Published in final edited form as:

Cancer Discov. 2017 August ; 7(8): 802–804. doi:10.1158/2159-8290.CD-17-0610.

Bap1 and *Pbrm1:* Determinants of Tumor Grade and mTOR Activation in VHL-Deficient Mouse Models of Renal Cell Carcinoma

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Summary:

Large genome sequencing efforts have identified frequent mutations in the histone-modifying and chromatin-remodeling genes *BAP1* and *PBRM1* in clear cell renal cell carcinoma (ccRCC). In this issue of *Cancer Discovery*, Gu and colleagues model these genetic events in mice and report that dual inactivation of *Vhl* with either *Bap1* or *Pbrm1* results in faithful genetically engineered murine models of ccRCC. Moreover, their work establishes that *Bap1* and *Pbrm1* are determinants of tumor grade and mTORC1 activation and provocatively suggests that the cell of origin of ccRCC may lie in PAX8-expressing Bowman capsule cells.

Renal cell carcinomas (RCC) are derived from the renal epithelium and can be categorized by histologic subtype. Clear cell RCC (ccRCC) is the most common histology, and multiple studies have now established that inactivation of the von Hippel–Lindau (*VHL*) tumor suppressor gene is a key event in the vast majority of ccRCCs (1). Large-scale cancer genomic projects within the past half decade have identified recurrent, previously unrecognized loss-of-function mutations in multiple genes, including polybromo-1 (*PBRM1*), encoding BRG1-associated factor (BAF) 180, and BRCA1-associated protein-1 (*BAP1*), encoding a histone deubiquitinating enzyme of the ubiquitin carboxyl-terminal hydrolase (UCH) family (2, 3). *PBRM1* and *BAP1* are mutated in 29% to 41% and 6% to 10% of ccRCCs, respectively, and mutations of *PBRM1* and *BAP1* are well documented to be mutually exclusive of each other, with *BAP1*-mutant ccRCC having a significant association with higher grade and worse prognosis (2, 3).

Numerous efforts over the past decade have inactivated *Vhl* in isolation or in combination with other genomic events in the mouse kidney. Until recently, despite attempts from multiple groups, these labors have largely failed to generate faithful genetically engineered

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

murine (GEM) models of ccRCC. Whether these unsuccessful efforts were a result of a failure to target the proper cell of origin (i.e., Cre strain), the spectrum of genomic events modeled, or a refractoriness of the mouse renal epithelium to transformation has been unclear.

In this issue of *Cancer Discovery*, Gu and colleagues provide provocative data that shed insight into some of the possible past barriers to success in the development of ccRCC GEM models (4). Prior work by Brugarolas and colleagues has shown that deletion of *Vhl* and *Bap1* using *Six2-Cre* in the kidney results in early lethality shortly after birth but that *Vhl*-null, *Bap1* heterozygous mice develop ccRCC with a longer latency. In their current work, in an attempt to improve the model, the authors chose to use the *Pax8-Cre* strain because of its more limited expression within the kidney epithelium and because it is activated later in development. Codeletion of *Vhl* together with deletion of either *Pbrm1* (*Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$) or *Bap1* (*Pax8-Cre; Vhl*$^{F/F}$*; Bap1*$^{F/F}$) resulted in kidney tumors with features that faithfully recapitulate human ccRCC, including expression of markers CD10, vimentin, CAIX, and PAX8 and cytoplasmic accumulation of glycogen and lipid (Fig. 1). Quite strikingly and recapitulating genotype/phenotype correlations seen in human ccRCC, *Pax8-Cre; Vhl*$^{F/F}$*; Bap1*$^{F/F}$ mice developed high-grade cystic ccRCCs and lived to around 3 months of age, whereas *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ mice developed solid, multifocal, predominantly low-grade tumors in their kidneys with long latency and a median survival of around 12 months. In general, the histologic features, penetrance, and latency of the tumors seen in the *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ mice were similar to recent work by Nargund and colleagues, who used the Ksp-Cre system to drive deletion of *Vhl* and *Pbrm1* (5).

