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Review

Molecular Classification of Renal Cell Carcinoma and Its Implication in Future Clinical Practice

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Abstract. Renal cell carcinoma (RCC) encompasses a wide spectrum of morphologically and molecularly distinct (>10) cancer subtypes originated from the kidney epithelium. Metastatic RCC (mRCC) is lethal and refractory to conventional chemotherapeutic agents. The incorporation of targeted therapies and immune checkpoint inhibitors into the current practice of mRCC has markedly improved the median overall survival of clear cell RCC (ccRCC) patients, the most common subtype, but not rare kidney cancer (RKC or non-ccRCC, nccRCC). Varied treatment response in mRCC patients is observed, which presents clinical challenges/opportunities at the modern mRCC therapeutic landscape consisting of 12 approved drugs representing 6 different effective mechanisms. Key contributing factors include inter- and intra-RCC heterogeneity. With the advances in pan-omics technologies, we now have a better understanding of the molecular pathobiology of individual RCC subtype. Here, we attempt to classify ccRCC based on contemporary molecular features with emphasis on their respective potential significance in clinical practice.

Keywords: Kidney cancer, genomics, transcriptomics, proteomics, metabolomics, therapeutics, molecular classification, biomarkers, precision medicine

INTRODUCTION

Renal cell carcinoma (RCC) is a heterogeneous group of cancers of the kidney parenchyma consisting of different subtypes with the most frequent and best studied being clear cell RCC (ccRCC, ∼75%), followed by papillary (pRCC, ∼15%), chromophobe (chRCC, ∼5%), unclassified RCC (uRCC, ∼4%) and even rarer (<1%) RCC entities such as medullary RCC (mdRCC), collecting duct RCC (cdRCC), MiT family translocation RCC (mtfRCC), and SDHB RCC (sdRCC) [1–12]. The classification of the numerous subtypes has been repeatedly revised in the past two decades, due to advances in the histological and molecular characterization of this disease [13–15]. Currently, the latest World Health Organization Classification of renal tumors from the year 2016 counts 12 recognized subtypes and several provisional entities

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awaiting to be fully recognized [1, 16]. Pan-RCC analysis demonstrated major genetic and molecular differences and minor similarities among major subtypes [17].

Localized RCC can potentially be treated with curative intent surgically by partial or radical removal of the involved kidney. However, 30% of the patients present with metastatic disease at time of diagnosis and further 30% will eventually develop metastases during the course of the disease [2]. The knowledge obtained from molecular characterization of ccRCC has led to the regulatory approval of 12 systemic therapeutic agents encompassing 6 different effective mechanisms [2]. Despite these marked advances, mRCC remains lethal and clinical benefit varies greatly among patients receiving the same therapeutic agents. Potential contributing factors include tumor/host heterogeneity and cancer evolution [18–21]. Facing the recent explosive growth in both molecular understandings and effective treatments of RCC [2, 22], further molecular sub-classification of RCC with emphasis on clinical association in addition to current prognostic models based on clinical parameters [23] could impact future clinical management of kidney cancer patients with either localized disease or distant metastasis. With these questions in mind, this review focuses on recent molecular analyses of ccRCC shown to influence clinical outcomes.

MOLECULAR CLASSIFICATION OF CLEAR CELL RENAL CELL CARCINOMA ACCORDING TO INDIVIDUAL OMICS

Genomic classification of ccRCC

Contemporary ccRCC genomic studies demonstrated that loss of heterozygosity (LOH) of chromosome 3p occurs at >90% and mutations/methylation of VHL, residing at 3p25, occurs at >70%/10–15%, confirming that inactivation of VHL serves as the fundamental driver event of human ccRCC [3, 24, 25]. The VHL E3 ligase complex targets hypoxia inducible factors (HIFs) for proteasome-mediated degradation. HIFs are transcription activator and upon stabilization/activation due to low oxygen stress such as tissue injury they trigger new blood vessel formation through activating vascular endothelial growth factor (VEGF) [26–28]. The pathological loss of VHL underlies the highly vascular nature of ccRCC and explains why anti-VEGF/VEGF receptor (VEGFR) drugs including bevacizumab, sorafenib, sunitinib, pazopanib, axitinib, lenvatinib, and cabozantinib constitute the most effective mainstream therapy for metastatic ccRCC patients [2]. However, most metastatic ccRCC patients eventually progressed and succumbed to their disease [22].

