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Biomarkers in renal cell carcinoma

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Summary We have entered a new era for patients with mRCC with multiple treatment options including VEGF(Vascular Endothelial Growth Factor)-, MET(Mesenchymal-eptihelial Transition)-, AXL(AXL-Rezeptortyrosinkinase)-, mTOR(mechanistic Target of Rapamycin)-targeted TKIs (tyrosinekinaseinhibitors) as well as PD-1(programmed cell death protein 1)-, PD-L1(Programmed cell death 1 ligand)- and CTLA4(cytotoxic T-lymphocyte-associated Protein 4)-targeted IOs and their combinations, respectively. However, the possibility to select patients based on predictive biomarkers for the different treatment options is still lacking. The TCGA (Cancer Genome Atlas) consortium conducted comprehensive analyses of genomic and metabolic features of RCC (renal cell carcinoma) and these findings demonstrated significant differences between the major histological subtypes of RCC like differences in immune signatures and their course of disease. The increasing knowledge on the genomic landscape of RCC supports stratification of patients for targeted therapies. Biomarker development for future therapeutic approaches will require integration of multiple biologic components like PD-L1 expression, tumor-infiltrating lymphocytes and mutations in addition to the prognostic risk scores. However, no single molecular marker has been shown to improve the accuracy of MSKCC (Memorial Sloan Kettering Cancer Center) or IMDC (International Metastatic RCC Database Consortium) prognostic risk scores. Large-scale biomarker-driven prospective trials with consensus methodologies on biomarker assessment and scoring are needed to obtain clinically validated new prognostic and predictive biomarkers as described above. The integration of routinely available parameters and new biomarkers could hold the key for personalized treatment strategies of patients with RCC.

Keywords Disease heterogeneity · Role of common risk scores · PD-L1 expression · Inflammation · Gene signature

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Introduction

Kidney cancer is the third most common genitourinary malignancy worldwide, representing the 7th most common cancer in men and the 10th most common cancer in women [2]. After over two decades of increased incidence rates of RCC (renal cell carcinoma), trends worldwide have shown a plateau in recent years. Furthermore, kidney cancer mortality rates overall have levelled. This is mainly due to incidental diagnosis and downward shift of tumor stage by the widespread use of noninvasive radiological techniques, e.g. ultrasonography and computed tomography.

Nephrectomy remains the curative treatment strategy for most patients with localized disease. However, one-third of patients present with metastatic disease upfront and one-quarter of all patients operated with curative intend ultimately experience disease relapse. Identifying the optimal treatment options and sequences for patients with metastatic disease are important goals. Up to now, treatment selection is still guided by the use of prognostic scores—Memorial Sloan Kettering Cancer Center (MSKCC) and International Metastatic RCC Database Consortium (IMDC) risk scores [3, 4]—based on clinical, radiological and

laboratory findings. The identification of drug-able targets which is necessary for precision medicine within the field of RCC is ongoing [1]. Progress has been made by the investigation of new prognostic and predictive biomarkers included as translational research studies in prospective clinical trials. Furthermore, large collaborative efforts to characterize tumor samples using multi-omics platforms represented in The Cancer Genome Atlas (TCGA) and more recently in the Pan Cancer Genome Atlas will help to identify new biomarkers to guide optimal treatment selection in the near future [5].

Disease heterogeneity

RCC comprises a heterogeneous group of 16 histologically distinct malignancies. In the last decade, genomic, transcriptomic, and metabolic features have been identified to delineate biological differences including alterations in tumor metabolism between these histologic subtypes of RCC. The three main histological subtypes are papillary RCC (pRCC), clear cell RCC (ccRCC) and chromophobe RCC (chRCC). Efforts in research to uncover the underlying genetic, molecular, and biological differences between heterogeneous disease subtypes are on the way and will help to employ specific treatment strategies for each subtype.

Molecular biomarkers of signal transduction pathways

Clear cell RCC

Genomic sequencing has paved the way to understand the specific patterns that characterize RCC tumor genesis, particularly that of ccRCC. This histologic subtype of RCC is the most common type and prominent features are clear cell histology, acinar growth patterns and a rich vascular network.

Loss of the short arm of chromosome 3 (3p) and inactivation of the von Hippel-Lindau (VHL) tumor suppressor gene on chromosome 3p25, which was identified through germline genetic studies in patients with the von Hippel-Lindau inherited ccRCC-susceptibility syndrome, are the most common molecular alteration. In sporadic ccRCC, VHL inactivation has been reported in 60–90% of cases [6, 7].

VHL in normal healthy tissue is a "multipurpose protein" which controls a variety of gene expression programs like the regulation of hypoxia inducible factor 1 α (HIF-1α). In RCC, a lack of VHL leads to an increase in HIF1α expression mediating increased transcription of genes involved in angiogenesis and tumor genesis such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDFGR). Furthermore, the loss of VHL unleashes the activation of phosphatidylinositol 3 kinase (PI3-K) and mTOR (mechanistic Target of Rapamycin) [8]. The understanding of these pathways in the pathogenesis of RCC has led to the development of VEGF-tyrosine kinase inhibitors (TKI) and mTOR inhibitors. In the search for predictive and prognostic biomarkers for VEGF-targeting compounds, a variety of markers have been explored including the expression of VEGF, HIF and carbonic anhydrase-9. None of these could be established as a robust biomarker in routine praxis nor have they improved the prognostic accuracy of the validated scores.