In keeping with their previous work demonstrating that BAP1 loss results in mTOR activation, the authors found that *Vhl-Bap1*–deficient tumors showed strong phospho-S6 (pS6) expression. pS6 staining of *Vhl-Pbrm1* tumors, however, demonstrated that although low-grade *Vhl-Pbrm1*–deficient tumors stained weakly for pS6, high-grade *Vhl-Pbrm1* tumors demonstrated strong pS6 expression, suggesting that mTOR activation is linked with tumor grade (Fig. 1). The authors go on to establish a causal role for aberrant mTORC1 activation as a determinant of tumor grade in *Vhl-Pbrm1*–deficient tumors. Specifically, deletion of a single allele of the Tuberous Sclerosis 1 gene, *Tsc1*, which negatively regulates mTORC1, in *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ mice (*Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$*; Tsc*$^{F/+}$) resulted in an increased rate of high-grade tumors and a shortened tumor latency relative to *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ mice.

There are now two FDA-approved allosteric inhibitors of mTOR, everolimus and temsirolimus. Interestingly, a clinical trial that enriched for patients with poor-prognosis metastatic RCC, which although not known, likely enriches for high-grade ccRCC tumors, demonstrated that patients treated with temsirolimus have a longer overall survival and progression-free survival compared with patients treated with standard IFNα, thus confirming the importance of mTOR activation and potential of mTOR targeting in poor prognosis ccRCC (6). Nonetheless, the mechanisms underlying how mTORC1 is activated by BAP1 loss and whether *BAP1* mutation truly imparts enhanced sensitivity to mTOR inhibition remain unclear. With respect to the latter point, analysis of a subset of patients from the RECORD-3 trial (randomized trial comparing first-line everolimus and sunitinib)

demonstrates that of the everolimus-treated patients with metastatic ccRCC, those with *BAP1* mutations had a shorter median progression-free survival than those with *PBRM1* mutations, the opposite of what would be predicted by the current work (7). Finally, precisely whether mTOR activation directly facilitates or is merely permissive for the transformation of low-grade to high-grade tumors deserves further investigation. This topic is especially interesting given that the spectrum of tumors that develop in patients with TSC are almost exclusively benign hamartomas.

Perhaps the most provocative aspect of the work presented by Gu and colleagues is the suggestion that the parietal cells of Bowman capsule are a potential cell of origin for ccRCC. Although it has long been held that the cell of origin for sporadic ccRCC exists within the proximal tubular epithelial compartment, the authors present several lines of evidence supporting their presumption. First, careful histologic analysis of *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ kidneys demonstrated alterations (cytoplasmic clearing, proliferative lesions) in the lining of parietal epithelial cells of Bowman capsule, which were also observed in uninvolved renal parenchyma adjacent to human ccRCC. Second, although inactivation of *Vhl*$^{F/F}$*; Bap1*$^{F/F}$ or *Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ using *Pax8-Cre* gave rise to ccRCC as above, inactivation of these same genes using Cre drivers expressed solely in proximal tubules (*Villin-Cre* and *Sglt2-Cre*) did not give rise to renal tumors (Fig. 1). Outside of the proximal tubule, *Pax8* is known to be expressed strongly in the distal renal tubules, collecting ducts and the epithelial cells of the Bowman capsule. This knowledge along with the findings that (i) ccRCC that develop in *Ksp-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ mice (as recently published by Hsieh and colleagues; ref. 5) have a similar phenotype to *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ tumors and (ii) *Ksp-Cre*, in addition to being expressed broadly in the renal tubular cells, is also known to be expressed in the parietal cells of Bowman capsule, suggest that at least in mice, the cell of origin of ccRCC is a *Pax8*-expressing cell outside of the proximal tubule. Indeed, a careful review of prior work by Creighton and colleagues (8) comparing the gene expression profiles of human ccRCC with an atlas of gene expression of microdissected human and mouse nephrons showed that ccRCCs (relative to papillary and chromophobe RCC) have high expression of genes found not only in proximal tubules but also in glomeruli (8). Nonetheless, as the authors acknowledge, the absence of tumors in *Villin-Cre* and *Sglt2-Cre* mice may be attributed to significantly fewer proximal tubular cells being targeted in these mice relative to mice expressing *Pax8-Cre*.

Progress in cancer research is greatly facilitated by having appropriate model systems. For many years, the kidney cancer research community has enviously sat on the sidelines while a plethora of GEM models of other cancers (i.e., lung, pancreas, sarcoma, to name a few) have been leveraged to better understand cancer biology and to expedite drug development. The faithful *Vhl-Bap1* and *Vhl-Pbrm1* GEM models developed by Gu and colleagues add to the recent salvo of GEM models of ccRCC based upon concurrent deletion of *Vhl, p53*, and *Rb1* by Frew and colleagues or the inactivation of *Vhl* and *Cdkn2a* with concurrent activation of *MYC* by our group (9, 10). The work by Gu and colleagues is a superb demonstration of how these GEM models can be leveraged to enhance our understanding of aspects of cancer biology (i.e., cell of origin) that cannot easily be examined in humans. Armed with these models, further important advances in kidney cancer should be swiftly forthcoming.

