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Review

Molecular Cancer Research

Insights into the Genetic Basis of the Renal Cell Carcinomas from The Cancer Genome Atlas

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Abstract

The renal cell carcinomas (RCC), clear cell, papillary, and chromophobe, have recently undergone an unmatched genomic characterization by The Cancer Genome Atlas. This analysis has revealed new insights into each of these malignancies and underscores the unique biology of clear cell, papillary, and chromophobe RCC. Themes that have emerged include distinct mechanisms of metabolic dysregulation and common mutations in chromatin modifier genes. Importantly, the papillary RCC classification encompasses a heterogeneous group of diseases, each with highly distinct genetic and molecular features. In conclusion, this review summarizes RCCs that represent a diverse set of malignancies, each with novel biologic programs that define new paradigms for cancer biology. *Mol Cancer Res; 14(7); 589–98. ©2016 AACR.*

Introduction

Cancers of the kidney have long fascinated physicians with their highly divergent phenotypes and patterns of behavior (1). Kidney tumors are conventionally grouped based upon their anatomic origin and cell type into four general categories: renal cell carcinomas (RCC), which arise from the renal cortex epithelial cell compartment; collecting duct carcinomas and renal medullary carcinomas, both of which arise from the renal medulla; and papillary urothelial carcinomas, which arise from the transitional epithelium lining the renal pelvis and ureter (2). These classifications roughly follow the anatomic groupings of the nephron and the route of passage toward the urinary bladder, with papillary urothelial carcinomas recognized as sharing more pathologic and histologic characteristics with transitional cell carcinoma of the urinary bladder and ureter than other types of kidney cancer (3).

The RCC classification is yet further broken down into three main pathologic subtypes defined by their histologic and morphologic characteristics: clear cell RCC (ccRCC), papillary RCC (pRCC), and chromophobe RCC (chRCC). Of these, ccRCC is by far the most common, comprising roughly 70% of all renal cortical tumors. Next most common is pRCC, making up about 10% to 15% of RCC cases, followed by chRCC, which is a rare carcinoma that accounts for only about 5% of RCCs (4). Marked differences in risk for development of metastatic disease and tumor aggressiveness are exhibited between all of these different classes of RCC tumors.

Although rare, there are yet additional cases of RCC that do not fit into these broad categories. These include renal medullary carcinoma and translocation carcinoma. It is anticipated that a revised World Health Organization (WHO) pathologic classification recognizing even more rare and largely low-risk entities, such as clear cell tubulopapillary RCC and multilocular cystic RCC, will be published in 2016 (5, 6). There are also a handful of related benign tumors that occur in the kidney, including angiomyolipoma, which shares some common features with ccRCC, and renal oncocytoma, which is genetically and histologically similar to chRCC, although with key differences (7).

One major challenge that has stymied the field has been classifying these tumors for therapeutic considerations. Modern clinical trials now dictate the histology of cases that are included, but until even a few years ago, it was commonplace for all RCC histologies to be lumped together in clinical studies. The result is that today, the FDA-approved treatments applied to the RCCs are identical for what we now know are highly disparate tumor types. However, thanks to the highly forward thinking of The Cancer Genome Atlas (TCGA) project, the three major RCC subtypes have each been examined independently in TCGA genomic profiling efforts. Had all RCCs been considered as a single group, the numbers of tumors for the important but rarer entities of chRCC and the type II pRCC (pRCC-II) would have been insufficient to draw new conclusions about these clinically distinct and meaningful diseases. Thus, in this review, we compare and discuss the unique findings observed by the TCGA for tumors from each of the three primary RCC classifications, as a more complete understanding of the biology and signaling of these different types of RCC is a major step forward in determining the best way to treat each of these distinct tumor types.

ccRCC

Background

The most commonly encountered malignancy in the cortex of the kidney is ccRCC (also referred to as KIRC in the TCGA studies), making up roughly 70% of RCCs. This tumor type is well known to overlap significantly with the ccRCC occurring in the setting of von Hippel–Lindau (VHL) disease, and indeed,

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mutation of the *VHL* gene, which causes VHL disease, is observed in up to 90% of sporadic cases of this cancer (8). These tumors are characterized by a histology pattern of cleared cytoplasm with an acentric nucleus and cells that are organized into small, tight vascular bundles. Although patients with ccRCC usually present with nonmetastatic disease, approximately 25% of patients have metastases at initial presentation, and another 30% of individuals with ccRCC will eventually develop distant metastases.

Historically, RCCs, and ccRCC specifically, display inherent resistance to conventional cytotoxic chemotherapies. As a result, despite the lack of actionable tumor cell intrinsic targets, the field has sought to take advantage of the unique biology imparted on this cancer as a result of *VHL* mutation, namely, VEGF-promoted angiogenesis (9, 10). Antiangiogenic agents targeting VEGF or the endothelial cell VEGFR have been approved for the treatment of ccRCC (11–14), and due to the dominance of this histologic subtype, these agents have been given broad approval for use against all of the RCCs. When used to treat ccRCC, although rarely curative, these agents have been shown to significantly reduce metastatic growth and enhance progression-free survival (11–13) and overall survival as determined by implicit meta-analysis (15). The benefit of these drugs as monotherapy in this setting, in contrast to many other tumors, is clearly tied to the central biologic feature of enhanced vascularity (16) driven by the deregulation of hypoxia-inducible factors (HIF) that is inherent in *VHL*-mutated ccRCC.

