Renal Cell Carcinoma and Genomics

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Abstract
A renal mass/Renal cell carcinoma has a range of histologies and tumour phenotypes that it presents with a particular challenge to treat them. A renal mass can range from benign (oncocytes) to a clinically indolent malignancy (papillary type I, chromophobe) to aggressive disease [papillary type II or high-grade clear cell renal cell carcinoma (ccRCC)]. Even among various subtypes, kidney cancers are genetically diverse with variable prognoses and treatment response rates. The key to proper management depends on the identification of these subtypes. Currently, a wide array of diagnostic, prognostic, and predictive biomarkers are available to help guide the individualized care of kidney cancer patients. This paper discusses the various serum, urine, and imaging biomarkers that are available in practice.

Keywords: Renal cell carcinoma • Genomics • Biomarkers • Genomic Alterations • Tumour

Introduction
Renal cell carcinoma (RCC), accounts for 2% to 3% of all adult malignancies, and is the most lethal of all the common urologic cancers. Approximately 73,750 new cases of RCC are diagnosed each year in the United States alone, and 14,830 patients are reported to die of the disease. RCC is primarily a disease seen in the older adults, with typical presentation between 65 and 75 years of age [1]. Most of the cases of RCC are sporadic and only 4% to 6% are believed to be familial in occurrence [1-3].

Several familial syndromes associated with RCC have been identified since the early 1990s. Similarly, the tumour suppressor genes and oncogenes that contribute to the development of sporadic and familial forms of this malignancy have also been characterized. The various subtypes of RCC and their distinct nature, has led to a major revision in the histologic classification of this malignancy based on advances in molecular genetics [3,4]. A beneficial impact on patient management has also been achieved with the development of targeted molecular agents (Figure 1) and these have shown an extended impact on patient management has also been achieved with the development of targeted molecular agents (Figure 1) and these have shown an extended survival for many patients with advanced RCC [3,5,6].

The familial forms of cancer may be holding the key to the identification of important regulatory elements such as the tumour suppressor genes. Knudson and Strong made certain observations in cases of retinoblastoma, which are known to be familial, multifocal and have an early onset and led them to propose a two-hit theory of carcinogenesis [7]. They proposed that there exists a gene product that suppresses the development of tumours and whenever both the alleles of this “tumour suppressor gene” is either mutated or inactivated, it leads to tumorigenesis. Knudson also postulated that patients with familial cancers were born with one mutant allele and that all cells in that organ or tissue were at risk, accounting for the early onset and multifocal nature of the disease. In contrast, sporadic tumours develop whenever a mutation occurs in both alleles within the same cell; because each event occurs with low frequency, most tumours develop late in life and in a unifocal manner [7,8].

Von Hippel-Lindau Disease, VHL Gene and Genetics of Clear Cell RCC
von Hippel-Lindau (VHL) disease is an autosomal dominant disorder that occurs with a frequency of 1 per 36,000 people. The familial form of clear cell RCC is seen in patients with von Hippel-Lindau (VHL) disease. Patients affected with this disease develop RCC, pheochromocytoma, retinal angiomias, and hemangioblastomas of the brainstem, cerebellum, or spinal cord. All these tumours are highly vascular and can lead to substantial morbidity. About 50% of patients affected with VHL disease develop RCC, which is seen at an early age (often in the third to fifth decades of life) and is bilateral and multifocal. RCC has now become the most common cause of mortality in patients with VHL disease as management of central nervous system manifestations have improved [3,9].

Cytogenetics provided the earliest clues regarding involvement of genetic elements in the development of RCC. Studies demonstrated that there was a common loss of chromosome 3 in patients with kidney cancer, particularly the clear cell variant, and this led to intensive efforts to find a tumour suppressor gene in this region. Kovacs and Cohen reported of translocations involving chromosome 3 which further implicated this chromosome as an important regulatory element [10-13]. Sophisticated molecular studies in patients with VHL disease eventually led to the identification of the VHL tumour suppressor gene [14]. The role of this gene as a tumour suppressor for sporadic and familial forms of clear cell RCC has been confirmed [3].

