Recent Genomic Advances in Renal Cell Carcinoma

Satish K. Tickoo, M.D.
Memorial Sloan Kettering Cancer Center
New York, NY

I have no disclosures
Renal cell carcinomas (RCC) comprise many different entities at clinicopathological level, and that these are distinct at molecular level as well, has been known for almost 3-4 decades now. Recently, many large-scale comprehensive, multi-platform genomic analyses projects have not only re-confirmed this knowledge, but also shown significant variability among the large sub-types of RCC.1-8

While many of these findings point toward biological implications of these differences, utilizing this information for further prognostic sub-classification among different sub-types of RCC is not recommended at this stage.

Three of the most comprehensively studied RCC sub-types at molecular level include clear cell RCC, chromophobe RCC and papillary RCC.3,4,6,7 Some insights into the molecular findings in renal cell tumors are discussed below, in brief:

**Clear cell RCC:**

Allelic loss of 3p is fundamental to clear cell RCC; loss of heterozygosity (LOH) at 3p is now known to occur in more than 90% of the sporadic tumors. LOH at 3p occurs through either simple 3p loss (78%) or copy-neutral LOH (uniparental disomy; 22%). These different mechanisms might explain the variable rates of 3p loss cited in the literature in the past. Biallelic loss of VHL, located on chromosome 3p25, occurs through 3p loss and concomitant VHL mutation or promoter methylation in up to 65-90% of clear cell RCC.5

In normoxemic conditions, hypoxia-inducible factor-1α (HIF1α) and HIF2α are hydroxylated on proline residues by Prolyl Hydroxylase 1 (PHD1), PHD2 and PHD3. Hydroxylated HIFα is recognized by the pVHL–elonginC–elonginB–culin2–RBX1 E3 ubiquitin ligase complex (VHL complex) and targeted for ubiquitination and proteasomal degradation. In hypoxemic conditions, PHD1, PHD2 and PHD3 are inactive (oxygen being an essential cofactor). Similarly, in the absence of pVHL, as is true for most clear cell RCCs, VHL complex is lacking. As a result, HIFα is not degraded, accumulates, and forms heterodimers with HIF1β. These heterodimers translocate to the nucleus, bind to hypoxia-response elements (HREs) and induce the transcription of multiple genes involved in adaptations to hypoxia, and likely tumorigenesis, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), erythropoietin (EPO), glucose transporter 1 (GLUT1), carbonic anhydrase 9 (CA9/CAIX), etc. HIF is also regulated by the mammalian target of rapamycin (mTOR) via an mTOR signaling motif in HIF molecule. Targeted therapies in clear cell RCC have extensively utilized tyrosine kinase inhibitors for VEGF/PDGF (sunitinib and related agents) and mTOR inhibitors, targeting these HIF and mTOR pathways.

The other top three mutated genes in clear cell RCC- PBRM1, SETD2 and BAP1- are located close to each other on chromosome 3p21.2-5 These histone modifying and chromatin remodeling genes are tumor suppressors. As is expected, one-copy loss of 3p simultaneously results in loss of one of the two copies of these genes, similar to VHL. Further, mutations in these 3 genes are
reported in approximately 40, 11 and 10% of the tumors.\textsuperscript{5} Mutations in \textit{BAP1} and \textit{SETD2} have been reported to have adverse prognostic significance, negatively affecting survival and tumor recurrence.\textsuperscript{5,9-11}

**Other clear cell RCC mimickers:**

**Clear cell papillary RCC**

WHO now recognizes clear cell papillary RCC as a distinct tumor, separate from clear cell RCC with which it has been confused in the past. The tumor is characterized by branching or non-branching tubular, cystic, acinar, and collapsed acinar architectural patterns usually in various combinations. The most characteristic feature is the diffuse, linear alignment of the nuclei, away from basement membrane.\textsuperscript{12-15} The characteristic immunophenotype consists of diffuse CK7 positivity (in almost 100% cells), with cup-shaped diffuse membranous reactivity for CA-IX. HMWCK is often positive, while CD10 and AMACR are usually negative. At genetic level unlike clear cell RCC, these tumors do not show 3p losses, or \textit{VHL} gene mutations or promoter hypermethylation.\textsuperscript{12-15} Rare described instances with LOH of 3p or \textit{VHL} mutations likely represent clear cell RCCs with areas resembling clear cell papillary RCC.\textsuperscript{16} Clear cell papillary RCC show elevated sorbitol levels, and these high sorbitol levels are believed to lead to high levels of HIF and activation of HIF pathway.

**TCEB1-mutated RCC**

TCEB1-mutated RCC was recently described as an entity distinct from clear cell RCC, with which it shares a number of morphological and some immunohistochemical features. The tumor shows prominent fibromuscular stroma, imparting it a vaguely nodular appearance. While immunohistochemical staining pattern for CA-IX is similar to that in clear cell RCC, these tumors are also CK7 positive. They show hotspot mutations in \textit{TCEB1} gene, along with loss of heterozygosity of chromosome 8q, the location of \textit{TCEB1} (8q21.11), in the other allele.\textsuperscript{17,18} All tumors lack 3p loss or mutations in \textit{VHL} and related genes. \textit{TCEB1} encodes for the protein elongin C, which is one of components required for the formation of VHL complex. This entire complex is essential for proteasomal degradation of hydroxylated HIF1\(\alpha\). The mutation almost always occurs at a single hotspot amino acid residue (Tyr 79) that is required for binding to pVHL. This explains why these tumors display overexpressed HIF1\(\alpha\) and its downstream signaling molecules such as CA-IX.

