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Intratumoral heterogeneity in kidney cancer

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Abstract

Clear cell renal cell carcinoma (CCRCC) is characterized by mutation of the *VHL* gene and loss of a segment of chromosome 3. A new study using multi-region exome sequencing has identified substantial intratumoral heterogeneity within large primary CCRCCs, which has profound implications for understanding tumor evolution and for developing effective therapies.

Kidney cancer affects nearly 300,000 individuals worldwide each year and is responsible for nearly 100,000 deaths annually. Clear cell kidney cancer, which represents 75% of cases, occurs in both a sporadic (non-hereditary) and a hereditary form. Mutations in the *VHL* gene, located on the short arm of chromosome 3, cause the inherited form associated with von Hippel-Lindau disease. *VHL* is also inactivated by mutation or hypermethylation in over 90% of sporadic clear cell kidney cancers¹. The fact that clear cell kidney cancers are slow growing, likely taking decades to reach 5 cm in size, and the observation that biallelic inactivation of the *VHL* gene is a very early event in the genesis of clear cell kidney cancer, make this malignancy an ideal model to study the genomic architecture and evolution of cancer. On page xxx of this issue, Charles Swanton and colleagues² report the application of multi-region exome sequencing (M-Seq) to investigate intratumoral heterogeneity in a collection of large (7–13 cm) clear cell renal cell carcinomas (CCRCCs). Notably, many of the mutations perceived to be cancer drivers were found to be present in only a segment of each tumor, and the genesis of the later mutations observed in the different regions appeared to be fundamentally different from the mechanisms responsible for the early genetic events. This work raises profound questions concerning the genetic landscape of cancer and how tumor heterogeneity may affect, and possibly confound, targeted therapeutic interventions.

Assessing cancer mutations

While tumor heterogeneity, assessed by mutation or copy number analysis, has been previously observed in a number of epithelial cancers, including breast, pancreatic and ovarian cancer^{3–5}, the study by Gerlinger *et al.*² highlights the variability of the mutational signatures that can occur during the development of large renal tumors. *VHL* gene mutation, combined with chromosome 3p loss, was universally found in each sample from every tumor, and this was considered to be a “truncal” (early) mutation. Truncal mutation of the

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chromatin remodeling gene *PBRM1* was also found in a subset of tumors. Conversely, other driver mutations—including alterations in the chromatin remodeling genes *SETD2*, *BAP1* and *KDM5C*, the *TP53* gene and genes in the PI3-kinase/mTOR pathway (*PTEN*, *PIK3CA*, *TSC2* and *MTOR*)—were found to be present only in segments of the tumor and were labeled “branch” mutations. In general, each assessed portion of tumor was found to have a unique spectrum of branch mutations. Thus, M-Seq analysis provides a view of the genomic architecture of a tumor that is not possible to ascertain from analysis of a single portion of a tumor.

Although The Cancer Genome Atlas clear cell kidney carcinoma (TCGA KIRC) single-sample mutational screen identified the same spectrum of driver mutations as the M-Seq analysis, the prevalence was considerably higher with M-Seq for *TP53* mutations and PI3-kinase/mTOR pathway mutations (5% and 18% versus 40% and 60%, respectively). Gerlinger *et al.*² also found that, as more areas were sampled, more heterogeneity was identified, suggesting that even this study underestimates the true extent of genomic heterogeneity in CCRCC. Another new finding of this study was the fundamental difference between truncal mutations and branch mutations. In particular, branch mutations were characterized by a high percentage of C>T transitions, specifically at CpG dinucleotides, and a lower percentage of A>G transitions when compared to truncal mutations, suggesting that specific mechanisms may underlie the later development of genomic heterogeneity in CCRCC. Further elucidation of CCRCC mutational signatures will hopefully facilitate the identification of druggable targets to alter tumor evolution or to prevent or slow the development of drug resistance.

Treating genomic heterogeneity

How do you develop a targeted therapeutic approach to a tumor with substantial genomic heterogeneity? One approach is to target truncal mutations⁶, such as *VHL*, which was found in every sample and is known to be an early event in CCRCC tumorigenesis. Studies of the *VHL* gene pathway over the past 20 years have provided the foundation for the development of seven targeted approaches that have been approved by the U.S. Food and Drug Administration (FDA) for treating individuals with advanced disease. The current targeted therapeutic approaches for the VHL/HIF pathway in CCRCC revolve around targeting downstream HIF pathway genes⁷, such as the vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) receptors. To date, this has been only partially successful, possibly due to the fact that only a small portion of the HIF pathway is being targeted. A more effective strategy may involve adopting a more global approach or developing combinatorial approaches to target the VHL/HIF pathway⁸. The increased availability of next-generation sequencing provides the opportunity for mutational analysis of multiple primary and metastatic biopsies to be performed either initially or after a VHL-based therapy (Fig. 1). This may identify multiple mutations associated with alternative targeted therapies that could be selected depending on the degree of tumor containing each mutation, their presence at the more aggressive metastatic sites or their increased presence after initial therapy.

Targeting CCRCC metabolism

Mutation of chromatin remodeling genes has been a consistent finding in single-sample analyses of CCRCC^{9–11}, and the study by Gerlinger *et al.*² emphasizes their importance by demonstrating a degree of parallel evolution occurring in the same chromatin remodeling genes, including *SETD2*, *BAP1*, *KDM5C* and *PBRM1*. Integrative analyses comparing patient outcome and *SETD2* and *BAP1* mutation have shown a correlation with decreased survival in CCRCC patients^{12,13}. In addition, an altered metabolic pattern, consistent with a Warburg shift to aerobic glycolysis with decreased oxidative phosphorylation and glutamine dependent reductive carboxylation¹⁴, was identified in the TCGA CCRCC study¹² in patients with advanced tumors and low survival. If multiple biopsies are taken for sequence analysis, metabolic analysis could also be performed on the same samples to correlate with the mutational spectrum as well as to potentially direct a targeted therapy. It is possible that gain of additional driver mutations and alteration of metabolism are fundamental to the development of advanced CCRCC and that targeting the metabolic basis of CCRCC¹⁵ could provide the foundation for the development of effective forms of therapy for this disease.

In summary, the findings of Swanton and colleagues have profound implications for understanding the genomic architecture and evolution of CCRCC—and potentially other forms of cancer—and for the development of therapeutic approaches for CCRCC as well as other malignancies.

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Figure 1.

Clear cell renal cell carcinoma is characterized by substantial genomic heterogeneity during tumor development. Potential approaches to identify targeted therapy could involve multi-region exome sequencing (M-Seq) of multiple primary tumor foci to identify common driver mutations. Alternatively, or in addition, tumors from metastatic sites could undergo sequence analysis to identify driver mutations essential for the development of metastases.