Genetic Heterogeneity of Kidney Cancer

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Key Points

- The observation that renal cell cancer patients often develop mixed responses to therapy has led to the hypothesis that intratumoral heterogeneity may exist within individual patient tumors.
- Advances in molecular phenotyping techniques have led to the identification of significant intratumoral genetic heterogeneity in renal cell cancers of clear cell and variant histologies.
- Phylogenetic trees constructed by inferring ancestral relationships of tumor subclones demonstrate branched rather than linear evolution patterns in individual renal cell cancers. The majority of known driver mutations in renal cell carcinoma map to branching and not to truncal portions of phylogenetic tree constructions.
- Individual renal cell cancers demonstrate evidence of convergent phenotypic evolution by tumor subclones. SETD2 and other tumor suppressor genes have undergone distinct genetic alterations in multiple spatially separated regions within a single tumor converging on loss of function.
- Intratumoral heterogeneity in renal cell cancer may confound clinical

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decision-making on therapeutic strategies, alter drug development strategies, and may require the identification of improved biomarkers to guide clinical practice.

5.1 Background

Clinicians have long suspected that significant heterogeneity may exist within individual tumors and their metastases [1]. Patients with metastatic renal cell cancer (RCC) are known to develop mixed responses to therapy suggesting the presence of tumor subclones and clonal selection [1, 2]. In the past, traditional laboratory techniques were employed to gain insights into the molecular basis of such heterogeneity. For example, chromosomal analysis has shown that a more complex cytogenetic pattern is found in more aggressive and advanced RCC, suggesting that sequential accumulation of chromosome changes may play a role in cancer progression [3]. An evaluation of chromosomal mutations and mitotic segregation patterns in RCC showed that in a subset of tumors, there were abnormally shortened telomere repeat sequences, chromosomal breakage-fusion-bridge events, multipolar configurations, and supernumerary centrosomes [4]. These observations suggested that changes in cell division machinery may be involved in the evolution of complex karyotypes and genetic intratumoral heterogeneity in a subgroup of RCC. Furthermore, Ljundberg et al. employed flow cytometry to evaluate DNA ploidy in 200 consecutive RCC specimens: these investigators reported that there was frequent heterogeneity in these specimens and concluded that "multiple samples must be investigated to evaluate properly the malignant character of renal cell carcinoma" [5]. Early investigations into the metastatic heterogeneity of RCC also involved the development of a nude mouse model for evaluating RCC metastasis [6]. Employing this mouse model, Fidler and colleagues used the SN12C RCC line which had a heterogeneous subpopulations of cells with varied metastatic potential, as well as cells derived from spontaneous lung metastases [6]. These investigations provided some early tools to individually study RCC variants with high metastatic potential and to develop models for dissecting tumor evolution and metastasis [7].

More recently, advances in molecular phenotyping techniques such as next-generation sequencing have allowed for a deeper understanding of RCC evolutionary biology through the detection of genetically distinct subclones within individual tumors and the characterization of clonal architecture [8]. This technology has subsequently been used to study intratumor heterogeneity not just in RCC but in a diverse range of tumor types including breast cancer [9, 10], pancreatic cancer [11], ovarian carcinoma [11], and acute leukemia, [13–15], among others. This chapter will summarize recent data that employed modern molecular techniques to shed light on RCC heterogeneity, clonal evolution, and the potential clinical implications of these findings.

5.2 Intratumor Heterogeneity

Employing whole-exome sequencing to study intratumoral heterogeneity, Gerlinger et al. analyzed multiple regions from ten primary tumors and their associated metastases in three cases [16, 17]. These investigators found that 67 % of identified somatic mutations were heterogeneous and not detectable across all sampled regions within an individual tumor. Mutational intratumoral heterogeneity was seen for multiple tumor suppressor genes converging on loss of function. In addition, these investigators applied a 110-gene signature shown to classify ccRCC into good prognostic and poor prognostic molecular subgroups on spatially distinct regions of one tumor sample. The metastatic tumors and one region of the primary tumor segregated into the good prognostic subgroup, while the remaining regions of the primary tumor segregated into the poor prognostic subgroup, further illustrating the significant molecular heterogeneity within an individual tumor.

Martinez et al. [18] further characterized the extent of intratumoral heterogeneity by comparing individual tumor samples of clear cell RCC with unrelated tumor samples collected from the Cancer Genome Atlas (TCGA). Twenty-five percent of tumor biopsies demonstrated greater genetic similarity with unrelated tumor samples than with samples originating from the same primary tumor.

