RENAL CANCER BIOLOGY AND NOVEL TARGETS:
Genetically Defined Groups in Renal Cancer

Katherine L. Nathanson, MD, Cancer Center University of Pennsylvania, Philadelphia, PA

Introduction

Much of our understanding of how to delineate types of renal cancers has come from studies of inherited cancer susceptibility syndromes. While such syndromes are estimated to account for <3% of all renal cancers, they have contributed greatly to our knowledge of the biological basis of sporadic disease. Cancer susceptibility syndromes with a high risk of renal cancer include:

- von Hippel Lindau disease (vHL)
- hereditary papillary renal cancer (HPRCC)
- hereditary leiomyomatosis and renal cancer (HLRCC), and
- Birt-Hogg-Dube (BHD)

Each of these inherited diseases is associated with a predominant type of renal cancer – clear cell (ccRCC), papillary type 1, papillary type 2 and hybrid chromophobe/oncocytoma cancers, respectively. The study of inherited disease has enabled the development and use of targeted therapeutics for all patients with renal cancer. In addition, genetic changes may serve as predictive or prognostic biomarkers for treatment efficacy, which has been most thoroughly explored in relationship to VHL mutation status in ccRCC.

Von Hippel Lindau disease and ccRCC

vHL is an autosomal dominant cancer susceptibility syndrome in which patients develop hemangioblastomas of the brain, spine and retina, clear cell renal cancer, pancreatic cysts, pancreatic neuroendocrine tumors, endolymphatic sac tumors and pheochromocytomas.[1] The gene responsible for inherited susceptibility to vHL, VHL, was found through the study of multiple case families.[2] VHL is mutated not only in inherited ccRCC, but also in the vast majority of sporadic ccRCCs, with both copies lost in 86% and genetic or epigenetic changes of one allele found in 96%.[3] The VHL protein comprises part

of a complex, the main function of which is to ubiquinate the alpha regulatory subunits of the hypoxia inducible factor (HIF) family and target them for degradation.[4] The HIFs are transcription factors that regulate adaptation to tissue hypoxia, and loss of VHL allows chronic activation of the hypoxic response, including upregulation of the vascular endothelial growth factors (VEGFs), even under normoxic conditions.[5] The link between VHL and HIF has provided the basis for development of VEGF-targeted therapies for ccRCC.

Mechanisms of VHL mutation as biomarkers in ccRCC

VHL can be altered through point mutation (either truncating or missense), promoter methylation and larger genomic deletions or rearrangements. The different types of mutations, as well as their location, have been studied as potential prognostic or predictive markers associated with response to VEGF-inhibitors in ccRCC. Many of the studies of VHL mutations as biomarkers are limited due to sample size, incomplete genetic characterization or as in studies of predictive markers by inclusion of multiple VEGF inhibitors. The studies of VHL mutational status as a prognostic marker have been inconsistent, with some suggesting that loss is associated with a

worse, and others a better, prognosis. The largest study by Choueiri et al. examining VHL mutational status as a predictive biomarker in

123 patients treated with a variety of VEGF-inhibitors suggested that loss of function mutations in VHL were associated with treatment response.[6] However, VHL mutation status did not appear to associate with progression-free and overall survival. In order to fully evaluate the potential role of VHL mutation status as predictive or prognostic biomarker, it needs to be a component of large scale prospective clinical trials with thorough genetic evaluation.

Hereditary papillary renal cancer

HPRCC is an autosomal dominant syndrome characterized by multifocal, bilateral type I papillary renal cell carcinomas without extra-renal manifestations.[7,8] The responsible mutated gene is MET.[9] However, MET is mutated in less than 10% of sporadic type papillary renal cancers. Clinical trials of MET inhibitors for type 1 papillary renal cancers are underway.[10]

Hereditary leiomyomatosis and renal cancer

HLRCC is an autosomal syndrome characterized by the development of cutaneous and uterine leiomyomas and renal cancer.[11,12] Papillary type 2 is the predominant pathological type associated with HLRCC and tends to be early onset, high grade and aggressive.[13] The mean age of diagnosis is 40; metastatic renal cancer has been observed in individuals as young as 17. The mutated gene in HLRCC is fumarate hydratase (FH), which encodes the enzyme that converts fumarate to malate in the Kreb's cycle.[14] Consistent with a postulated role as a tumor suppressor gene, loss of the wild type allele is observed in renal cancer from individuals with FH mutations. However, mutations have not been observed in patients with sporadic RCC, but in part the lack of this observation may arise due to the limited number of papillary type 2 tumors included in the screening series.[15,16]