The VHL gene encodes a protein of 213 amino acids and consists of three exons. A large number of common mutations or “hot spots” in the gene have been identified, and a direct correlation between genotype and phenotype has been established in some cases [4,15]. The identification of this tumour suppressor gene represented a major advance in the field and required close collaboration between urologic oncologists and molecular geneticists [3]. The VHL protein is known to bind to elongins B and C and other proteins to form an E3 ubiquitin ligase complex and thereby modulates the degradation of important regulatory proteins (Figure 2). The most important function of the VHL protein complex is to target the hypoxia-inducible factors-1α and -2α (HIF-1α and HIF-2α) for ubiquitin-mediated degradation, keeping the levels of HIFs low under normal conditions. Inactivation or mutation of the VHL gene leads to accumulation of HIFs, most notably HIF-2α [3,16]. Accumulation of HIF-2α leads to a several-fold upregulation of the expression of vascular endothelial growth factor (VEGF), the primary angiogenic growth factor in RCC, contributing to the pronounced neovascularity associated with clear cell RCC [16]. HIF-2α also upregulates the expression of transforming growth factor-β platelet-derived growth factor, glucose transporter 1, erythropoietin, and carbonic anhydrase IX, which also promote tumorigenesis. VHL also upregulates HIF-1α which also plays a regulatory role, and this remains an important area of investigation [18].

The three most commonly mutated genes (PBRM1, BAP1, and SETD2) responsible in the development of sporadic RCC are also located on the short arm of chromosome 3, which is affected in more than 90% of clear cell RCC cases. These genes are involved in chromatin remodelling and histone methylation [17]. BAP1 mutations occur in up to 14% of sporadic clear cell

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RCC and are associated with poor prognosis. Germline mutations of BAP1 have been identified in some individuals with early onset, multifocal clear cell RCC and should be considered in such patients who are wild type for VHL [18].

Hereditary Papillary Renal Carcinoma Syndrome

Several studies have suggested an alternate genetic basis for non-clear cell RCC. Papillary RCC, which is the second most common histologic subtype of RCC, is characterized by trisomy for chromosomes 7 and 17 as well as abnormalities on chromosomes 1, 12, 18, 20, and Y [3]. In 1995 Zbar et al. reported on a familial syndrome of RCC-Hereditary Papillary Renal Carcinoma (HPRC), in 41 affected members from 10 families, the median age was 45 years, most of the patients developed multifocal and bilateral papillary RCC, and the mean survival in affected individuals was only 52 years. Unlike VHL disease, most patients with HPRC typically do not develop tumours in other organ systems [19,3].

HPRC demonstrates an autosomal dominant mode of transmission, similar to all of the other familial RCC syndromes [3]. Molecular linkage analysis revealed that the involved gene was localized to chromosome 7q31. In this case however the inciting event was activation of a proto-oncogene, rather than inactivation of a tumour suppressor gene, action research to appraise professional development plan usage.

Hereditary Leiomyomatosis and Renal Cell Carcinoma

In 2001 a familial renal cancer syndrome was described by Launonen in which patients commonly develop cutaneous and uterine leiomyomas and type 2 papillary RCC [20]. Patients at diagnosis are in the early 40s. Renal tumours are unusual for familial RCC in that they are often solitary and unilateral, and they are more likely to be aggressive than other forms of familial RCC. Collecting duct carcinoma, another highly malignant variant of RCC, has also been observed in this syndrome, which was named hereditary leiomyomatosis and renal cell carcinoma (HLRCC) syndrome. The HLRCC locus has been mapped to a region on 1q42-43, and this has been later shown to be the site of the fumarate hydratase (FH) gene. In this tumour autosomal dominant inheritance has been observed, and FH appears to be a tumor suppressor gene rather than an oncogene. FH is an essential enzyme in the Krebs cycle of oxidative metabolism [3,21]. The exact mechanism by which loss of FH expression leads to malignancy is still under investigation. The other familial subtypes of RCC have been described in Table 1.

Cancer Genome

In 2013 the Cancer Genome Atlas Research Network provided a more comprehensive molecular characterization of clear cell RCC. Next generation sequencing (NGS) was used to evaluate the whole genome of 22 tumours and whole-exome sequencing of 417 additional tumors. DNA copy number and genotype, CpG DNA methylation, messenger RNA expression, microRNA expression, and protein expression were also analysed in these same tumours, providing a wealth of information about the molecular features of clear cell RCC. The main findings included the identification of alterations in genes controlling cellular oxygen sensing, such as VHL, and regulation of chromatin status, such as PBRM1, BAP1, and SETD2. Overall, the analyses provided strong evidence for a metabolic shift in aggressive renal cancers, with down regulation of genes involved in the tricarboxylic acid (TCA) cycle, up regulation...
Relevant Genomic Alterations

The research in relation to RCC has focused primarily on identifying the clinical relevance of individual genes. Advances in whole-genome sequencing techniques have enabled researchers to examine a larger number of genes and their prognostic and predictive relevance in large cohorts of localized and metastatic RCC. The studies have shown that the genomic profile and clinical relevance of individual genes differed based on the histological subtype of RCC.

