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The roles of chromatin-remodelers and epigenetic modifiers in kidney cancer

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Clear cell renal cell carcinoma (ccRCC) is the major subtype of kidney cancer that is characterized by frequent inactivation of the von Hippel-Lindau (*VHL*) gene in 80–90% of the tumors. Recent reports using massive parallel sequencing technologies have discovered additional cancer driver genes. *PBRM1* was found to be mutated in about 40% of ccRCC tumors, whereas *BAP1* and *SETD2* were each mutated in about 10–15% of ccRCC tumors. *JARID1C* and *UTX*, two histone H3 demethylases, were also found to harbor mutations in ccRCC, albeit at lower rates. ccRCC tumors display a high degree of intra-tumoral heterogeneity, with some mutations present in all cancer cells (ubiquitous), whereas others are subclonal. The *VHL* mutations were always ubiquitous in the tumors; *PBRM1* mutations were also ubiquitous but to a lesser extent. On the contrary, mutations in *BAP1*, *SETD2*, *JARID1C*, and *UTX* were all subclonal, meaning that they were present in a subset of cancer cells in a tumor. The prognostic value of *PBRM1* mutations in ccRCC is still controversial, whereas *BAP1* mutations were tightly linked to worse clinical outcomes in multiple studies. The molecular functions of these newly identified cancer driver genes are discussed, and they were known readers, writers, or erasers of histone marks on histone H2 and H3 tails that are very close to each other, suggesting that these factors might functionally interact and affect common pathways. The studies on these newly identified tumor suppressors will shed light on ccRCC tumorigenesis and development, and will likely lead to development of novel therapeutic interventions for ccRCC patients.

Keywords *VHL*, *PBRM1*, *BAP1*, *SETD2*, *JARID1C/KDM5A*

VHL is the most frequently mutated gene in ccRCC

Inactivation of the *VHL* tumor suppressor gene is the causal event in the pathogenesis of clear cell renal cell carcinoma (ccRCC). Approximately 75% of the RCCs are of the clear cell type. Among them, 70–80% of ccRCC tumors harbor biallelic inactivation of *VHL* through mutation, deletion, or hypermethylation of its promoter that results in loss of expression (1). In hereditary kidney cancer patients, a germline *VHL* mutation predisposes individuals to earlier onset bilateral kidney cancer. Because one allele is already defective, only the remaining copy of *VHL* must be altered for biallelic inactivation to occur. pVHL, the protein product of the *VHL* tumor suppressor gene, encodes the substrate recognition unit of an E3 ubiquitin ligase complex, which also includes Cul2 and Rbx1. This complex targets the α subunits of the heterodimeric transcription factor hypoxia-inducible factor (HIF) for poly-ubiquitylation and proteasomal destruction. pVHL recognizes HIFα only when HIFα is hydroxylated on either of two prolyl residues by members of the Egl nine homolog family (also called prolyl hydroxylase domain-containing proteins or HIF prolyl hydroxylases) (Figure 1). When pVHL is inactivated, HIFα proteins are synthesized, and accumulate and form a complex with HIF1β protein. The complex then activates a transcriptional response to hypoxia in the nucleus. The constitutively active HIF activity subsequently drives tumorigenesis and growth of ccRCC tumors (2). Interestingly, not all HIF-induced genes are tumor-promoting (3,4). pVHL also has HIF-independent functions, but their relevance to tumor suppression remains unclear (5–7).

Restoration of pVHL expression and function in *VHL*-defective kidney cancer cells suppresses their ability to form

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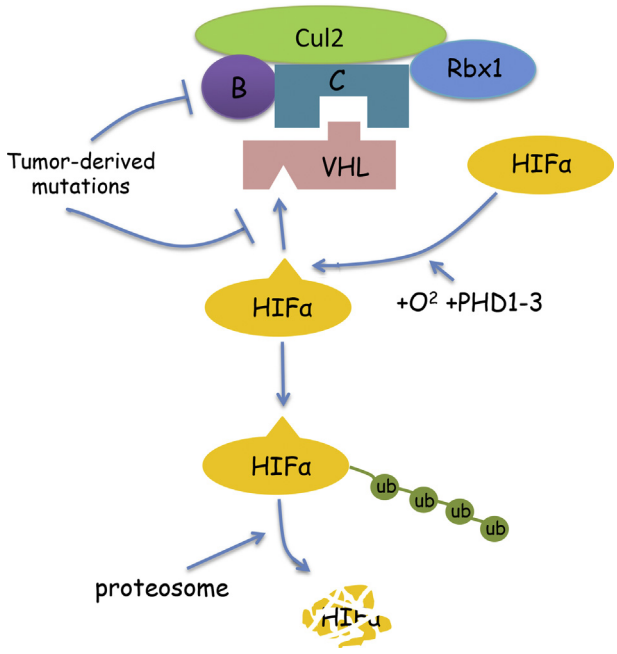