Despite the quintessential role of VHL loss during the cancer initiation process and its subsequent impact on therapeutics, genetic evidence from studying human VHL syndrome [29] and mouse VHL deficiency model [30] suggest the involvement of additional genetic drivers. Large efforts have been undertaken in the past >10 years to discover additional genetic and epigenetic events leading to metastatic ccRCC [31]. These efforts were made possible by the rapid progress in next-generation sequencing technologies and large-scale consortium efforts, revealing several novel gene mutations in ccRCC, including the tumor suppressor genes *PBRM1* (40%), *SETD2* (15%), *BAP1* (15%), and *KDM5C* (7%), and the oncogene *MTOR* (5%) [3, 24, 32–35]. Of note, *PBRM1*, SETD2, and *BAP1* reside at 3p21, and *VHL* at 3p25, thereby through the loss of one copy of chromosome 3p, 4 tumor suppressor genes are lost simultaneously [35]. Furthermore, *KDM5C* resides at X chromosome, thereby mutations on the sole wild type allele in male patients cause complete loss of function of KDM5C, which could contribute to the 2 : 1 male predominance in ccRCC [2].

Several retrospective studies have reported interesting clinical correlation between cancer somatic gene mutations and clinical/therapeutic outcome in ccRCC, which offers insights regarding outcome-based molecular classification of ccRCC and warrants future validations. Ten genes, *VHL, PBRM1, BAP1, SETD2, KDM5C, TP53, PIK3CA, MTOR, TSC1,* and *NF2* altogether mutated in 93% of ccRCC, are included for the discussion/classification in this review (Fig. 1). VHL inactivation is the fundamental driver event and VHL mutation occurs in >70% [24, 25, 33]. Accordingly, *VHL* mutation can serve as a good diagnostic tool but carries no clinical impact for ccRCC in subset analysis [2].

BAP1 mutations are associated with high grade, large tumors, and poor overall clinical outcome even on targeted therapy [25, 33, 36–38]. For example, progression free survival (PFS) of BAP1 mutant (MT) vs. BAP1 wild-type (WT) patients on first line sunitinib, a VEGFR TKI, was 8.1 vs. 11.0 months, and on first line everolimus, an MTORC1 inhibitor, was 4.9 vs. 10.5 months in RECORD-3 trial, one large randomized phase II study [25], suggesting that