Acknowledgments

Grant Support

This work was supported by NIH R01-CA202053 (to W.Y. Kim) and R21-CA194987 (to J.Y. Leung).

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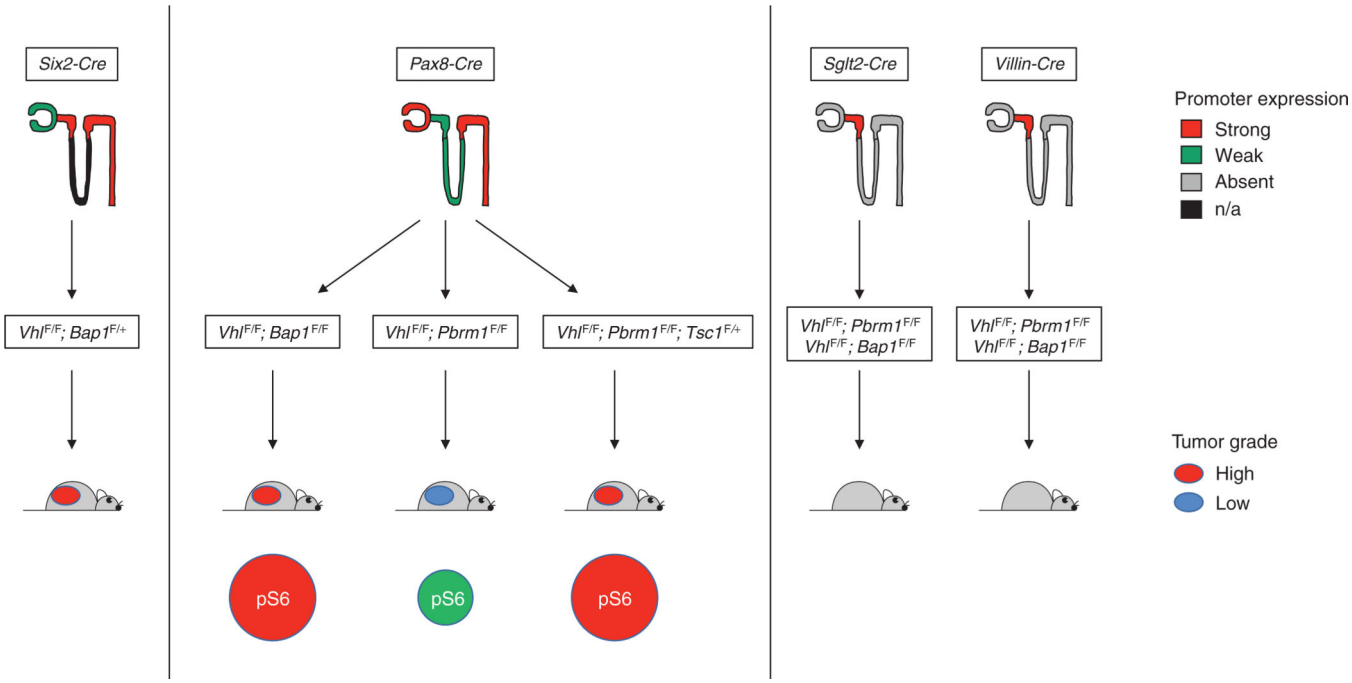

Figure 1.

Cre-specific phenotypes and determinants of tumor grade in RCC mouse models. *Six2*, *Pax8*, *Villin*, and *Sglt2* Cre strains all have unique expression patterns within the nephron. Inactivation of *Vhl* and *Bap1* by either *Six2-Cre* or *Pax8-Cre* results in high-grade ccRCC with high levels of mTORC1 activation, whereas inactivation of *Vhl* and *Pbrm1* by *Pax8-Cre* results in low-grade ccRCC with low levels of pS6. *Pax8-Cre; Vhl*$^{F/F}$*; Pbrm1*$^{F/F}$ tumors can be transformed into high-grade tumors by additional heterozygous deletion of *Tsc1* and resultant mTORC1 activation. Deletion of *Vhl* and *Bap1* or *Vhl* and *Pbrm1* using the proximal tubule–specific promoters *Villin-Cre* or *Sglt2-Cre* did not result in tumors, suggesting that the cell of origin of ccRCC in mice is a *Pax8*-expressing cell that lies outside of the proximal tubule.