**Tuberous Sclerosis Complex (TSC)-associated RCC**

Some renal cell carcinomas in Tuberous Sclerosis Complex (TSC) may be mistaken for clear cell RCC. The tumors are often multifocal and bilateral, are accompanied by multiple cysts in the renal parenchyma usually lined by large cells with eosinophilic cytoplasm, and may be associated with angiomyolipoma in the kidney or regional lymph nodes.

TSC is associated with inactivating mutations in Tuberous Sclerosis Complex (\textit{TSC1} and \textit{TSC2})
tumor suppressor genes. TSC1 is localized to chromosome 9q34 and TSC2 to 16p13.3. Prevalence of mutations in TSC1 and TSC2 genes are roughly equal in TSC syndrome. Proteins encoded by TSC1 (hamartin) and TSC2 (tuberin) function as complex to negatively regulate mTOR signaling; the negative regulation is performed through activation of Ras homologue expressed in brain (Rheb). Abnormal/absent hamartin/tuberin complex leads to increase in activated mTOR and tumorigenesis. About 2/3 of cases of TSC are sporadic, as result of new mutations; therefore a family history may be absent.

MiTF/TFE Family Translocation-Associated Carcinoma

These translocation-associated carcinomas are characterized by translocations involving MiTF/TFE family genes (TFE3 or TFEB) to mostly well-characterized genes at a number of different chromosomal locations, and includes renal tumors with:

TFE3 gene (located at chromosome Xp11.2) translocated to 17q25 (ASPS/CR1 or ASPL), 1q21 (PRCC), 1p34 (PSF), Xq12 (nonO), 17q23 (CLTC), 17q25 (RCC17), or 3q23 (unknown), or TFEB gene (located at chromosome 6p21) translocated to 11q12 (MALAT1 or α).19,20

t(X;17)(p11.2;q25) (ASPS/CR1 or ASPL-TFE3) translocation is similar to that in alveolar soft part sarcoma (ASPS), but the translocation is balanced in TFE3 renal carcinoma, and unbalanced (loss of some genetic material) in ASPS.

Different TFE3 gene fusions consistently lead to overexpression of fusion protein relative to native TFE3, such that protein becomes detectable by immunohistochemical (IHC) assay. Similarly, MALAT1-TFEB fusion gene results in dysregulated expression of full-length TFEB protein detectable by IHC. However, because of technical and fixation related issues with IHC assays that may lead to non-specific, weak or inconsistent results, TFE3 and TFEB break-apart FISH assays are regarded to be more specific and useful for the diagnosis.

Chromophobe renal cell carcinoma

Chromophobe renal cell carcinoma shows loss of one copy of six chromosomes in more than 85% of the tumors tested, and these are chr. 1, 2, 6, 10, 13, and 17.6 Losses of other chromosomes, including 3, 5, 8, 9, 11, 18, 21, and Y, are also seen in a smaller proportion of cases. As a matter of fact, multiple chromosomal losses are present in all classic variants of the tumor, whereas such losses are less frequent, or rarely even absent, in the eosinophilic variants.

Single somatic mutations of TP53 and PTEN are other significant genetic alterations (in 32% and 9% cases, respectively), as observed in The Cancer Genome Atlas (TCGA) cohort of 66 chromophobe RCCs. In addition, mutations of different genes in the mammalian target of rapamycin (mTOR) pathway (in approximately 20% tumors), and recurrent rearrangements in promoter region of telomerase reverse transcriptase (TERT) were also noted.6

Another interesting aspect of the tumor is the presence of mutations in mitochondrial DNA (mtDNA), that mostly involve electron transport chain complex I, in approximately 20% of the
tumors. Such mutations are observed predominantly in the eosinophilic variants of the tumor. mtDNA mutations are also well known in renal oncocytoma, and these recent observation in predominantly eosinophilic chromophobe RCCs raise interesting issues about these 2 tumor types.

Papillary renal cell carcinoma

Both the pathological and molecular classifications of papillary RCC are beset with controversies and lack of clarity. While WHO divides papillary RCC into types 1 and 2, with well-defined histological features, a number of entities that are not papillary RCC have been included among type 2 papillary RCC category in numerous publications in the literature. Some of such tumors include what we know now as HLRCC-associated RCC, collecting duct carcinoma, MiTF/TFE family translocation-associated carcinoma, etc. As a result, while the genetic features of type 1 papillary RCC are well defined and consistent, a variety of findings have been reported among the so-called type 2 tumors. To address some of these issues, the 2016 WHO has defined papillary RCC as, “...tumor with papillary or tubulopapillary architecture and is often well circumscribed”, and further states, “Tumours that have papillary architecture but show features of recognized morphotypes of RCC (i.e. MiT family translocation RCC, hereditary leiomyomatosis and RCC–associated RCC, collecting duct carcinoma, and mucinous tubular and spindle cell carcinoma) should not be diagnosed as PRCC.”

Papillary RCC shows multiple chromosomal gains (of at least one complete copy of the chromosome), including frequent gains of chromosomes 7 and 17 and less frequently of chromosomes 2, 3, 12, 16, and 20, most often in type 1. Some tumors included among type 2 group also show such changes. Also, MET mutations are seen in approximately 17% of type 1 papillary RCC, and rarely in type 2. Trisomy 7 or MET mutations are present in an overwhelming majority of type 1 tumors (>80%). Type 2 papillary RCC, in addition, shows CDKN2A mutations in approximately 1/4th of the tumors.

Other genetic alterations described in type 2 tumors include CpG Island Methylator Phenotype (CIMP), FH gene mutations, TFE translocations, and NF2 mutations. These alterations clearly indicate that a number of other tumor types are being included among type 2 papillary RCCs, and as suggested by some authors this group includes several independent diseases. Therefore, type 2 papillary RCC as a group needs to be better defined before consistent genetic alterations can be ascribed to the tumor type.

BIBLIOGRAPHY