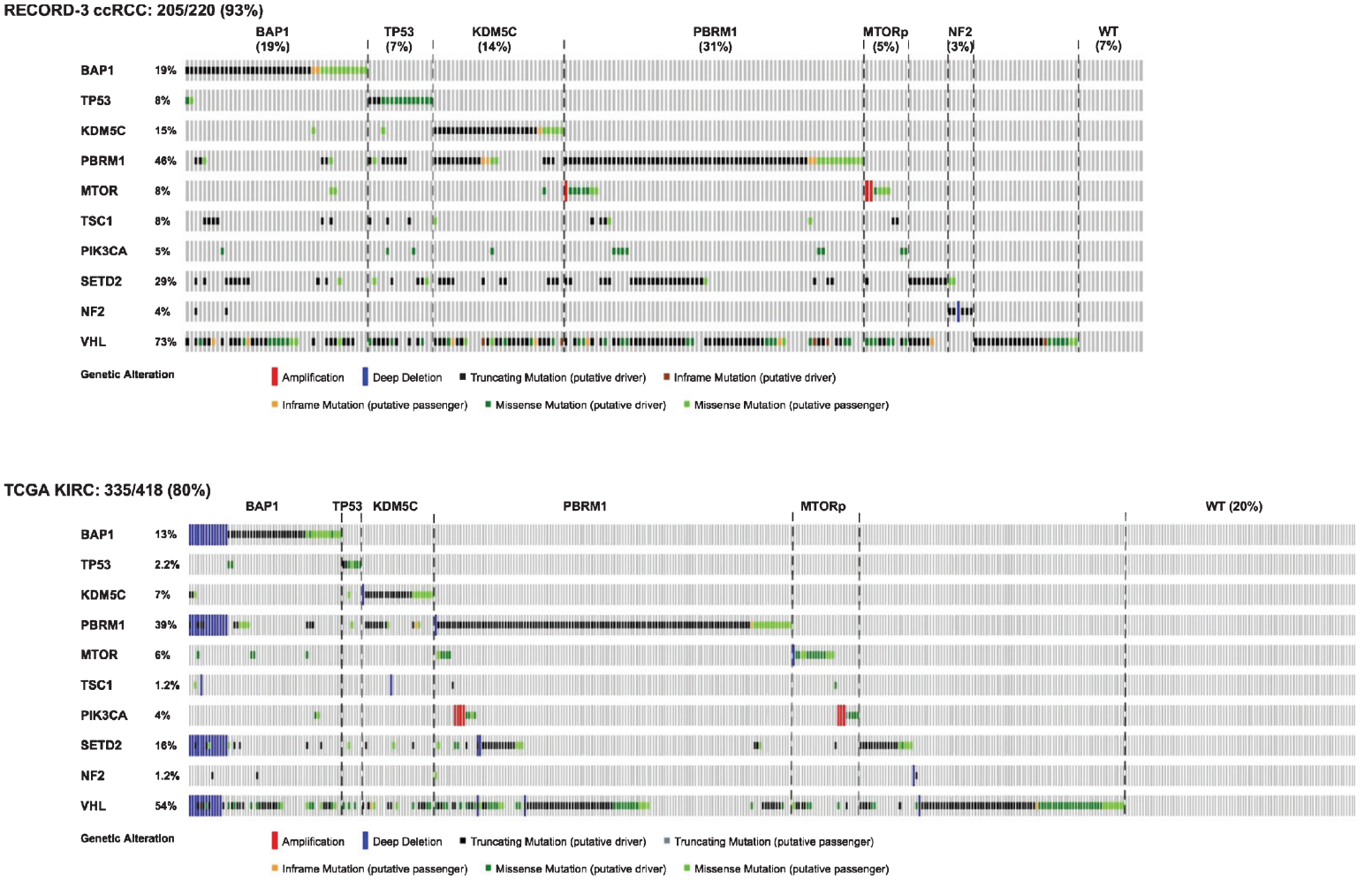

Fig. 1. Somatic mutation landscape of ccRCC based on 10 genes that are selected for either prevalent mutations or shown prognostic/therapeutic significances. Top panel represents 220 metastatic ccRCC patients in the reported NGS cohort of RECORD3 [25], and bottom panel represents 418 ccRCC patients of all stages in the reported TCGA KIRC cohort [3]. MTORp denotes MTORC1 pathway.

BAP1 mutant patients may not benefit from everolimus. Furthermore, *BAP1* mutated mouse ccRCC tumors exhibited high grade features [39].

TP53 mutations occur at lower frequency (2.2%) in the primary tumors of non-metastatic ccRCC patients [3], at higher frequency (8%) in primaries of metastatic ccRCC [25], and at even higher frequencies (>10%) when multiple regions [40] or matched primary-metastasis pairs were sequenced (Hsieh et al. unpublished data). *TP53* mutation in ccRCC associate with high grade in both human [40] and mouse [41] ccRCC tumors and significantly decreased cancer specific survival even after correction for SSIGN (stage, size, grade, and necrosis) score [42].