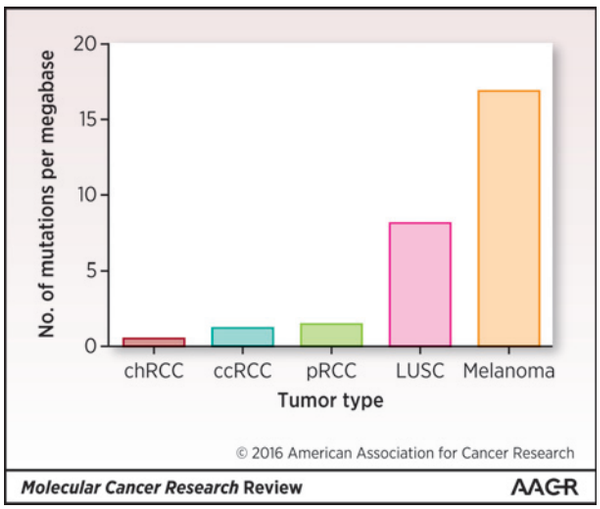

Figure 1.
Mutational burden across RCC spectrum in comparison with other solid tumors. The RCC TCGA projects identified lower mutational burdens relative to TCGA projects in classic, carcinogen-associated solid tumors, such as squamous lung cancer (LUSC) and melanoma.

TCGA analysis of ccRCC

Even though ccRCC is the most studied of the RCCs, the large set of more than 400 ccRCC tumors examined by TCGA provided additional insight into the genetic and molecular makeup of this disease (17). As with each of the types of RCCs detailed below, the TCGA applied a multiplatform analysis consisting of copy number analysis, whole-exome sequencing (WES), RNA sequencing, miRNA sequencing, methylation analysis, and proteomic analysis (RPPA). In addition, selected cases also underwent whole-genome sequencing (WGS). All samples were annotated with demographic, outcome, and treatment information.

ccRCC genomics and emerging biomarkers. The copy number analysis of ccRCC samples revealed a strong signature of 3p deletion and 5q gains, with other modifications largely scattered across the genome. Some alterations of interest included frequent deletions of *CDKN2A (*p16/INK4A tumor suppressors) and *RB* loci, as well as *MYC* amplification. In general, the number of focal and arm level alterations was much smaller on a per-tumor basis than that commonly observed in other types of cancer (Fig. 1). WES revealed common *VHL* mutations, as expected, which when coupled with promoter hypermethylation, indicated *VHL* inactivation occurred in more than 60% of cases. The next most commonly mutated genes were all chromatin modifiers: *PBRM1* (*BAF180*), *SETD2*, *KDM5C (JARID1C)*, and *BAP1*. These proteins all play diverse roles in chromatin maintenance, ranging from Swi/Snf nucleosome repositioning (PBRM1) to histone modification, such as deubiquitination (BAP1), methylation (SETD2), and demethylation (KDM5C). Intriguingly, three of these genes (*PBRM1*, *SETD2*, and *BAP1*) reside in the commonly deleted 3p region, which includes the *VHL* gene. 3p loss occurs in >95% of ccRCC, and this clustering of genes may account for some of the unusual aspects of this tumor type and is explored in greater detail in a recent review (18). Subsequent studies have suggested that mutations in *BAP1* may identify a subgroup of tumors with a higher malignant potential (19, 20). Another pathway recurrently mutated in the ccRCC tumors were genes in the PI3K/AKT/mTOR signaling pathway. Alterations in one or more components of this pathway were present in approximately 28% of TCGA ccRCC samples. Recently, study of aggressive sarcomatoid RCCs found an association between mTOR pathway activation and increased proliferation, and tumors with mutations in this pathway are, in some instances, more sensitive to mTOR inhibitors (21).

Although sequencing analysis of ccRCC did not reveal distinct genetic subgroupings, gene expression and microRNA analyses did identify four unique subgroups (m1–m4 and mi1–mi4, respectively), including predicted mRNA targets for the miRNA subgroups. With the emergence of mRNA-based prognostic and predictive biomarker panels in breast and colon cancer (22, 23) as well as lymphoma (24), expression-based subgroups are becoming more widely accepted for diagnostic use. It is now clear that gene expression biomarkers also may be useful for the classification of ccRCC. Interestingly, the gene expression clusters identified by the TCGA overlap clearly with the previously reported subtypes of ccRCC known as ccA (corresponding to m1) and ccB (corresponding to m2 and m3; ref. 25). Likewise, a distinct overall survival advantage was associated with the m1 subtype, as was previously observed for the corresponding ccA tumors (25). Thus, continued study of these subgroups as potential biomarkers remains an area of active investigation.

Metabolic features contribute to the overall outcome of ccRCC. Dysregulation of metabolic pathways is a common feature of ccRCC, owing to the upregulation of numerous key enzymes

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of glycolysis downstream of HIF transcriptional activation (26, 27). Examination of the HIF family members has demonstrated that changes in the glycolytic genes are largely driven by a HIF1α-specific transcription (28), which has both direct and indirect influences on metabolism (29). Similar changes in the expression of metabolic pathway genes have also been observed in a HIF1α-mutant mouse model of RCC (30). The interactions between HIF and metabolism are complex and bidirectional, with recent studies suggesting that expression of one glycolytic enzyme, fructose-1,6-bisphosphatase, has tumor-suppressive activity via direct repression of HIF functionality, while loss of this enzyme is associated with the progression of disease (31).