To further assess intratumoral genetics that underlie the mutational spectrum of clear cell RCC, Xu et al. performed single-cell exome sequencing using material from a kidney cancer and its adjacent normal kidney tissue [19]. These investigations revealed that the kidney tumor was unlikely to have evolved from mutations in VHL and PBRM1. Quantitative population genetic analysis interestingly showed that the tumor did not contain any significant clonal subpopulations. However, this analysis revealed that mutations with different allele frequencies within the population had different mutational spectra, suggesting that clear cell RCC "may be more genetically complex than previously thought" [19]. Novel algorithms to construct phylogenetic models of tumor progression at the cellular level – incorporating copy number changes at the scale of single genes, entire chromosomes, and the whole genome – are currently under development and may help shed additional light on the implications of single-cell sequencing [20].

5.3 Heterogeneity in Variant RCC Histologies

Investigations of RCC heterogeneity extend beyond that of clear cell histology into that of less common variant subtypes. Using next-generation sequencing (NGS), Durinck et al. analyzed exome, transcriptome, and copy number alteration data from 167 primary human tumors that included renal oncocytomas and non-clear cell RCC consisting of papillary (pRCC), chromophobe (chRCC), and translocation (tRCC) subtypes [21]. Within the non-clear cell subtypes, these investigators found that pRCCs had a higher mutation rate than chRCCs and renal oncocytomas and that genes altered in non-clear cell RCC were distinct from that reported with clear cell histology. Ten significantly mutated genes were identified in pRCC, including MET, NF2, SLC5A3, PNKD, and CPQ. In chRCC, the following genes were found to be significantly mutated: TP53, PTEN, FAAH2, PDHB, PDXDC1, and ZNF765. Interestingly, gene expression analysis identified a five-gene set that molecularly classified chRCC, renal oncocytoma, and pRCC.

Malouf et al. described the genomic and epigenetic characteristics of translocation renal cell carcinoma (tRCC), a rare subtype of kidney cancer involving the TFEB/TFE3 genes [22]. These investigators reported moderate cytogenetic heterogeneity in this rare tumor type, with 31.2 % and 18.7 % of cases presenting similarities with clear cell and pRCC profiles, respectively. The most common alterations seen were 17q gain in 44 % and 9p loss in 37 %. Exome sequencing of tRCC revealed a distinct mutational spectrum with frequent mutations in chromatin-remodeling genes [23].

A study of molecular heterogeneity in RCC with sarcomatoid differentiation using X-chromosome inactivation analysis suggested that both clear cell and sarcomatoid components of renal cell carcinomas were derived from the same progenitor cell [24]. Additionally, different patterns of allelic loss in multiple chromosomal regions were reported in clear cell and sarcomatoid elements from the same patient, suggesting divergence during RCC clonal evolution.

5.4 Branching Evolution

Gerlinger et al. utilized genetic analyses to construct phylogenetic trees by inferring ancestral relationships of tumor subclones [17]. These phylogenetic trees of ccRCC demonstrated branched rather than linear evolutionary patterns in all ten samples analyzed. Early ubiquitous genetic alterations were mapped to the truncal portion of the phylogenetic trees, while later heterogeneous alterations occurring in separate spatial regions composed the branches. Known driver mutations of ccRCC were mapped onto the phylogenetic trees to determine whether specific

driver genes were predominantly altered on truncal or branch portions [25]. Alterations in the von Hippel-Lindau (VHL) tumor suppressor gene were identified ubiquitously on the truncal portions of each phylogenetic tree consistent with its role as a critical founder event in the pathogenesis of ccRCC. However, the majority of known driver mutations were mapped onto the branches of the phylogenetic trees with 73 % of driver mutations identified in subclonal populations. These mutations included alterations in PTEN, SETD2, KDM5C, PBRM1, and BAP1 expression identified in spatially separate subclones.

Tumor subclones frequently displayed evidence of convergent phenotypic evolution. Three distinct alterations of SETD2 were identified with different regional distributions in one patient tumor. Splice-site mutations were carried in one biopsy site, a missense mutation was identified in metastatic sites, and a two-base-pair frameshift deletion was detected in all other tumor sites. Convergent evolution was also observed for KDM5C, PIK3CA, BAP1, and PBRM1 with different disruptive mutations identified in regionally separate tumor sites.