KDM5C mutations occurred mainly in male patients and were associated with much longer 1st line PFS (PFS1L) on sunitinib (20.6 months) than everolimus (9.8 months) whereas *KDM5C* wild-type patients exhibited similar PFS1L on either sunitinib (8.3 months) or everolimus (8.2 months) [25]. Enrichment of KDM5C mutation tumors in responders to anti-VEGF/VEGFR agents was also reported by independent researchers [43]. Notably, *KDM5C* mutations tend to co-occur with *PBRM1* mutations [25], whereas both KDM5C and PBRM1 mutations tend to occur in a mutually exclusive manner with BAP1 mutations [25, 33].

PBRM1, a SWI/SNF chromatin remodeling complex protein, is the second most commonly mutated (∼40%) gene in ccRCC [2, 25, 32]. *PBRM1* mutations in small renal masses (<4 cm) were associated with tumor invasiveness [34], and mice bearing mutations in *VHL* and *PBRM1* (*VHL–/–;PBRM1–/–*) developed ccRCC [44]. Mechanistic and mouse genetic studies of PBRM1 further unveiled the tumor suppressor activity of PBRM1 in preventing the self-perpetuating over-amplification of HIF1 oncogenic signals [44, 45]. Of note, a long latency period for mouse kidney epithelium with deletion of both *VHL* and *PBRM1* to develop ccRCC was observed, and the preferred third driver event for the development of ccRCC was the activation of MTORC1 [44]. Of note, PBRM1 mutant patients experienced longer PFS1L on everolimus (12.8 months) than the whole cohort receiving everolimus (8.3 months) [25]. Remarkably, data from both human cancer genomics [44] and therapeutics [25] further support a three-driver event orchestrating the step-by-step pathogenesis of ccRCC, entailing VHL loss (1st), PBRM1 loss (2nd), and MTORC1 activation (3rd).

MTOR gene mutations occur in ∼5% of ccRCC [3] that cluster at conserve domains [46] and result in enhanced MTORC1 signaling [46, 47]. MTORC1 is a key regulator of kidney cancer cell growth [44, 46, 48], functioning downstream to the PI3K activating and the TSC1/TSC2 repressing signals [49]. Accordingly, mutations in *MTOR*, *TSC1*, *TSC2*, and *PI3K* were enriched in ccRCC patients most benefited from treatment with mTORC1 inhibitors everolimus and temsirolimus [50–52]. However, several factors are important in predicting sensitivity to targeted therapy [48], e.g. mutations detected in metastasis were shown to be superior to primary in treatment prediction and should be weighed differently for precision cancer therapy [21].

SETD2 mutations in ccRCC are the best example highlighting intra-tumor heterogeneity and convergent evolution [18], i.e. through multi-region sequencing of tumors from the same patient multiple variants of SETD2 mutation were identified [40]. In fact, despite seemingly chaotic branched evolution of cancer acquiring random mutations, convergent evolution takes root during tumorigenesis and occurs at gene, pathway, function, and phenotype levels that could be exploited for therapeutic interventions [21]. Several lines of evidence indicate the importance of SETD2 mutations in ccRCC progression, especially in metastasis, thereby impacting cancer survival. Genomic analysis of the primary tumors from TCGA 421 patients and MSK 188 patients that consists patients of all stages, SETD2 mutations (11.6% TCGA, 7.4% MSK) were associated with worse cancer specific survival [37], which was later validated with a combined cohort of 1049 patients [42]. Furthermore, examination of the primary tumors of metastatic ccRCC patients demonstrated enrichment of SETD2 mutations to 30% [25]. Of note, although *SETD2* mutations were associated with metastasis of ccRCC, they were not associated poor targeted treatment outcome contrasting patients with *BAP1* mutations [25, 38]. Mechanistically, it was reported that SETD2 loss promotes renal cancer branched evolution through replication stress and impaired DNA repair [53]. Interestingly, SETD2 mutations also occur in other RCC types [17], and co-occur with NF2 mutations in unclassified RCC [6]. Moreover, two cases of cancer of unknown primary carry concurrent SETD2 and NF2 mutations and exhibit clear cell morphology and positivity for CA9 [54].