Surprisingly, through the integrated analysis of hundreds of tumors in the TCGA study, it was revealed that the upregulation of genes involved in glycolysis and fatty acid synthesis were associated with a significantly worse survival outcome in ccRCC patients, whereas genes involved in Krebs cycle or AMPK signaling were associated with an overall better survival outcome. These findings suggest that there are either inherent metabolic differences that may influence the progression of ccRCC tumors or that a metabolic switch occurs favoring HIF-targeted glycolytic gene expression and higher rates of glycolysis during tumor progression, the latter of which has been suggested by studies showing fructose bisphosphatase 1 downregulation, and commensurate increase in glucose uptake in tumor samples displaying loss of this enzyme and patterns of poor risk gene expression (31, 32). Although the mechanisms by which these metabolic differences contribute to tumor progression have not yet been elucidated, these findings may help to explain some of the heterogeneity that is observed between ccRCC cases.

Linking chromatin modifier gene mutations to the progression of ccRCC. As indicated above, after *VHL*, the most commonly mutated genes in ccRCC, *PBRM1*, *SETD2*, *KDM5C*, and *BAP1*, are all involved in regulation of chromatin. Overall, methylation patterns in ccRCC tumors in the TCGA favored hypermethylation when compared with normal kidney. In addition, the extent of hypermethylated promoter sites increased with both stage and grade, suggesting that hypermethylation was a feature associated with progression. In contrast, the *SETD2*-mutant tumors displayed a unique signature pattern of global DNA hypomethylation at nonpromoter regions that distinguished them from other tumors. The implications of this remain uncertain, although recent studies have demonstrated that *SETD2* mutations alter chromatin accessibility (33) and that expanded methylation coordinated with changes in histone methylation across the genome (34). As mutations in these chromatin regulators were associated with altered expression patterns in a large number of other genes, chromatin remodeling appears to play a key role in the progression of ccRCC.

Summary of TCGA analysis of ccRCC

Analysis of more than 400 ccRCC tumors by the TCGA revealed that the primary genetic changes contributing to this tumor type include those underlying cellular oxygen sensing, including *VHL* and its signaling pathways, and those involved in the maintenance of chromatin states, specifically *PBRM1*, *SETD2*, *KDM5C*, and *BAP1*. Importantly, a metabolic shift in ccRCC appears to be associated with disease progression, as more aggressive tumors demonstrated upregulation of genes involved in glycolysis and fatty acid synthesis and down regulation of genes involved in Krebs (TCA) cycle and AMPK pathway signaling. Likewise, overall changes in promoter hypermethylation were correlated with higher grade tumors. These findings, combined with the clustering of tumors into distinct subsets using gene expression data, have provided a foundation for future identification of subtype- and pathway-specific diagnostics and treatments.

pRCC

Background

pRCC is the second most common histologic subtype of RCC, representing 10% to 15% of cases (4). On the basis of histology, pRCC can be further divided approximately 1:1 into type I (pRCC-I) and type II (pRCC-II) tumors (35, 36). pRCC-I tumors feature small, basophilic cells forming distinct papillae, whereas pRCC-II tumors exhibit large, eosinophilic cells with pseudostratification (35). The pRCC-II tumors are larger, more likely to metastasize, and have an inferior prognosis (37).

The clinical care of pRCC remains largely uninformed by the tumor's biology, especially among metastatic patients. These patients are typically treated with VEGFR-directed therapies developed predominantly for ccRCC, a biologically and genetically divergent disease. Outcomes for pRCC patients treated with these medications are, predictably, inferior relative to ccRCC (38). If clinical care is to be advanced for these patients, a more complete understanding of the molecular biology of these tumors is needed.

TCGA analysis of pRCC

Prior to the TCGA report on pRCC, no single large study had systematically examined the sporadic form of this disease. The TCGA examined 161 pRCC tumors, which after expert pathology review were classified as pRCC-I (39), pRCC-II (40), and pRCC not otherwise specified (26). A number of significantly mutated genes were identified in these tumors. Of particular note were alterations in several genes previously known to be commonly mutated in other cancers. These included *NF1*, involved in the Hippo signaling pathway, and *SMARCB1*, *PBRM1*, *SETD2*, *KDM6A*, and *BAP1*, all involved in chromatin modification pathways. *TFE3* and *TFEB* gene fusions were also found to occur frequently in pRCC, resulting in higher levels of expression of their transcriptional targets.