Birt Hogg Dube syndrome

BHD is an autosomal dominant syndrome characterized by the development of fibrofolliculomas (dysplastic hair follicles), lung cysts and pneumothoracies, and renal cancer, predominantly hybrid oncocytic tumors.[17,18] The gene in which mutations cause BHD is named folliculin (FLCN).[19] The FLCN protein has no homology to previously identified proteins, and its function is still largely unknown. A wide spectrum of renal cancers has been observed in patients with BHD, even within the same kidney.[20] The most common type of tumor is an unusual hybrid oncocytic tumor (mixed oncocytoma and chromophobe). Observation of a hybrid oncocytic tumor in any patient should prompt an evaluation for BHD, as it is so characteristic of this disease. In BHD, FCLN functions as a tumor suppressor gene, and, unusually, the second allele of FCLN is most frequently inactivated by point mutation rather than loss.[21] However, mutations in FLCN are rarely identified in sporadic renal cancers, most commonly in chromophobe tumors.[22,23]

Molecular profiling to define sub-groups of renal cancers

Both DNA and RNA-based molecular profiling in renal cancers has been done as proof of concept to demonstrate that these methods can differentiate between different types, such as clear cell and papillary renal cancers. However, more recent studies have focused on delineating sub-types within genetically defined groups of renal cancers, with most studies focusing on ccRCCs.

Innovations and Challenges in Renal Cancer

Gordan et al. recently demonstrated that within the group of ccRCCs with pVHL loss caused by mutation or methylation, two sub-types exist, those expressing HIF1α and HIF2α (termed 'H1H2') and expressing HIF2α only (H2).[24] Whereas H1H2 tumors show increased activation of Akt/mTOR and MAPK signaling pathways, H2 tumors have greater c-Myc activity. H2 tumors demonstrate increased expression of genes involved in double strand break repair, such as BRCA1 and BARD1, and consequently decreased levels of DNA damage, as measured by γH2AX and genomic copy number changes. In addition, they have higher levels of proliferation, and H2-only expressing cell lines progress more quickly through S-phase. Additional studies are necessary to delineate whether these sub-types of ccRCC are of prognostic or predictive significance in relationship to treatment with VEGF inhibitors.

Recent copy number analyses of ccRCCs, sporadic and associated with VHL disease, showed a similar profile between both groups, although the sporadic tumors were more heterogeneous with more events per tumor.[25] Unsupervised clustering of expression profiles could not distinguish between the two groups. Standard karotyping has been performed in 282 ccRCCs in patients with nephrectomies to examine whether cytogenetic changes were prognostic.[26] Deletion of 3p was associated with a better prognosis (p=0.03), whereas 4p (p<0.001), 9p (p<0.01) and 14q (p<0.01) loss were associated with a worse prognosis. In multivariate analysis, loss of 9p emerged, along with stage and grade as associated with poor survival.

Expression profiling has been used to delineate sub groups of ccRCC. In 177 tumors obtained at the time of nephrectomy using an array of 3,674 genes, Zhao at al. identified two major sub-groups, which encompassed two and three smaller groups, respectively.[27] These groups were associated with significant survival differences, and the activation of distinct pathways. More recently, two studies by Skubitz et al. and Brannon et al. have been performd on smaller sample sets of ccRCCs (16 and 48), but using much larger gene sets.[28, 29] Both analyses also identify two groups of ccRCCs, one of which is dominated by metabolism genes, the other by wound healing and epithelial to mesenchymal transition genes; the former group appears to be associated with a significant survival advantage. Expression analysis in 75 ccRCCs, a sub-set of 101 that underwent whole exome sequencing, also showed two groups – hypoxic and non-hypoxic.[30] In the former group, most (65%) carried a point mutation in VHL; the latter group was associated with NF2 mutations. JARID1C, SETD2 and UTX, histone modification genes, were each mutated in 3% of ccRCCs. A signature expression profile was associated with JARID1C and SETD2 mutations, but each of these account for a very small percentage of ccRCCs overall.

Conclusion

These different approaches all suggest that there are distinct molecularly defined sub-types of ccRCC, however additional work needs to be done to integrate them together. Future studies should combine HIF status, copy number, mutational data and expression profiling for optimal sub-grouping of ccRCC.

Discussion

Dr. Atkins: An interesting question is whether these tumors evolve. As we talk about immune therapy and then angiogenic therapy and then TOR inhibition therapy, is the mechanism of resistance or escape in those settings somehow related to selection of a different subset of tumors that may have profiles different from the primary tumor? Or is resistance related to a physiologic adaptation that can reverse once the selective pressure is removed? I do not think we know the answer, but I think that it is clinically relevant.