Altogether, mutation profiles between RECORD-3 and TCGA ccRCC cohorts are similar (Fig. 1), sug-

gesting that ccRCC might be classified based on a specific subset of genes that carry prognostic and therapeutic significance.

Transcriptomic classification of ccRCC

In addition to the understanding of the mutational landscape of ccRCC, approaches based on the quantification of mRNA transcripts have also been developed at a high-throughput level.

ccA/ccB & ClearCode 34 transcription classification of ccRCC were initially proposed by Rose Brannon and colleagues based on gene expression microarray data [55]. They performed an unsupervised consensus clustering in a discovery cohort of 48 RCC which allowed to identify two robust subtypes ccA and ccB with differentiating biological signatures and distinct prognoses. Then, they identified a small gene set by logical analysis of data (LAD), which allowed to assign individual tumors within the ccA/ccB classification. Finally they validated it in an independent cohort of 177 patients with RCC and confirmed that ccA tumors had a markedly better prognosis than ccB. Moreover molecular subtype was found to be significantly associated with survival in both univariate and multivariate analysis. The ccA/ccB classification was subsequently validated in a meta-analysis of 480 ccRCC tumors, suggesting this profile may have value for risk stratification [56]. The gene set used to classify RCC tumors in ccA/ccB was then optimized and simplified by Brooks and colleagues using a centroid-based classification algorithm (called prediction analysis of microarray (PAM)) to end with a 34-gene expression classifier named ClearCode34 [57]. The classifier was applied to RNA-sequencing data from 380 non-metastatic ccRCC samples from the Cancer Genome Atlas (TCGA), and to 157 formalin-fixed (FFPE) clinical samples collected at the University of North Carolina. Finally, the authors developed a recurrence risk model with the addition of stage and Fuhrman grade to the ClearCode34 sub-classification. C-index analysis showed that ClearCode34 model better predict disease-specific events compared to the University of California, Los Angeles (UCLA) Integrated Staging System (UISS) [58] and the Mayo Clinic Stage, Size, Grade, and Necrosis (SSIGN) score [59]. Moreover, this model was also additive independently of both UISS and SSIGN indicating added prognostic information for disease-specific outcomes. Thus, using ClearCode34 enhances risk stratification, which may guide future clinical planning regarding patient surveillance and adjuvant therapy. Recently de Velasco and colleagues assessed the predictive power of ClearCode34 in the setting of systemic therapy for metastatic ccRCC [60]. They found that ccB remained independently associated with a worse OS ($p = 0.044$) after adjusting for IMDC groups. They found also that the joint model of ccA/ccB and IMDC had higher accuracy (C-Index 0.63, 95%CI = 0.51–0.75) than a model with IMDC alone (0.60, 95%CI = 0.47–0.72).

Although ClearCode34 might be ready to be used prospectively in a clinical trial, a major limitation has emerged: the intra-tumoral heterogeneity (ITH). Regarding driver mutations and somatic copy number aberrations, Marco Gerlinger and colleagues from Charles Swanton's group has already shown the high frequency of ITH toward intra-tumor multi-region sequencing. They also found a high ITH of the ccA/ccB signature. They reanalysed their published gene expression data of 63 tumor regions from 10 stage II–IV ccRCCs [18, 40] and mapped the results onto the phylogenetic trees previously published for these tumors [40]. Only two tumors homogeneously expressed the ccA signature; the other eight tumors were heterogeneous with ccA and ccB components detectable, suggesting the need to sample multiple tumor regions to reliably detect poor prognostic clones [61]. More recently a team from the Mayo Clinic has evaluated both intra- and inter-tumor molecular heterogeneity in a large cohort of resected metastatic ccRCC tumors [62]. The authors found that ccA or ccB subtype differed across longitudinal metastatic tumors from the same patient in 23% (7/30) of patients and across patient-matched primary and metastatic tumors in 43% (35/80) of patients. Among these 35 patients, 80% had a primary tumor that was classified as ccA and at least one metastatic tumor classified as ccB. Conversely, seven (20%) patients had a primary tumor classified as ccB and at least one metastatic tumor classified as ccA. These data suggest that molecular classification performed on primary tumor does not reflect the biology of the metastasis in a large proportion of cases.