Similar to prior gene expression studies (41), the multiple molecular platforms used in the TCGA, including those evaluating somatic copy number, miRNA, and mRNA expression, were able to confirm the divergent biology of pRCC-I and pRCC-II tumors. In addition, correlation with clinical data demonstrated a higher stage and poorer survival for the pRCC-II patients, as observed in previous studies (37). Moreover, strategies integrating these molecular platforms (42, 43) not only reinforced the pRCC-I versus pRCC-II classification schema but further resolved pRCC-II into three distinct subtypes: pRCC-IIa, pRCC-IIb, and CpG island methylator phenotype (CIMP). Thus, pRCC represents at least four molecularly distinct subtypes (Table 1).

pRCC type I. Tumors classified as pRCC-I demonstrated a lower clinical stage and better survival relative to pRCC-II tumors. Consistent with prior observations (44, 45), pRCC-I tumors were enriched for *MET* mutations: of the 14 observed somatic MET

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Table 1. Clinical and molecular distinctions among the pRCC molecular subtypes

	pRCC-I	pRCC-IIa	pRCC-IIb	pRCC-IIc or CIMP
Survival	Good	Good	Intermediate	Poor
MET activation	++++	−	−	−
NRF2/ARE activation	−	+	+	+++
CDKN2A/RB alterations	−/+	++	++	+++
SETD2 mutation	−	−	++	−
SWI/SNF alteration	+	+	++	+
DNA hypermethylation	−	−	+	++++
FH mutation	−	−	−	++++

NOTE: A total of 161 pRCC tumor samples were collected with clinical correlative data, subtyped as pRCC-I or -II during central pathology review, and molecularly profiled. *MET* activation corresponds to a diverse set of molecular perturbations, including trisomy 7, promoter and exon mutations, RNA overexpression, and others. NRF2/ARE activation includes *NQO1* overexpression. *CDKN2A* inactivation includes focal deletion, promoter methylation, and mutations. *SETD2* was significantly mutated in type II papillary tumors and enriched within the pRCC-IIb subtype. SWI/SNF pathway activation as determined by HotNet analysis was significant across all tumors but enriched within the pRCC-IIb subtype. The CIMP subtype was unique in terms of poor survival, young age of onset, extensive DNA hypermethylation, and *FH* mutations, as well as evidence of hypoxia and Warburg-like metabolism −, not observed; +, observed occasionally; ++, observed; +++, observed frequently; ++++, observed universally; +/−, equivocal.

mutations, only one was in a pRCC-II tumor. In addition to somatic tumor mutations, several of the samples contained a previously described germline *MET* mutation reported in hereditary pRCC (46). Other molecular mechanisms of *MET* activation were also observed in the pRCC-I patients. For example, about 10% of pRCC-I patients contained a novel *MET* RNA transcript lacking exons 1 and 2. As these exons encode the HGF ligand-binding domain of the *MET*-encoded HGF receptor, the transcribed protein is hypothesized to exhibit ligand-independent activation. Furthermore, four tumors that underwent WGS were observed to have *MET* promoter mutations that were predicted to be functional (47). In addition to MET mutations, pRCC-I tumors were frequently observed to have chromosomal gains of the *MET*-encoding chromosome 7. Thus, trisomy 7 may also contribute to the overall increase in *MET* mRNA expression and HGF receptor activation observed in pRCC-I relative to pRCC-II tumors. When considering these various molecular events, the role of *MET* as a driver in pRCC-I tumors was reinforced.

pRCC type II. As described previously, pRCC type I and II tumors are distinct in terms of histology, clinical stage, and patient survival. As such, it is not surprising that several molecular features emerged that distinguished pRCC-II tumors from pRCC-I. For example, alterations in *CDKN2A*, which encodes p16 (INK4A), were more common amongst pRCC-II samples, resulting in proliferation-associated increases in expression of phosphorylated Rb and cell-cycle genes. In addition, as seen for ccRCC, mutations in the chromosome modifier genes *SETD2*, *BAP1*, and *PBRM1* were also more commonly observed in pRCC-II tumors.

When examining differential gene expression between pRCC-I and pRCC-II tumors, one of the most divergent gene groups corresponded to the NRF2/antioxidant response element (ARE) pathway. This observation is consistent with reports that have documented mutations in NRF2/ARE pathway genes in pRCC-II tumors (48). *NFE2L2*, the canonical NRF2 gene, is a transcription factor that, when stable, triggers a cellular response that is protective against oxidative and electrophilic stresses. Although initially thought to act as a tumor suppressor by inhibiting carcinogenesis, recent reports implicate this pathway as oncogenic. Indeed, inactivating mutations in genes involved in *NFE2L2* degradation, such as *KEAP1* and *CUL3*, as well *NFE2L2* mutations that render it resistant to degradation, have been observed in cancers and are associated with poor outcomes (49–51). Thus, the NRF2/ARE pathway has been suggested to be oncogenic, conferring protection against the stress incurred by rapid proliferation and chemotherapy. Expression of *NQO1*, one of the chief transcriptional targets of *NFE2L2*, was observed to be significantly higher in pRCC-II tumors in the TCGA study, underscoring the fact that the NRF2/ARE pathway seems to play a particularly important role in these tumors.

As previously mentioned, TCGA analysis of the pRCC tumors further subdivided the pRCC-II tumors into three additional subtypes: pRCC-IIa, pRCC-IIb, and CIMP. These subgroups were distinguished from one another based upon combined differences in histology, genetic alterations, gene expression and methylation patterns, and clinical outcomes.

pRCC type IIa and IIb. Among the pRCC-II tumors, patients with pRCC-IIa tumors trend toward the best survival, whereas those with pRCC-IIb tumors have intermediate survival. Similarly, patients with pRCC-IIa tumors tend to have more clinical stage I and II disease, whereas pRCC-IIb patients have more clinical stage III and IV disease. In addition, the pRCC-IIb tumors harbored the majority of the *SETD2* mutations identified among all the pRCC samples.