5.5 Implications for Clinical Practice

The presence of significant intratumoral heterogeneity in RCC presents several challenges to clinical practice. In addition, the presence of branching evolution can influence biomarker identification and validation, evaluation of prognosis, and even therapy resistance [26]. Current therapeutic decision-making is frequently based on characteristics of a single tissue biopsy of a primary tumor or a metastatic site. The genetic profile of the biopsy is assumed to be uniformly expressed in all other sites of disease. The presence of intratumoral heterogeneity confounds this assumption and may lead clinicians to wonder whether multiple biopsies will be necessary to accurately characterize a tumor [27]. There are several barriers to performing multiple biopsies in a patient. Multiple procedures may be associated with significant physical and psychological morbidity, and access to metastatic sites may be technically difficult or impossible. In addition, it is unknown how many biopsies are necessary to accurately characterize a tumor. Gerlinger et al. attempted to identify the optimal number of biopsies to reliably detect the majority of somatic mutations in a tumor but reported that a persistent increase in the number of detected mutations was observed with each additional biopsy in a majority of cases [16]. This observation casts doubt on the assertion that multiple biopsy attempts can accurately characterize a patient's tumor. A different perspective was offered by Sankin et al. who obtained core needle biopsies from three to five different regions of resected renal tumors and performed targeted DNA sequencing on five genes associated with ccRCC (VHL, PBRM1, SETD2, BAP1, and KDM5C) [28]. These investigators estimated that sampling three different tumor regions was sufficient to detect mutations in PBRM1, SETD2, BAP1, and/or KDM5C with 90 % certainty but noted that the mutational burden of renal tumors varied by region sampled.

The branched evolutionary pattern of ccRCC genetic alterations also poses additional challenges to the clinician and to drug development. Somatic alterations that may be theoretically "actionable" may not be ubiquitously present in all tumor subclones and thus may represent an inadequate therapeutic target. To date, there are no therapeutic drugs that directly and fully address the consequences of VHL tumor suppressor inactivation, even though this alteration represents the only ubiquitous "truncal" event in ccRCC. The identification of intratumoral heterogeneity may spur additional research into the development of agents that can target "truncal" alterations or increase interest in combinatorial drug therapy that can target several subclonal driver mutations.

The detection of intratumoral heterogeneity in RCC also raises the question of whether improved biomarkers or detection modalities may be necessary to fully characterize this heterogeneity. We may end up determining that characterization of the dominant tumor subclone is sufficient for guiding clinical therapy. The development of technologies that detect circulating serum biomarkers such as free tumor DNA (cfDNA) may

hold potential to detect and characterize the dominant tumor subclone at a given time in therapy but will require further research and validation in RCC [29–31].

Additionally, intratumoral heterogeneity can also affect the pharmacodynamic properties of anticancer therapies. A recent review article noted that the concentration of many anticancer drugs in human solid tumors is low, with strong variation in different parts of the tumor [32]. This scenario mirrors the genetic heterogeneity discussed in detail above. There is strong likelihood that in some malignancies such as RCC, therapy resistance may result from insufficient and/or heterogeneous exposure of cancer cells to effective drug levels. More sensitive analytical methods to assess drug distribution within tumors coupled with novel noninvasive imaging techniques such as imaging mass spectrometry and fluorescence microscopy may allow for real-time drug localization in relation to the microscopic structure of the tumor. These newer techniques may provide insights into the relative contribution of tumor architecture on drug distribution [32].

Clinical Vignette

A 63-year-old female with medical history significant for hypertension and diabetes mellitus presented with dysuria, gross hematuria, and lower abdominal pain. Her primary care physician prescribed a course of oral antibiotics for a urinary tract infection, but symptoms persisted. A complete blood count showed anemia with a hemoglobin concentration of 9 g/dL. Computed tomography (CT) scan revealed a large 9 cm solid mass involving the lower pole of the left kidney, a 3 cm right liver lobe metastasis, and multiple 1–2 cm bilateral pulmonary metastases.

A preoperative assessment placed the patient at low risk for surgery. The patient underwent a left radical nephrectomy and was found to have clear cell renal cell cancer, Fuhrman grade 4. The patient is then referred to a medical oncologist.

Four weeks later, the patient is recovering well from surgery. She sees her medical oncologist who discusses treatment options including therapy with sunitinib. The patient agrees to therapy with sunitinib and begins a regimen of 50 mg orally once daily on a 4 weeks-on, 2 weeks-off schedule. Restaging CT scans performed after 12 weeks of total therapy reveal interval improvement in her bilateral pulmonary metastases that are now ranging from 0.5 to 1.5 cm in greatest dimension. However, her right liver lobe lesion has now increased to 4.6 cm in size. She asks her oncologist to explain to her why she appeared to have a good response to therapy in her lungs but not in her liver.

This case is an illustration of a common clinical scenario. This patient with metastatic renal cell cancer has developed a mixed response to therapy with antiangiogenic therapy. The tumor biology of the liver metastasis apparently differs from the pulmonary metastases. If advanced molecular profiling were performed on each metastatic tumor, differences in expression of key driver mutations may be discovered that explain the variable response to therapy.

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