The TCGA m1-m4 mRNA classification of ccRCC was reported by the KIRC analysis working group [3]. In this study, multi-platform analyses were performed to identify somatic alterations, DNA methylation status and mRNA/miRNA expression signatures on 446 patients. Regarding mRNA expression, an unsupervised clustering method identified four subsets

(m1–m4). The m1 subtype was characterized by gene sets associated with chromatin remodeling processes and a higher frequency of PBRM1 mutations (39% in m1 vs. 27% in others, $P = 0.027$). Deletion of CDKN2A (53% vs. 26%; $P < 0.0001$) and mutations in PTEN (11% vs 1%; $P < 0.0001$) were more frequent in m3 tumours. The m4 group showed higher frequencies of BAP1 mutations (17% vs. 7%; $P = 0.002$) and base-excision repair; however, this group also harboured more mTOR mutations (12% vs. 4%; $P = 0.01$) and ribosomal gene sets. Supervised clustering revealed an overlap between these new subsets to the previously reported ccA and ccB expression subtypes, with cluster m1 corresponding to ccA and ccB divided between m2 and m3. Cluster m4 probably accounts for the roughly 15% of tumors previously unclassified in the ccA/ccB classification scheme. Similarly, the survival advantage previously observed for ccA cases was again identified for m1 tumours.

CC-e.1, 2, 3 classification of ccRCC was recently reported by Chen et al., reported the comprehensive molecular analysis of 894 primary renal cell carcinomas [17]. The authors identified nine subtypes defined by systematic analysis of five genomic data platforms (mRNA expression, DNA methylation, DNA copy, microRNA (miRNA) expression, and protein expression). ccRCC clustered into 3 different subtypes designated as CC-e.1, 2 and 3 associated with intermediate, better and worse patient survival. Genomic subtypes made across TCGA ccRCC cases showed high concordance with other subtype designations previously called for the same samples, on the basis of gene expression profiles. The previously reported [55] ccA and ccB clear cell RCC expression subtypes corresponded to CC-e.2 (better prognosis) and CC-e.3 (worse prognosis), respectively. Of the four mRNA-expression-based subtypes, m1/m2/m3/m4, defined previously in the original KIRC study, m1 and m3 overlapped with CC-e.2 and CC-e.3, respectively, while CC-e.1 overlapped significantly with m2 and m4. All collaborative efforts made by The Cancer Genome Atlas Research Network were dedicated to provide a comprehensive characterization of RCC and to bring some molecular insights to identify the opportunities for disease treatment. Thus, the TCGA mRNA signature has not been yet assessed in a prospective clinical trial. Indeed, mRNA expression analyses were performed on frozen tumor samples using mainly RNA sequencing which is a major limitation for its use within a clinical trial.

