

regulates Brk1 and HSPC300, however, remains unclear.

VHL AND PULMONARY ARTERIAL HYPERTENSION

Pulmonary artery hypertension (PAH) is a devastating disease that results in a progressive increase in pulmonary vascular resistance, right ventricular failure, and ultimately death^[41,42]. Despite recent advances in management of PAH, there is currently no cure for PAH. PAH is characterized by pulmonary arterial remodeling that includes vascular cell proliferation^[41,42]. Hypoxia is a well established stimulus for the induction of pulmonary hypertension (PH) in several animal models that exhibit pulmonary artery smooth muscle cell proliferation and de-differentiation after exposure to hypoxia^[43]. HIF is a master transcription factor that regulates cellular adaptation in hypoxia^[44,45] and has been implicated in PH^[46,47]. Furthermore, inhibition of HIF by Digoxin prevents and reverses hypoxia-induced PH^[48].

Distinct from the classic VHL-associated inherited cancer syndrome, Chuvash polycythemia is defined as a new form of VHL-associated disease which is caused by the homozygosity for the C598T mutation of the VHL gene^[49]. The C598T VHL mutation increases the HIF- α level and the expression of several HIF target genes including EPO and VEGF, leading to the development of polycythemia^[49]. Smith *et al.*^[50] reported that a small group of patients with Chuvash polycythemia showed striking abnormalities in the respiratory and pulmonary vascular systems. Compared to control individuals, Chuvash polycythemia patients displayed elevated basal ventilation and pulmonary vascular tone as well as increased ventilatory, pulmonary vasoconstrictive, and heart rate responses to acute hypoxia, suggesting a role of pVHL/HIF signaling in calibration and homeostasis of the respiratory and cardiovascular system^[50]. Other studies have also shown that patients with Chuvash polycythemia had a higher systolic pulmonary artery pressure and plasma concentration of endothelin-1 and VEGF than control individuals^[51,52], suggesting that, in Chuvash polycythemia patients, the VHL mutation may activate HIF and upregulate endothelin-1 and VEGF, leading to pulmonary hypertension.

In a strain of mice harboring the C598T VHL mutation (VhlR/R mice) as a model for Chuvash disease, Hickey *et al.*^[53] found that lungs from VhlR/R mice showed pulmonary vascular remodeling, hemorrhage, edema, and macrophage infiltration resembling pulmonary hypertension. Interestingly, they found that the C598T VHL mutation in mouse lungs did not change the expression of HIF-1 α and its targets whereas the expression of HIF-2 α protein and HIF-2 α -regulated genes such as *Serpine1* were induced^[53]. Moreover, heterozygosity of HIF-2 α (HIF-2 α +/-), but not HIF-1 α (HIF-1 α +/-), suppressed both polycythemia and pulmonary hypertension in the VhlR/R mice and attenuated vascular remodeling in VhlR/R lungs, suggesting a selective and critical role for HIF-2 α in the pulmonary pathology in

C598T VHL mutants^[53]. This concept is strengthened by the study of Formenti *et al.*^[54] in which they showed that patients with HIF-2 α gain-of-function mutations developed pulmonary hypertension with increased cardiac output, heart rate, and pulmonary ventilation. Recently, we found that HIF-2 α , but not HIF-1 α induced expression of FoxM1, leading to the induction of Aurora A kinase and Cyclin D1 and cell cycle progression which resulted in the proliferation of pulmonary artery smooth muscle cells^[55]. A recent study reported novel VHL mutations in exon2 (G376A) and exon3 (C548T) of the lungs in a 2-month-old boy with severe polycythemia and pulmonary arterial hypertension^[56]. Together, these studies suggest that, in Chuvash polycythemia, VHL mutations activate HIF, particularly HIF-2 α , and contribute to the development of pulmonary arterial hypertension.

VHL AND LUNG CANCER

Lung cancer is the most common cause of cancer-related death in the United States^[57]. There are two main types of lung cancer, small cell lung cancer (SCLC), and non-small cell lung cancer (NSCLC)^[57]. SCLC is more aggressive and is strongly associated with cigarette smoking. SCLC arises in the larger airways: the primary and secondary bronchi. NSCLC accounts for 80% of lung cancer cases and is divided into three subcategories: squamous cell carcinoma, large cell carcinoma, and adenocarcinomas. Adenocarcinomas account for 30% of NSCLC cases and usually arise from epithelial cells in peripheral lung tissue. Recently, the population of adenocarcinomas cases in nonsmokers has been rising^[58,59]. As in other solid tumors, lung cancer cells proliferate at a rate that exceeds the oxygen supply, resulting in regions of low oxygen tension (hypoxia)^[60,61]. Tumor cells adapt to hypoxia by inducing genes involved in angiogenesis or glucose metabolism *via* HIF^[61-63]. Hypoxia induces epithelial-mesenchymal transition (EMT) in tumor cells. EMT is a molecular and cellular process during which epithelial cells lose cell-cell interactions and apico-basal polarity and acquire mesenchymal and migratory properties^[64-67]. EMT is featured with changes on cell morphology and genetic markers including the disappearance of an epithelial marker such as E-cadherin and acquisition of mesenchymal markers such as α -smooth muscle actin (α -SMA) and vimentin^[68]. The significance of EMT in tumor metastasis is recently evidenced in a few tumor models^[69]. Many researchers observed the loss of epithelial characteristics paired with the gain of mesenchymal markers in the invasive front of various cancers, pointing to a possible contribution of EMT in tumor metastasis^[70]. EMT leads to increased tumor cell invasiveness and metastatic potential and resistance to radiation-induced cell death, which are the main causes of cancer death^[61-63,66,71].