Dr. Stadler: I am convinced that we are underestimating the complexity. We call one disease renal cancer, but we know that it is not one disease to start with. We have a couple of different histologic subtypes starting out, and then we have a couple of different clear-cell subtypes. And then we have selective pressure with regard to metastases, that allow certain things to grow out, and then we introduce selective pressures of therapy. Furthermore, we have not even talked about the complexities within the stroma.

Dr. Kaelin: I would argue that as bad as it is in kidney cancer it is worse in many other tumors. To a first approximation kidney cancer is a disease caused by VHL loss. You can assume that and you will be right 90-percent of the time.

Dr. Stadler: I want to know what makes a clear cell a clear cell. I mean, clearly VHL loss is a critical step, but I think we need to put some names to some of the other lesions seen in these tumors, and then we will have the "Vogel-gram" for clear cell, and then we can ask these more sophisticated questions of, well, if the tumor evolves or is put under drug selection, what comes out?

Dr. Choueiri: In essence we don't know the reason we have all this heterogeneity in RCC including that 10-percent of clear cell RCC has wild type for VHL. Some of this may be technical and they may be clear cell, some of them may be some other cancer. It becomes even more complex when you start talking about other changes such as sarcomatoid differentiation because the tumor might have been clear cell to start with or it may have been something else completely different.

Dr. Kaelin: It looks to me that about 70–75-percent of clear cell patients get at least some benefit from VEGF pathway inhibition, which is consistent with having about 75-percent of clear cell as VHL-defective tumors. Now, I do not know that anyone has gone back to look to see whether people who are not getting any tumor shrinkage whatsoever are in fact molecularly clear-cell carcinomas.

Dr. Choueiri: We had 100-140 VHL patients and looked at CAIX status but we did not have a strong correlation with either response or progression free survival. VHL mutation was independently associated with response but not with survival or progression free survival. But the real issue is much, larger. What has happened in the past 7-10 years in this field was that all the large clinical studies were not required to collect tissue, and we as investigators did not push enough on industry to require tissue and to fund those studies. So here we are with drugs that we don't understand which population they work best in. The next generation of studies absolutely needs funding allocated for tissue collection to get these analyses going.

Dr. Atkins: Yes. The cooperative groups may be our opportunity to ask biology questions if industry does not fund them. But there is one study which I am hoping will help us address this question, and that is the RECORD 3 Study. This study looks at sunitinib versus everolimus first line with a switch to the alternative drug at the time of progression. I believe there will be extensive tumor tissue collection and hopefully we will learn something about who responds to an mTOR inhibitor versus a VEGF pathway inhibitor from that study.

Dr. Nathanson: Phospho-proteomic arrays, particularly RPPA, where you want to actually look at big proteomic efforts, really need fresh tissue and I think that the importance of fresh tissue has been really undervalued. We need to push for fresh tissue that can be used for a variety of studies that we just cannot do adequately on paraffin. Paraffin embedded tissue is really second best. This is an important issue for the kidney cancer community.