ccrcc1-4 classification was proposed in 2007 when the French association „La Ligue contre le Cancer“ launched the Tumor Identity Card („Carte d'Identité des Tumeurs“) to molecularly characterize solid tumors. Stéphane Oudard's team together with Hervé Fridman's team took advantage of this program to propose the characterization of molecular features of metastatic ccRCC patients. Transcriptomic analysis (microarray gene expression) of the primary tumor from 53 metastatic ccRCC patients treated with sunitinib was performed [63]. Using an unsupervised clustering analysis, 4 molecular groups (ccrcc1 to 4) were identified with distinct pathological features and mutational profiles. For example, ccrcc4 tumors had a significant higher inflammation score, a higher frequency of sarcomatoid component, a low frequency of *VHL* mutation and the absence of *PBRM1* mutation. At the opposite ccrcc3 had the lower inflammation score and the lower frequency of sarcomatoid component but has also low frequencies of *VHL* and *PBRM1* mutations. Importantly, the 4 groups appeared to have significantly different prognoses with ccrcc 1 and 4 having reduced progression free, overall survival and the poorer response to sunitinib, suggesting to be a good tool to predict response to TKI. A classifier of 35 genes was constructed using a step-by-step strategy with a first centroid-based predictor to assign a sample one of the three following groups ccrcc1&4/ccrcc2/ccrcc3; a second centroid-based predictor was used for samples predicted as ccrcc1&4. The classifier was tested on the 51 patients with available microarray data and using qRT-PCR and then validated on additional 47 patients with qRT-PCR only. Finally the gene set was tested in the TCGA samples. Somatic *PBRM1* mutations were most frequently identified in ccrcc1/ccrcc2 tumors but rarely found in ccrcc3/ccrcc4 tumors. In both series, somatic *VHL* mutations were more frequently distributed in ccrcc1/ccrcc2 tumors. The *BAP1* and *SETD2* mutations also showed association with the molecular subtypes: *BAP1* was most mutated in the ccrcc4 tumors ($p = 0.0098$) and *SETD2* was most mutated in the ccrcc1 tumors ($p = 0.06$). As in the „in-house“ dataset ccrcc2/ccrcc3 tumors display the best survival, ccrcc1 tumors an intermediate survival, and ccrcc4 tumors the poorest survival ($p < 0.0001$).

A 16-gene assay to predict recurrence after surgery in localized RCC was reported by Rini et al. in 2015 [64]. In this study, the expression of 732 genes was measured in 942 patients presented with Stage I-III diseases, and correlated with the risk

of recurrence cancer-specific survival. Among 516 significantly altered genes, 11 genes that involve vascular, cell growth/division, immune response, and inflammation were selected along with 5 reference genes to develop the recurrence score, which was then validated in another 626 patients. In multivariable analyses, the 16-gene score was significantly associated with recurrence ($p < 0.0001$) after stratification by stage and adjustment for size, grade, or Leibovich score.

With the rapid development of checkpoint inhibitors in RCC, interestingly we found that some immune cell infiltrates, evaluated with mRNA expression, were associated with molecular groups [65]. For instance ccrcc4 tumors, which were associated with sunitinib resistance, were the most highly infiltrated tumors by T cells. In addition ccrcc4 had the highly expression of immunosuppressive markers such as PD-L1, PD-1, LAG-3, TIM-3, suggesting a high fraction of exhausted T cells within these tumors. Conversely, ccrcc1 tumors, which were also associated with poor prognosis, had the poorest T cell infiltration and a global low expression of T cell inhibition markers. ccrcc1 could be considered as immune-desert or immune-cold tumors.

Based on these results, a French academic and multicentric molecular-driven randomized phase 2 trial named Bionikk (phase 2 BIOmarker driven trial with Nivolumab and Ipilimumab or VEGFR TKI in naïve metastatic Kidney cancer, NCT02960906) was recently launched. Main objective is to evaluate the ability of the CIT classification to select patients to have either a TKI, or a checkpoint inhibitor alone or in combination. Primary endpoint is overall response rate within each arm. 150 patients with a metastatic ccRCC naïve of any systemic therapy with available frozen tumor are planned to be included. Molecular classification is determined within 2 weeks and patients are then randomized according to their group: ccrcc1 and 4 are randomized between nivolumab plus ipilimumab and nivolumab alone, and ccrcc2 and 3 are randomized between nivolumab plus ipilimumab and TKI (sunitinib or pazopanib). Among main secondary objectives including overall survival and progression free survival, according to treatment arm and group, many exploratory biomarkers will be evaluated. One of them is the comparison between molecular groups obtained from FFPE samples versus those obtained from frozen samples. Completion of the study is planned to be around mid 2020.