pRCC type-II CIMP. The pRCC-II CIMP subtype is of particular interest, as it is associated with the extremes in terms of age of onset, prognosis, and several oncogenic pathways. The CIMP subgroup in the TCGA analysis was relatively small (9 tumors or 6% of all pRCC) and was identified primarily from the DNA methylation data as having significant, genome-wide hypermethylation. The CIMP patients were the youngest of the pRCC-II subtypes, with a median age of 42 years and had the worst overall survival. The previously described NRF2/ARE pathway was highly dysregulated in these tumors, as was the CDKN2A/RB tumor suppressor pathway. In addition, the CIMP tumors demonstrated marked metabolic dysregulation highlighted by the presence of either somatic or germline mutations in the gene fumarate hydratase (*FH*) in 6 of the 9 CIMP tumors and low *FH* expression in all CIMP tumors. Overall, the CIMP tumors demonstrated a metabolic shift toward increased glycolysis, with the upregulation of hypoxia-related and glycolytic pathway genes, accompanied by lower expression of Krebs (TCA) cycle and AMPK complex genes.

Summary of TCGA analysis of pRCC

The powerful and integrated analysis of pRCC performed by the TCGA provided important insights into this disease's genetic basis. This study not only validated previous disease classifications and their genetic drivers but also identified new differences between tumor types that may prove valuable in treating this disease.

Known biologic distinctions, such as pRCC-I versus pRCC-II, were validated and described in greater molecular detail than was previously possible. *MET* was validated as a likely oncogenic driver in many pRCC-I tumors, but other oncogenic pathways

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were also identified as playing an important role in pRCC-I tumorigenesis. For example, while the NRF2/ARE pathway is activated more frequently in pRCC-II, a small minority of pRCC-I tumors also demonstrated high levels of NRF2/ARE target gene expression. The more aggressive pRCC-II tumors are a heterogeneous group that were further resolved through the multiplatform TCGA analysis into pRCC-IIa, pRCC-IIb, and CIMP. Collectively, these tumors exhibit activated NRF2/ARE as well as inactivation of the tumor suppressor *CDKN2A*. The CIMP tumors were identified as a group with especially poor prognosis and early onset, characterized by genome-wide hypermethylation, *FH* mutations or low expression, and a shift to a Warburg-like metabolism.

With the knowledge gained, investigators are now presented with the challenge of designing logical strategies to therapeutically target the diverse biology and signaling patterns exhibited by these tumor subtypes and to select for patients most likely to benefit from these therapies.

chRCC

Background

chRCC is traditionally an indolent disease, with tumors characterized by cells with mildly granular to pale and finely reticular cytoplasm with central clearing and irregular nuclear borders. The disease shares many histologic features of the benign condition oncocytoma, but unlike oncocytoma, does carry risk for metastasis, and can transform with sarcomatoid features, rendering it highly aggressive and lethal. Only about 5% of RCC cases are classified as chRCC, making it the kidney cancer subgroup occurring at the lowest frequency among those included in TCGA studies. Interestingly, however, this normally rare cancer is more common than pRCC among young women with non–clear cell histology tumors. This raises questions about how gender differences, such as hormonal factors or specific pathway dysregulation, could be involved in the development of chRCC (52).

The inclusion of chRCC in the TCGA marked the first commitment to mapping the integrated genome of rare tumor types (53). Although the number of deaths annually attributed to chRCC is undoubtedly low, the argument for interrogating this rare disease was 2-fold: (i) to reveal important features of chRCC, so that these patients could benefit from appropriate treatment advances, and (ii) to allow a rare and unusual, but homogeneous, set of tumors to inform new aspects of tumor biology, which may be relevant for other more common diseases.

Mutational burden and profiles

One of the most characteristic features of chRCC is monosomy of many chromosomes. Originally described in 1992 (54, 55), chromophobe tumors show almost complete uniformity in the wholesale loss of chromosomes 1, 2, 6, 10, 13, 17, and often 21. This signature was confirmed by the TCGA analysis, which also demonstrated that other minor copy number changes are generally absent in this tumor type. Such a fingerprint of whole chromosome loss, in the absence of other evidence of genomic instability, is unprecedented. Unfortunately, the genomic error or process that allows for this pattern of chromosomal missegregation to occur remains unknown.

The TP53 axis and PTEN pathway. Tumor suppressor gene alterations are prominent in chRCC. Unlike either ccRCC or pRCC tumors, the most commonly mutated gene in chRCC is *TP53*. As anticipated, TP53 mutations are largely inactivating and, combined with chromosome 17 deletion, results in loss of function of this important tumor suppressor. Thus, complete inactivation of p53 signaling is likely a major driving event in chRCC tumorigenesis. This is an important feature to consider, as it distinguishes these tumors from all other forms of RCC, and aligns them more closely (in genetic terms) with breast and ovarian cancers. Similarly, the next most commonly mutated gene in chRCC is *PTEN*. Again, pairing these mutations with the near ubiquitous loss of chromosome 10 results in complete loss of function of this tumor suppressor, which acts as a brake on the PI3K signaling pathway. The expected activation of mTOR signaling downstream from PI3K has been previously observed in small studies and provides additional validation for the expanded use of mTOR inhibitors in this disease (21, 56). Together, the frequent loss of these tumor suppressors has important therapeutic implications as the current standard practice focuses on antiangiogenic therapies (57).