Clear cell RCC: Clear cell renal carcinoma forms 75%-80% of all renal cancers. The majority of information on RCC genomics has been obtained from studies of this RCC subtype. VHL gene has been the most commonly studied gene owing to its historical association with VHL syndrome, as well as chromatin remodelling genes residing in close proximity to VHL in the short arm of chromosome 3. Investigations have also demonstrated a link as chromatin remodelling genes residing in close proximity to VHL in the short arm of chromosome 3. Investigations have also demonstrated a link between somatic VHL mutations and the pathophysiology of sporadic ccRCC.

ccRCC pathogenesis begins with a chromothripsis event, which occurs in epithelial cells, to be the cell of origin in ccRCC, however some reports have suggested that these tumours could also originate in the Bowman’s capsule.

Other tumors

of pentose phosphate and glutamine transporter genes, decreased AMPK and PTEN protein levels, and increased acetyl coenzyme A carboxylase protein [22].

Table 1. Subtypes of familial renal cell carcinoma.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Predisposing gene (Chromosome)</th>
<th>Renal tumor histology and other major clinical manifestations</th>
<th>Recommended management for renal tumors</th>
<th>Potential therapeutic targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTF-associated cancer syndrome</td>
<td>MTF (3p14.1-p12.3)</td>
<td>Not defined</td>
<td>To be determined</td>
<td>To be determined</td>
</tr>
<tr>
<td>BAP1 tumor predisposition syndrome</td>
<td>BAP1 (3p21.2)</td>
<td>Clear cell RCC, can be high grade</td>
<td>Surgical excision, preference for nephron-sparing approaches</td>
<td>To be determined</td>
</tr>
<tr>
<td>Tuberous sclerosis complex (TSC)</td>
<td>TSC1 (9q34) or TSC2 (16p13.3)</td>
<td>Multiple renal angiomyolipomas Clear cell RCC (2%-3% incidence) Renal cysts / polycystic kidney disease Cardiac rhabdomyomas Cutaneous angiolipomas Pulmonary lymphangioleiomyomatosis Neuropsychiatric disorders, including autism spectrum disorder and cognitive disability</td>
<td>AML: surveillance for &lt;3 cm, everolimus for 3-5 cm, consideration for embolization or excision for ≥ 5 cm, preference for nephron-sparing approaches</td>
<td>mTOR pathway</td>
</tr>
<tr>
<td>PTEN hamartoma tumor (Cowden syndrome)</td>
<td>PTEN (10q23)</td>
<td>Papillary RCC or other histology Breast tumours (malignant nad benign) Epithelial thyroid carcinoma</td>
<td>Active surveillance &lt;3 cm</td>
<td>To be determined</td>
</tr>
<tr>
<td>Birt-Hogg Dube syndrome (BHD)</td>
<td>Folliculin (17p11.2)</td>
<td>Multiple chromophobe RCC, hybrid oncocytic tumors, oncocytomas Clear cell RCC (occasionally) Papillary RCC (occasionally) Facial fibrofolliculomas Lung cysts Spontaneous pneumothorax</td>
<td>Active surveillance &lt;3 cm</td>
<td>mTOR pathway</td>
</tr>
<tr>
<td>Succinate dehydrogenase deficient RCC (SDH-RCC)</td>
<td>SDHA (1p36.13), SDHB (1q23.3), SDHD (11q23.1), SHDAF2</td>
<td>SDH-associated RCC (chromophobe, clear cell, type 2 papillary RCC; or oncocytoma), variable aggressiveness Paragangliomas (benign and malignant) Papillary thyroid carcinoma</td>
<td>Surgical excision, preference for PN, but only when wide margins can be achieved</td>
<td>HIF-VEGF pathway; reductive carboxylation pathway</td>
</tr>
<tr>
<td>Hereditary leiomyomatosis and renal cell carcinoma (HLRCC)</td>
<td>Fumarate hydratase (FH) (1q24-23)</td>
<td>Type 2 papillary RCC most common Collecting duct carcinoma Leiomyomas of skin or uterus Uterine leiomyosarcomas</td>
<td>Surgical excision, preference for PN, but only when wide margins can be achieved</td>
<td>HIF-VEGF pathway; antioxidant response pathway; reductive carboxylation pathway</td>
</tr>
<tr>
<td>Hereditary papillary renal carcinoma (HPRC)</td>
<td>MET (7q31)</td>
<td>Multiple, bilateral type 1 papillary RCC</td>
<td>Surgical excision &lt;3 cm</td>
<td>MET kinase</td>
</tr>
<tr>
<td>von Hippel-Lindau disease VHL</td>
<td>3p25 (VHL)</td>
<td>Clear cell RCC, often multifocal Retinal angiomas Central nervous system hemangioblastomas Pheochromocytoma Other tumors</td>
<td>Surgical excision ≥ 3 cm</td>
<td>HIF-VEGF pathway</td>
</tr>
</tbody>
</table>
Accumulated HIFs, in turn, increase the production of several growth factors that have key roles in the progression of RCC [28]. A study from The Cancer Genome Atlas (TCGA) showed that 52% of the cohort of 478 patients with ccRCC had genomic alterations in VHL [14, 27]. However, clinical outcomes did not co-relate with the presence of VHL mutations in this cohort. Current evidence suggests that, overall, VHL alterations are not associated with clinical outcomes [28,29].