Metabolomic classification of ccRCC

mCluster 1–4 (metabolomics cluster) of ccRCC with distinct metabolic features and clinical outcomes were identified when global profiling of 877 metabolites was performed on 138 primary kidney tumor and adjacent normal pairs [66]. Worse clinical outcomes were associated with high glutathione-related metabolites observed in mCluster 2 or with high dipeptides in mCluster 3, whereas better clinical outcomes were associated with low glutathione in mCluster 4 and low dipeptide in mCluster 1, which may present therapeutic opportunity through further disrupting the redox or lysosome pathways, respectively [66]. Furthermore, grade-dependent metabolic reprogramming of ccRCC was also observed when a combined metabolomics and proteomics approach was performed [67]. The intra-tumor heterogeneity concerning ccRCC metabolism was recently reported through profiling multiple spatially separated samples [68]. In this small cohort study, global metabolomics was performed on 32 kidney tumor samples and 12 adjacent normal tissues representing 12 patients in conjunction with tissue tracer studies, demonstrating that different regions of primary RCC tumor tissues possess different metabolic characteristics and might contribute to intratumor treatment heterogeneity.

Immunogenomic profiling of ccRCC

As immune checkpoint inhibition has shown great promise in ccRCC, many efforts have been devoted to analyze tumor transcriptome and estimate the composition of the tumor microenvironment [69]. Recent methods aim at providing highly precise quantitative information about the cell content of heterogeneous samples using deconvolution techniques and has provided an immune atlas of ccRCC [70]. Furthermore, stringent and robust gene signatures for 8 immune cell types, as well as fibroblasts and vessels have recently been reported [65, 71] and used them in a method called MCP-counter, which scores are proportional to the cell amounts within the samples. As checkpoint inhibitors act primarily on immune cells, these new techniques that allow accurately quantify these cells could help us to predict response to therapy. Encouragingly, in a recently reported IMmotion150 phase II clinical trial where 305 metastatic patients were randomized to Sunitinib, Atezolimumab (anti-PD-L1), or Atezolimumab plus Bevacizumab (anti-VEGF), PD-L1+ was detected in 54% of patients, and among

these patients the PFS HR form atezolimumab plus bevacizumab vs. sunitinib was 0.64 but did not reach statistical significance ($p = 0.095$) [72]. Notably, in a small ($n = 13$) cohort study, the intratumoral balance between metabolic and immunologic gene expression was shown to associate with anti-PD-1 treatment response [73].

CONCLUSION

Given the unpredictability on the efficacy of currently available drugs for treating individual metastatic kidney cancer patients, there is an unmet medical need to improve the therapeutic approach for patients affected by this disease. Although the mutational landscape of ccRCC has dramatically evolved in the past 10 years, major limitations dampen their use to select the right therapy for the right patient. First, most of mutated genes are tumor suppressor genes and mutations lead to loss of function. Consequently these mutated genes are not directly targetable. Second, several events can inactivate a gene such as methylation, copy number loss, miRNA regulation and thus may lead to a loss of function in wild type (WT) genes that would evade select DNA mutation platforms. Third, ccRCC are highly heterogeneous tumors and even early events, e.g. *PBRM1* or *BAP1* mutations could be different when sequencing primary vs. metastatic tumors [21]. Hence, improvement may be achieved, such as through the development and the employment of methods to perform tumor-specific molecular stratification of renal cell carcinoma, and offers the most effective treatment to the select patient based on a combination of different molecular characteristics.

Novel insights into the molecular underpinnings of renal cell carcinoma have unraveled a far more complex classification than those simply based on histopathological criteria. Recent results on gene expression and mutation analysis were able to provide new subgroups within clear cell, but also papillary and chromophobe renal cell carcinoma [2, 74, 75]. The described subgroups define not only on different clinical risk groups, but also, to a lesser extent, on predictive biomarkers for current treatments of ccRCC. Prior to the tumor omics era, serum protein profiles and IHC constituted major biomarker efforts in kidney cancer translational research [76]. Currently, we are not yet ready to benchmark classification with biomarkers. Nevertheless, it is foreseeable that a combination strategy consisting of serum analysis, gene expression analysis, and mutation analysis platforms could be developed in the future to stratify patients with either localized or metastatic RCC, which aims at defining the characteristics and prognosis of an individual tumor or metastasis in a single patient in order to provide the best possible follow-up and treatment plan.

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RELEVANT DISCLOSURES

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