TERT fusions. A highly unique finding in the chRCC TCGA evaluation arose from the extended analysis of whole genomes in this set of tumors. WGS identified a number of genomic rearrangements in the *TERT* promoter region. This finding was also coupled with the observation that these same tumors displayed elevated *TERT* gene expression, suggesting a functional role for these gene fusions, and selection for these events in tumor progression. Mutations of the *TERT* promoter were also identified, as had been previously described in melanoma (58, 59), although tumors harboring these mutations had less robust surges in *TERT* gene expression levels. Collectively, genomic alterations leading to increased *TERT* expression represents a novel mechanism of RCC tumor promotion. High expression of TERT and the occurrence of these gene fusions were also associated with regional kataegis, a pattern of highly localized substitutions observed in a subset of chRCC tumors. Tumors displaying kataegis also had a mutation pattern consistent with APOBEC cytidine deaminase–mediated mutagenesis (60). This new finding remains an incompletely understood set of events guiding the mutational remodeling of the chRCC genome, but which is being increasingly observed in other cancers (60).

chRCC: A distinct metabolic disease

Mutations in the electron transport chain. Another special feature of the chRCC TCGA dataset was the inclusion of mitochondrial gene sequencing. chRCCs and oncocytomas have previously been reported to harbor mitochondrial gene mutations, but the frequency of these events, or the association with other features of chRCC, were unknown due to the rarity of this cancer. This analysis revealed a surprisingly high rate of mutations in genes encoding proteins involved in electron transport chain complex I. In particular, mutations in *MT-ND5*, a key component of this large complex, dominated the mutation landscape. No mutations were observed in other metabolic regulatory elements, such as glucose transport or the Krebs cycle. The association between gene expression of glycolytic enzymes and clinical outcome was not observed as it had been for ccRCC. However, the overall favorable outcome of chRCC tumors in this cohort limited analyses linked with survival-based outcomes.

Contributions to the eosinophilic variant subtype. A tight correlation was observed between mitochondrial gene mutations

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and the eosinophilic phenotype as identified by the TCGA expert pathology group members. An independent set of tumors was examined for eosinophilic histology, and measured for mitochondrial mass, clearly tying this phenotype to an accumulation of mitochondrial density. It remains uncertain whether these mutations promote a setting in which mitochondrial electron transport is hindered to such an extent that compensatory mitochondrial function (and mitogenesis) is needed to allow survival of these cells or whether the accumulation of mitochondria represents an alternate metabolic program fueling the growth of these cells. A recent assessment of the metabolic blockade in these tumors favors the former explanation (61).

Summary of TCGA analysis of chRCC

Overall, TCGA analysis of this unique and rare tumor type identified several interesting facets of tumor biology that render it highly distinct from the more common RCCs and also sheds light on the range of tumor biologic features that exist to drive cancer. The major findings in this cancer are (i) a highly stochastic copy number profile, indicative of a cellular genomic event that results in aneuploidy and massive elimination of chromosomal material; (ii) a program of *TP53* and *PTEN* mutations more aligned with breast and ovarian tumors than the classical tumors of the kidney cortex; (iii) high frequency gene fusions involving the *TERT* promoter that are associated with increased gene expression, and presumably contribute to the self-renewing phenotype of these cells as well as kataegis, and an APOBEC-type mutational spectrum in a subset of tumors; and (iv) a unique phenotype of mitochondrial perturbation resulting from inactivating mutations in key members of the electron transport chain. Although these tumors may be finding compensatory mechanisms of mitochondrial overduplication, the selection for these events suggests a growth advantage associated with alternative metabolic fuel utilization and/or resource generation.

Comparative Features of RCCs

The comprehensive molecular profiling of the major RCC subtypes achieved by TCGA allows for more extensive analysis and comparison of the biology across the RCC spectrum. Such an analysis was previously difficult and highlights both similarities and important distinctions among these related but distinct diseases (Fig. 2).

Using comparative features to reveal the origin of RCCs

Macroscopically, the kidney is divided into two distinct regions: the outer cortex and the inner medulla. Microscopically, each kidney consists of about a million complex, multicellular units called nephrons. The portion of the nephron that dips deepest into the medulla (the loop of Henle) divides the nephron into the proximal segment (including the proximal convoluted tubule) and the distal segment (including the distal convoluted tubule and collecting duct; ref. 60). Cells of the nephron are morphologically distinct in different regions, with unique gene expression patterns (62) reflecting their often nonoverlapping and specialized physiological roles. Indeed, distinct kidney cancers may arise from cells of these distinct portions of the nephron. At the level of gene expression, pRCC and ccRCC seem to be most similar to the proximal nephron as opposed to chRCC, which appears most similar to the distal nephron (53). These findings suggest that some portion of the biologic divergences among kidney cancer subtypes likely stems from their unique sites of origin within the nephron.

Mutation spectrum

The mutation rate in RCC tumors is generally low. For example, WES in the pRCC dataset revealed an average of 1.45 nonsilent mutations per megabase pair (MBP; ref. 63), comparable with the rate of 1.1 mutations/MBP observed in ccRCC (17) and significantly higher than the 0.4 mutations/MBP seen in most of the chRCC tumors (Fig. 1; ref. 53). Despite these differences among subtypes, overall, RCC tumors seem to have a significantly lower mutation rate than classic mutagen-associated cancers, such as lung squamous and melanoma (8 and 17 mutations/MBP, respectively; refs. 64, 65). This is important to note when considering the roughly 20% response rate in RCC to checkpoint inhibitor immunotherapy (66) and emerging evidence supporting a mutational burden association with response to checkpoint inhibition in other cancers (67).