It is well established that in renal carcinoma, mutation of pVHL leads to stabilization of HIF, which drives angiogenesis and cancer development. However, the role of pVHL in lung cancer is less known. Lungs express levels

of pVHL comparable to kidneys^[72,73], and loss of *VHL* allele frequently occurs in patients with NSCLC^[74-76], suggesting that pVHL likely contributes to lung cancer development. Indeed, we showed that suppression of pVHL in lung cancer cells induced EMT and increased migration and invasion^[77], which is consistent with the observation that loss of pVHL promotes EMT and invasion in renal carcinoma cells^[78-80]. We have shown that hypoxia induces lung cancer cell EMT and invasion and migration *via* a HIF-dependent pathway^[81,82]. Therefore, loss of pVHL may mediate EMT through HIF signaling. However, we have found that HIF activation alone is not sufficient to induce EMT in lung cancer cells^[81], indicating that a HIF independent pathway is also contributing to EMT when pVHL is absent. Surprisingly, our study suggests that loss of pVHL repressed lung cancer cell proliferation *in vitro* and decreased colonization *in vivo*^[77], suggesting that loss of pVHL may limit lung cancer development. Consistently, loss of pVHL also reduces cell proliferation in fibroblasts, mammary epithelial cells, and chondrocytes and inhibits fibrosarcoma^[83-85]. Since loss of pVHL induces HIF, we investigated the role of HIF in lung cancer cell proliferation and colonization. We found that constitutively active HIF increased lung cancer cell colonization whereas dominant negative HIF inhibited lung cancer colonization^[77]. Thus, loss of pVHL causes reduced lung cancer cell proliferation and colonization in a HIF-independent pathway. We showed that suppression of pVHL decreased integrin and phosphorylated focal adhesion kinase (FAK) levels, suggesting that knockdown of pVHL represses lung cancer cell proliferation and colonization *via* decreased integrin/FAK signaling. Taking these together, we conclude that the role of pVHL/HIF in lung cancer development and progression may be determined by the status of HIF activity and pVHL status and stage of the lung cancer. Further studies with spatially and temporally controlled pVHL expression are warranted.

VHL AND PULMONARY FIBROSIS

Idiopathic pulmonary fibrosis (IPF) is another devastating lung disease with poor patient survival. It is characterized by progressive scarring of the lungs and elevated respiratory failure^[86-89]. The etiology of IPF is unknown, and currently there is no effective treatment^[90,91]. Pulmonary fibrosis is characterized by proliferation of lung fibroblasts and exaggerated deposition of ECM proteins, especially collagen and fibronectin^[86,89]. ECM proteins interact with integrins, which act as their cell membrane receptors and thus initiate FAK signaling cascades involved in cell proliferation^[92,93]. The proper assembly of fibronectin and collagen matrix requires the presence of pVHL, and loss of pVHL prevents fibroblast proliferation^[24,28,29,83], suggesting that pVHL may play a role in the pathogenesis of pulmonary fibrosis.

A microarray study showed that lungs of IPF patients expressed higher levels of pVHL mRNA than lungs of

control individuals^[94]. We have reported that lungs of fibrotic patients expressed elevated levels of pVHL in fibroblastic foci. In an experimental lung fibrosis model induced by Bleomycin, pVHL expression is also elevated in mouse lung fibroblasts but not in alveolar type II cells. Ectopic overexpression of pVHL in lung fibroblasts increased the expression of fibronectin, collagen, and the $\alpha 5$ integrin subunit as well as lung fibroblast proliferation^[95]. In addition, overexpression of pVHL induced FAK phosphorylation and activation^[95]. Consistently, inhibition of the fibronectin/integrin $\alpha 5 \beta 1$ /FAK signaling pathway diminished pVHL mediated cell proliferation while activation of $\alpha 5$ and $\beta 1$ integrin fibronectin/integrin $\alpha 5 \beta 1$ /FAK signaling pathway increased proliferation of fibroblasts. Moreover, pVHL is necessary for fibroblast proliferation after treatment of TGF- $\beta 1$, a potent pro-fibrotic cytokine. These results suggest that elevated expression of pVHL results in the aberrant fibronectin expression and activation of integrin/FAK signaling, leading to fibroblast proliferation and fibrosis^[95]. Although fibroblast activation (increased expression of collagen) and differentiation to myofibroblast (elevated levels of α -smooth muscle actin) are critical steps in fibrogenesis, we found that pVHL increased collagen expression but not the expression of α -smooth muscle actin, indicating pVHL may participate in earlier events in the formation of the fibroblastic foci, such as fibroblast proliferation^[95]. Interestingly, this gain of function of pVHL did not alter HIF activity, suggesting that pVHL plays a HIF-independent role in the pathogenesis of pulmonary fibrosis. However, Hickey and colleagues have recently reported that the mutation of pVHL at codon 200 (R200W) causes Chuvash disease with pulmonary vascular remodeling and hypertension^[53]. Older mice with this mutation contain elevated ECM deposition and develop fibrosis. In terms of the mechanism underlying the fibrosis in R200W mutation mice, the authors speculated that fibrosis is partially secondary to hemorrhage, edema, and impaired endothelial integrity and partially due to HIF2 activity^[53]. Thus, additional studies are warranted to elucidate the HIF-dependent and HIF-independent roles of pVHL in pulmonary fibrosis.