References
Genetically Defined Groups in Renal Cancer

1. Richards FM, Phipps ME, Latif F, et al: Mapping the Von Hippel-Lindau disease tumour suppressor gene: identification of germline deletions by pulsed field gel electrophoresis. Human Molecular Genetics. 1993; 2: 879-82.
2. Latif F, Tory K, Gnarra J, et al: Identification of the von Hippel-Lindau disease tumor suppressor gene. Science. 1993; 260: 1317-20.
3. Young AC, Craven RA, Cohen D, et al: Analysis of VHL Gene Alterations and their Relationship to Clinical Parameters in Sporadic Conventional Renal Cell Carcinoma. Clin Cancer Res. 2009; 15: 7582-7592.
4. Stebbins CE, Kaelin WG, Jr., Pavletich NP: Structure of the VHL-ElonginC-ElonginB complex: implications for VHL tumor suppressor function. Science. 1999; 284: 455-61.
5. Gordan JD, Simon MC: Hypoxia-inducible factors: central regulators of the tumor phenotype. Curr Opin Genet Dev. 2007; 17:71-7.
6. Choueiri TK, Vaziri SA, Rini BI, et al: Use of Von-Hippel Lindau (VHL) mutation status to predict objective response to vascular endothelial growth factor (VEGF)-targeted therapy in metastatic renal cell carcinoma (RCC). J Clin Oncol. 2007; 25s: Abstract 5012.
7. Zbar B, Tory K, Merino M, et al: Hereditary papillary renal cell carcinoma. J Urol. 1994; 151: 561-6.
8. Zbar B, Glenn G, Lubensky I, et al: Hereditary papillary renal cell carcinoma: clinical studies in 10 families. J Urol. 1995; 153: 907-12.
9. Schmidt L, Duh FM, Chen F, et al: Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet. 2997; 16:68-73.
10. Eder JP, Shapiro GI, Appleman LJ, et al: A phase I study of foretinib, a multi-targeted inhibitor of c-Met and vascular endothelial growth factor receptor 2. Clin Cancer Res. 2010; 16: 3507-16.
11. Toro JR, Nickerson ML, Wei MH, et al: Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. Am J Hum Genet. 2003; 73:95-106.
12. Alam NA, Rowan AJ, Wortham NC, et al: Genetic and functional analyses of FH mutations in multiple cutaneous and uterine leiomyomatosis, hereditary leiomyomatosis and renal cancer, and fumarate hydratase deficiency. Hum Mol Genet. 2003; 12:1241-52.
13. Merino MJ, Torres-Cabala C, Pinto P, et al: The morphologic spectrum of kidney tumors in hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. Am J Surg Pathol. 2007; 31: 1578-85.
14. Wei MH, Toure O, Glenn GM, et al: Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. J Med Genet. 2006; 43:18-27.
15. Morris MR, Maina E, Morgan NV, et al: Molecular genetic analysis of FIH-1, FH, and SDHB candidate tumour suppressor genes in renal cell carcinoma. J Clin Pathol 57:706-11, 2004
16. Lehtonen R, Kiuru M, Vanharanta S, et al: Biallelic inactivation of fumarate hydratase (FH) occurs in nonsyndromic uterine leiomyomas but is rare in other tumors. Am J Pathol. 2004; 164:17-22.
17. Khoo SK, Bradley M, Wong FK, et al: Birt-Hogg-Dube syndrome: mapping of a novel hereditary neoplasia gene to chromosome 17p12-q11.2. Oncogene. 2001; 20:5239-42.
18. Schmidt LS, Warren MB, Nickerson ML, et al: Birt-Hogg-Dube syndrome, a genodermatosis associated with spontaneous pneumothorax and kidney neoplasia, maps to chromosome 17p11.2. Am J Hum Genet. 2001; 69:876-82.
19. Nickerson ML, Warren MB, Toro JR, et al: Mutations in a novel gene lead to kidney tumors, lung wall defects, and benign tumors of the hair follicle in patients with the Birt-Hogg-Dube syndrome. Cancer Cell. 2002; 2:157-64.
20. Pavlovich CP, Grubb RL, 3rd, Hurley K, et al: Evaluation and management of renal tumors in the Birt-Hogg-Dube syndrome. J Urol. 2005; 173:1482-6.
21. Vocke CD, Yang Y, Pavlovich CP, et al: High frequency of somatic frameshift BHD gene mutations in Birt-Hogg-Dube-associated renal tumors. J Natl Cancer Inst. 2005; 97:931-5.
22. Gad S, Lefevre SH, Khoo SK, et al: Mutations in BHD and TP53 genes, but not in HNF1beta gene, in a large series of sporadic chromophobe renal cell carcinoma. Br J Cancer. 2007; 96:336-40.
23. da Silva NF, Gentle D, Hesson LB, et al: Analysis of the Birt-Hogg-Dube (BHD) tumour suppressor gene in sporadic renal cell carcinoma and colorectal cancer. J Med Genet. 2003; 40: 820-4.
24. Gordan JD, Lal P, Dondeti VR, et al: HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. Cancer Cell. 2008;14:435-46.
25. Beroukhim R, Brunet JP, Di Napoli A, et al: Patterns of gene expression and copy-number alterations in von-hippel lindau disease-associated and sporadic clear cell carcinoma of the kidney. Cancer Res. 2009; 69:4674-81.
26. Klatte T, Rao PN, de Martino M, et al: Cytogenetic profile predicts prognosis of patients with clear cell renal cell carcinoma. J Clin Oncol. 2009; 27: 746-53.
27. Zhao H, Ljungberg B, Grankvist K, et al: Gene expression profiling predicts survival in conventional renal cell carcinoma. PLoS Med. 2006; 3: e13.
28. Skubitz KM, Zimmermann W, Kammerer R, et al: Differential gene expression identifies subgroups of renal cell carcinoma. J Lab Clin Med. 2006; 147: 250-67.
29. Brannon AR, Reddy A, Seiler M, et al: Molecular Stratification of Clear Cell Renal Cell Carcinoma by Consensus Clustering Reveals Distinct Subtypes and Survival Patterns Genes & Cancer. 2010; 1: 152-163.
30. Dalgliesh GL, Furge K, Greenman C, et al: Systematic sequencing of renal carcinoma reveals inactivation of histone modifying genes. Nature. 2010; 463: 360-3.