Among the significantly mutated genes identified in each RCC subtype were those anticipated from the genetic syndromes known to predispose individuals to RCC, including the enrichment of *VHL* mutations in ccRCC, *MET* mutations in pRCC-I, and *FH* mutations in pRCC-II. However, mutations in genes previously not associated with specific subtypes of RCC also emerged. Although *NFE2L2* mutations were observed in some ccRCC tumors, they did not reach statistical significance as they did in pRCC tumors. No mutations in the NRF2 pathway were identified in the chRCC tumors. Thus, mutations in *NRF2/ARE* genes may be most important in pRCC, especially type II. Similarly, the relatively high rate of *TP53* mutations in chRCC was not shared by ccRCC or pRCC tumors. In contrast, other pathways were mutated across all three subtypes. For example, mutations in mTOR pathway genes, including those shown to correlate with robust mTOR inhibitor response in RCC (68), were seen in all three subtypes (clear cell 14%, papillary 7%, and chromophobe 14%). Other examples of mutated pathways across the subtypes are described in greater detail below.

The common theme of chromatin modifier mutations

ccRCC tumors had frequent mutations in the chromatin modifier pathway (35%), as did pRCC-I and -II (35% and 38%, respectively). chRCC, however, consistent with its overall relatively low mutational burden, had far less (3%). Similarly, components of the chromatin-remodeling complex SWI/SNF were frequently mutated in both papillary subtypes (20% and 27%, respectively), a frequency intermediate between ccRCC (43%) and chRCC (3%). Interestingly, in an unbiased, network-driven analysis that evaluates significantly mutated genes, as well as less frequently mutated genes (HotNet analysis), SWI/SNF emerged as a significant pathway in pRCC and ccRCC. How mutations in chromatin modifier mutations impact growth, metastasis, and drug sensitivity of these tumors remains an intense area of research.

CDKN2A/RB pathway

The TCGA analysis also identified alterations in *CDKN2A* as an oncogenic pathway of importance across the RCC spectrum. A variety of mechanisms were identified that could inactivate *CDKN2A*, including mutations in the gene, focal deletions in 9p21, and hypermethylation of the *CDKN2A* promoter. Among

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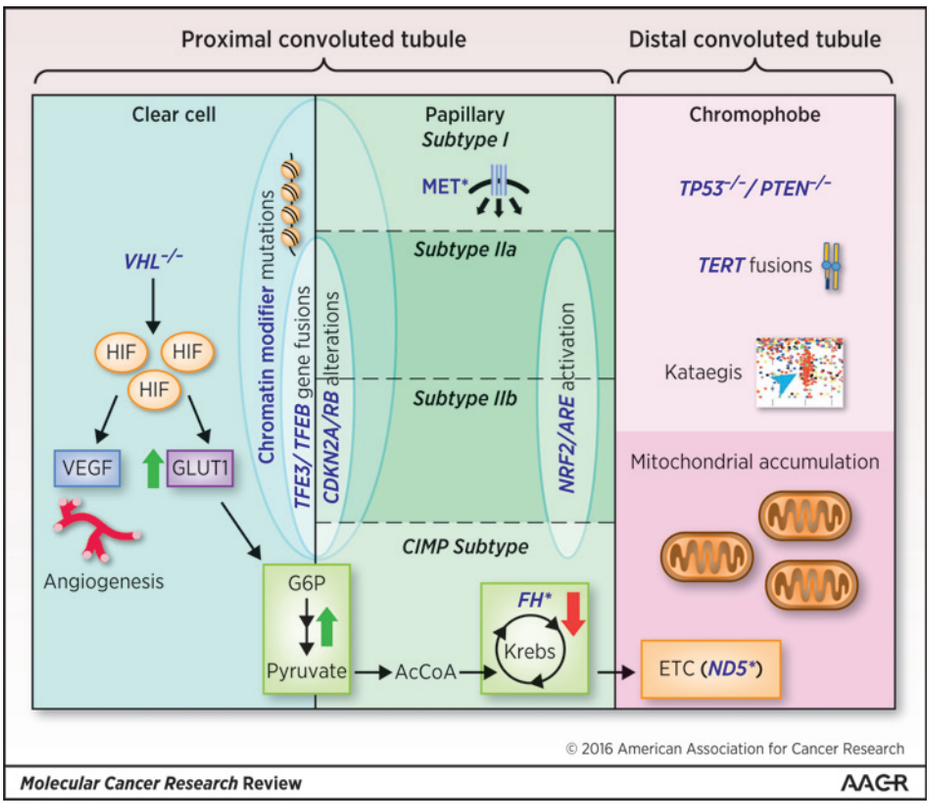

Figure 2.