VHL AND ACUTE RESPIRATORY DISTRESS SYNDROME

The acute respiratory distress syndrome (ARDS) is a key cause of acute respiratory failure. ARDS can be caused by pneumonia, sepsis, aspiration of gastric contents, and major trauma, and it is characterized by pulmonary edema and severe hypoxemia^[96]. Most patients with ARDS who cannot clear alveolar edema efficiently have worse outcomes^[97-99]. Alveolar fluid clearance is effected by active Na^+ transport across the alveolar epithelium through apical Na^+ channels and basolateral Na, K-ATPase^[100]. Na^+ enters alveolar epithelial cells *via* the apical Na^+ channels and is extruded by the basolateral Na, K-ATPase, with water following the osmotic gradient into the intersti-

tium and pulmonary circulation, leading to the clearance of edema^[100-103]. Over-expression of Na, K-ATPase has been shown to increase alveolar fluid clearance^[104-107]. In contrast, in several models of lung injury, Na, K-ATPase activity in alveolar epithelial cells is decreased, resulting in decreased lung fluid clearance^[97,102,108-111].

Hypoxia is common in the lungs of patients with ARDS and inhibits Na, K-ATPase activity, thereby decreasing the rate of alveolar fluid reabsorption and worsening the outcomes of ARDS patients^[110-112]. Dada *et al.*^[113] reported that hypoxia increases mitochondrial reactive oxygen species (ROS) production, which activates protein kinase C (PKC). PKC phosphorylates Ser18 of Na-K-ATPase $\alpha 1$ subunit, triggering the endocytosis of Na-K-ATPase^[113]. Although short-term effects of hypoxia appear to be reversible upon reoxygenation with no significant degradation of total Na-K-ATPase in the whole cell lysate, prolonged hypoxia results in degradation of alveolar epithelial Na-K-ATPase *via* the ubiquitination/proteasome pathway^[113,114].

We found that alveolar epithelial Na-K-ATPase is downregulated in a pVHL-dependent manner^[115]. In the presence of pVHL, hypoxia promoted the degradation of plasma membrane Na-K-ATPase; in the absence of pVHL, hypoxia is unable to degrade plasma membrane Na-K-ATPase. pVHL mutants and dominant-negative Ubc5 (an ubiquitin conjugating enzyme, E2) prevented Na-K-ATPase from degradation, suggesting a functional pVHL E3 ligase is essential for Na-K-ATPase degradation during hypoxia^[115]. Interestingly, HIF overexpression is not sufficient to induce Na-K-ATPase degradation, and loss of HIF does not prevent hypoxia-mediated degradation of Na-K-ATPase. Therefore, pVHL-mediated Na-K-ATPase degradation is likely HIF independent^[115]. Moreover, generation of reactive oxygen species was necessary for pVHL-mediated Na-K-ATPase degradation during hypoxia^[115].

CONCLUSION

Although pVHL was first identified to be a tumor suppressor in renal carcinoma, accumulating evidence suggests that pVHL has a much broader spectrum of functions and is key to many organ dysfunctions. In this review, we summarized recent evidence that pVHL plays an essential role in a few respiratory diseases, including pulmonary arterial hypertension, lung cancer, pulmonary fibrosis, and ARDS. As in other tissues, pVHL functions in two distinct pathway in the lungs: HIF independent (fibrosis, lung cancer, and ARDS) and HIF dependent (lung cancer and pulmonary arterial hypertension). Certainly, this is only the beginning of uncovering the complex role of pVHL in lung diseases, as evidenced by a recent report which suggests pVHL expression is elevated in skeletal muscles of patients with chronic obstructive pulmonary disease (COPD)^[116]. However, the challenge to dissect the function of VHL disease is to investigate the role of pVHL in a cell-type-specific, context-specific,

and temporal fashion. A more challenging task is to elucidate in detail the molecular mechanisms by which pVHL regulate these functions in the lungs as an E3 ligase and adaptor protein.

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