Figure 1 Schematic representation of HIF regulation by pVHL. pVHL is the substrate recognition subunit of an E3 ubiquitin ligase complex that contains Elongin B, C, Cul2, and Rbx1. HIFα subunits are constantly translated. In the presence of oxygen, prolyl hydroxylases promote proline hydroxylation on HIFα proteins, which leads to recognition by pVHL and proteasome-mediated degradation. Tumor-derived *VHL* mutations would either destroy the domain that mediates its interaction with the hydroxyl-prolines on HIFα or disrupt the domain that is responsible for Elongin C binding, leading to HIFα stabilization and constitutive activation of HIF. This, in turn, leads to ccRCC initiation and development.

tumors in nude mice (8), while stabilization of HIF2α in *VHL*-proficient cancer cells drives tumor growth (9,10). Conversely, reduction of high-level endogenous HIF2α expression in *VHL*-defective kidney cancer cells severely blocks the cancer cells' ability to form tumors in a xenograft model (11,12). Consistent with the notion that an abnormally activated HIF pathway is critical to tumor growth and maintenance, sunitinib, a small molecule antagonist against the VEGF and PDGF receptors (which are activated by two HIF-responsive genes), generates a 30–40% response rate and a 5-month increase in overall survival (OS) of kidney cancer patients (13). Other anti-angiogenesis drugs such as sorafenib, pazopanib, and axitinib hit the same targets and have similar effects. However, only one third of the patients respond strongly to these drugs, and almost all the tumors will develop drug resistance over time. Given that so many drugs are directed at the same molecular targets and that a large portion of ccRCC patients are not benefiting from them, other drug target(s) are urgently needed.

PBRM1 is another key tumor suppressor in kidney cancer

Although *VHL* is frequently mutated in ccRCC and has been shown to be essential to development in this disease, it is a well-known fact that most types of cancer harbor multiple driver mutations, sometimes dozens of them, which collectively establish the hallmarks of cancer that are too numerous for one mutation to achieve (14,15). Large-scale sequencing efforts have been undertaken to uncover genes mutated at a significant level in addition to *VHL* mutations in ccRCC. Varela et al. reported that 41% of ccRCC tumors harbor inactivating mutations in a SWI/SNF chromatin remodeling complex gene known as *PBRM1* (16). Interestingly, *PBRM1* is also mutated in bladder carcinoma, and in chromophobe and papillary RCC (Table 1), although at lower frequencies. The high rate of *PBRM1* mutations in ccRCC have been confirmed by multiple studies, along with mutations in several other tumor suppressor genes, including *BAP1*, *SETD2*, *JARID1C*, and *PTEN,* as well as the histone H3 lysine 27 demethylase gene *UTX* (*KMD6A*) (17,22–25). However, the mutations of these other genes occur at much lower rates than that of *PBRM1*.

PBRM1 is likely a tumor suppressor because the reported mutations are mostly inactivating ones; the main *PBRM1* mutations that have been observed are missense, frameshift truncation, frameshift insertion, and nonsense mutations, all of which are inactivating in nature. Knockdown of PBRM1 has been shown to significantly increase cell proliferation, cell migration, and colony formation in soft agar, indicating that *PBRM1* loss leads to a transformed phenotype. Transcriptionally, *PBRM1* suppression led to altered pathways governing chromosome instability and cell proliferation (16). The importance of *PBRM1* mutations to ccRCC development is further supported by the following discovery: *PBRM1* mutations, similar to those of *VHL*, occur early in ccRCC tumorigenesis, with mutations being present in all cancer cells within a tumor in many cases. Notably, however, it was discovered through multiregion exome sequencing that ccRCCs display profound intratumor heterogeneity (26–28). The majority of the driver mutations, including those of *BAP1*, *SETD2*, and *JARID1C*, are subclonal, meaning that they occur later during tumor development and are present only in a subpopulation of the cancer cells. Based on the mutations identified from different regions of a tumor, phylogenetic trees of mutations were constructed and the driver mutations were mapped onto them. *VHL* mutations were ubiquitous and mapped to the trunk of the mutational phylogenetic trees in the all cases, suggesting that it is the fundamental event of ccRCC development. *PBRM1* mutations were mapped onto the trunk in approximately 50% of cases when mutated, suggesting that, in many cases, *PBRM1* mutations also happen very early. The mutations of all the other genes mentioned previously were mapped onto the branches, suggesting that they occur later during tumor development (27). Collectively, these lines of evidence strongly suggest that, similar to *VHL*, *PBRM1* is a key tumor suppressor in ccRCC.

To date, the contribution of *PBRM1* mutations to the clinical outcome of ccRCC patients has been controversial. Some groups reported that *PBRM1* mutations occurred at a similar rate in different tumor stages, and these mutations did not seem to correlate with adverse patient survival (29,30). However, other groups have reported that *PBRM1* mutations are positively linked to tumor invasiveness (31) and, based on immunohistochemistry findings, loss of the PBRM1 protein was associated with advanced tumor stage, high