Molecular comparison of ccRCC, pRCC, and chRCC. The molecular annotation of RCC subtypes enhances our ability to compare these tumors at the molecular level. ccRCC and pRCC exhibit gene expression profiles most similar to the proximal convoluted tubule, whereas chRCC is most similar to the distal convoluted tubule. The loss of *VHL* with resultant HIF stabilization is unique to ccRCC. However, *CDKN2A/RB* alterations and *TFE3/TFEB* gene fusions were identified in both clear cell and papillary type II tumors. Mutations (*) of *MET* were specific to papillary type I. The chRCC tumors included *TERT* fusions and overexpression, kataegis, homozygous loss of *TP53* and *PTEN*, and an eosinophilic subtype characterized by mitochondrial accumulation and mutations in electron transport chain (ETC) genes (most notably MT-*ND5*). Across the spectrum, significant metabolic reprogramming was observed. Both ccRCC and pRCC featured increased glycolysis and decreased oxidative phosphorylation, including the Krebs cycle, as measured by gene and protein expression. The CIMP subtype of type II pRCC was unique in that it featured mutated and/or decreased expression of the Krebs cycle enzyme *FH* as well as dramatic Warburg effect. Conversely, the chRCC tumors demonstrate upregulation of the Krebs cycle and electron transport chain as well as mitochondrial accumulation.

the 23 pRCC where these alterations were observed, most were the aggressive pRCC-II tumors. Among these *CDKN2A*-altered tumors, consistent with loss of function of the p16/INK4A tumor suppressor, RB phosphorylation and expression of cell-cycle genes were significantly higher and survival was decreased. Among the ccRCC tumors, 9p21 deletions were enriched among the mRNA cluster referred to as "m3." This m3 cluster, contained within the poor prognostic "ccB" subtype referenced in other ccRCC literature, had poor survival (17, 69, 70). Thus, in both papillary and clear cell RCC, tumors with *CDKN2A* alterations correlate with aggressive subtypes. Although no mutations were observed, four of the chRCC tumors displayed epigenetic silencing of *CDKN2A*; therefore, strategies to target *CDKN2A* biology may prove useful across the kidney cancer spectrum.

RCC is a metabolic disease in many different ways

The kidneys are large organs with a very high blood flow (~400 mL/minure per 100 g of tissue relative to ~80 mL/minute for the heart; ref. 40). This blood flow is in excess of what is needed for their metabolic needs and instead facilitates their role in regulating water and electrolyte balance. This regulation, especially sodium reabsorption, requires extensive energy as reflected by the high oxygen consumption by the kidneys, second only to the heart. Given this high metabolic activity of the native organ, it is perhaps not surprising to find metabolic reprogramming to be a common phenomenon across RCC subtypes. For example, FH is a Krebs cycle enzyme as well as the gene responsible for the inherited cancer predisposition syndrome of hereditary leiomyomatosis and RCC, including pRCC-II (71). The accumulation of fumarate in *FH*-mutated RCC may cause multiple oncogenic sequelae, including activation of the NRF2/ARE pathway (72–74), as well as HIF stabilization with upregulation of hypoxia-related genes (39, 75). These, and possibly other mechanisms, likely contribute to the dramatic metabolic shift toward Warburg-like metabolism seen in the pRCC-II tumors (especially the CIMP subtype). Similar metabolic trends were seen in the ccRCC tumors driven by loss of *VHL* function and subsequent HIF

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stabilization. In both of these groups, high expression of genes and proteins involved in glycolysis, pentose phosphate pathway, and fatty acid synthesis, and low expression of those involved in Krebs cycle and AMPK signaling, correlated with poor survival. This is in stark contrast to the chromophobe tumors, where evidence of enhanced cellular respiration, including increased expression of Krebs cycle and oxidative phosphorylation genes, was observed. Thus, although the types of metabolic defects differ among the subtypes, metabolic reprogramming continues to emerge as a core principle in RCC biology.

Translocation RCC

A molecular event once thought to be unique to pRCC-II tumors are the cytogenetic translocations referred to as *TFE3* and *TFEB* fusions. *TFE3* is a transcription factor from the MiT family located on Xp11.2. Tumors with *TFE3* fusions are rare and referred to as translocation RCC. They represent one third of the approximately 25 pediatric RCC cases diagnosed in the United States per year (43) and are recognized by the WHO as a distinct RCC subtype (2) but are rarely seen in adults (76). How *TFE3* fusions promote oncogenesis in RCC is incompletely understood, although *TFE3* is known to regulate several oncogenic pathways involved in cell growth and metabolism, including the mTOR and TGFβ signaling pathways, MET, and AMPK (77). Although the disease often follows an indolent clinical course in pediatric patients (78), it can be very aggressive and metastasize early, especially in adults. In one retrospective analysis of adults with translocation RCC receiving antiangiogenesis therapies, the median overall survival was a mere 14.3 months (79). Interestingly, the true estimate of translocation RCC incidence in adults may be underestimated. Among the TCGA samples, in addition to seven *TFE3/TFEB* translocations identified in pRCC, five were observed in ccRCC cases. Thus, translocation RCC may be histologically indistinguishable from other RCC types and identifiable only at the genetic level. This finding suggests that it is important to be alert to the possibility that translocation tumors may present with different phenotypical characteristics.

Conclusions

The three major projects in RCC conducted by TCGA have revealed new insights into this heretofore enigmatic disease. The details summarized above are available in the three published TCGA index articles (17, 53, 63). These findings provide a foundation of genetic and molecular evidence, which combined with histologic and morphologic data, demonstrate that subtypes of RCCs are quite biologically distinct. Collectively, this information will be useful for improving both diagnosis and treatment of RCC patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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