**PBRM1**

PBRM1 mutations are the second most common somatic alteration in ccRCC and are detected in 30–40% of patients. PBRM1 is located in the short arm of chromosome 3 in close proximity to VHL, SETD2 and BAP1 [28,30]. Accordingly, loss of heterozygosity in chromosome 3p, which is the key tumour driver event in RCC pathogenesis, is also linked to loss of PBRM1 function [31,32].

BAP1: BAP1 is a two-hit tumour suppressor gene (that is, inactivation of both alleles is required to induce a change in the phenotype of the cell) with histone deubiquitinase activity that acts as an essential chromatin regulator and suppresses cell proliferation. Similar to PBRM1, BAP1 is located on the short arm of chromosome 3 and can thus also be affected by the most common RCC pathogenesis event - loss of the short arm of chromosome 3. The frequency of BAP1 mutations in ccRCC ranges from 5%-18%. BAP1 mutations have been associated with metastatic disease at diagnosis, high Fuhrman grade and the presence of necrosis [33,34].

**SETD2**

The histone-lysine N-methyltransferase SETD2 modulates alternative splicing and active transcription. Beyond its role in chromatin regulation, SETD2 contributes to DDR-loss of SETD2 and subsequent loss of trimethylated histone 3 lysine 36 (H3K36me3) in ccRCC cell lines resulted in microsatellite instability [35,36]. SETD2 is also located on chromosome 3p and is therefore prone to alterations in the context of ccRCC.

EZH2. The histone methyl transferase EZH2 is a component of the polycomb repressive complex 2 (PRC2) - which is involved in chromatin remodeling and functions as an important epigenetic modulator in oncogenesis. Activation of EZH2 leads to epigenetic silencing of various genes, including tumour suppressor genes [37,38].

**Genomic Signatures**

Despite the research efforts that have led to the discovery of promising associations between clinical outcomes and individual genomic alterations, no single alteration has been shown to directly modulate survival or benefit from RCC therapeutics. The treatment of localized RCC comprises surgery, with complete resection of malignant tissue, and subsequent follow-up to monitor for disease recurrence. Trials of adjuvant therapies to prevent tumour recurrence have been largely unsuccessful in both the cytokine era and in the targeted therapy era. However, the S-TRAC trial demonstrated that 12 months of sunitinib treatment delayed tumor recurrence for ~1 year. [39] Current ongoing efforts include the use of targeted therapies or immunotherapies in both the adjuvant and neoadjuvant settings.

Combination therapies represent the latest breakthrough in the treatment of metastatic RCC. Treatment with nivolumab and ipilimumab was the first breakthrough and was associated with compelling overall survival benefit over sunitinib (not reached versus 26 months, respectively; hazard ratio=0.83, P<0.001) in patients in the intermediate and poor risk International Metastatic Renal Cell Carcinoma Database Consortium categories [40]. Given the increasing number of first-line treatment options, investigators have attempted to develop predictive gene expression signatures to help guide treatment choices [41].

**Conclusion**

Current scientific knowledge is based largely on studies of genome-wide sequencing data from tissue samples obtained via surgery or biopsies. The genomic features of primary tumour tissues have been largely detailed and the next step is to track the natural genomic evolution of RCC throughout the course of the disease- the TRACERx Renal study has provided compelling details of these changes over time.