It has been reported recently that mutations of chromatin remodeling genes are common in ccRCC including PBM1, SETD2, and BAP1. Especially mutation of BAP1 as well as CDKN2A loss were shown to be associated with poor survival [9].

Large-scale transcriptome profiling of pretreatment ccRCC tissues has contributed to the recent identification of transcriptional signatures that might be useful in predicting clinical benefit from VEGF-targeted agents. An exploratory analysis of the IMmotion150 trial revealed that the expression of six angiogenesis-associated genes was predictive for response to sunitinib [10]. A different angiogenesis gene signature was associated with longer progression-free survival (PFS) and overall survival (OS) in patients treated with first-line sunitinib or pazopanib as part of the COMPARZ trial [11]. Brian Rini reported data from the IMmotion151 phase III study at the ESMO 2018 Congress in Munich suggesting that angiogenesis gene expression signatures (GES) T effector (T_{eff}), and T myeloid gene expression signatures, were linked with differential outcomes to treatment with atezolizumab plus bevacizumab vs. sunitinib. In patients receiving sunitinib, high angiogenesis GES were associated with improved PFS. Interestingly, prognostic risk groups and sarcomatoid histology were related to specific GES [12].

However, only the MSKCC and the IMDC risk scores have been validated as prognostic tools and are included in the most relevant international guidelines, like European Society of Medical Oncology (ESMO) guidelines on RCC and the National Comprehensive Cancer Network Clinical Practice Guidelines (NCCN) in kidney cancer [3, 4]. (Table 1).

Non-clear cell RCC

Non-clear cell RCC (nccRCC) comprise a heterogeneous group of tumors, which is differentiated mainly by characteristic histologic features as well as specific genetic alterations. Furthermore, in many patients with non-clear cell histologies, germline evaluation of cancer susceptibility genes may identify genomic alterations with relevance to therapeutic decision-making. *Type I pRCCs* show mesenchymal-epithelial transition (MET) alterations in overall 81.3%, which may convey efficacy to MET-targeted agents like cabozantinib [13, 14]. Loss of chromosome 22 occurred consistently as a specific copy number alter-

Table 1 Comparison of the different risk classifications

MSKCC (Motzer) score	IMDC prognostic score
Karnofsky Performance Status (KPS) < 80%	KPS
Time from diagnosis to treatment <1 year	Time from diagnosis to treatment <1 year
Hemoglobin level below the lower limit of normal	Hemoglobin level below the lower limit of normal
Corrected calcium above the upper limit of normal >2.5 mmol/l (>10 mg/dl)	Corrected calcium above the upper limit of normal >2.5 mmol/l (>10 mg/dl)
LDH > 1.5 of the upper limit of normal	Neutrophils greater than the upper limit of normal
	Platelets greater than the upper limit of normal

MSKCC Memorial Sloan Kettering Cancer Center, *IMDC* International Metastatic Renal Cell Carcinoma Database Consortium, *LDH* lactate dehydrogenase

ation in *type 2 pRCCs* and some show TFE3 or TFEB gene fusion causing uncontrolled activation of MiT family target genes [9, 15]. Furthermore, analysis of the TCGA could identify a subset of tumors within pRCC with a specific, genome-wide *CpG island methylator phenotype (CIMP)* and these tumors were considered as a new individual RCC subtype [16]. Patients with CIMP RCC are diagnosed in a late stage of disease (90% stage III/IV) and have the poorest survival of all subtypes [5]. In addition, the newly identified CIMP RCC tumors could be particularly sensitive to demethylating agents.

Chromophobe RCC (chRCC) comprise 6–11% of all RCCs and is characterized by frequent loss of a whole copy of chromosomes 1, 2, 6, 10, 13 and 17 [17]. Patients with this subtype show significant response to immune checkpoint inhibitors (IOs).

The *MiT family translocation RCC* is a special subtype that accounts for less than 5% of all renal neoplasms in children, but it is the most common type of RCC in this age group [18].

In summary, it must be emphasized that there is currently a lack of validated therapy options for nccRCC and identifying important oncogenic pathways and predictive markers as suggested above may lead to better treatment selection and development of new treatment strategies.

Biomarkers in the area of immune checkpoint inhibitors

The outstanding achievement in the last decade was the development of novel IOs targeting CTLA-4 (cytotoxic T-lymphocyte-associated Protein 4) and PD-1 (programmed cell death protein 1) pathways. The discovery that tumors can exploit immune-inhibitory signals (immune checkpoints) to evade the immune system has changed the landscape of treatment in RCC. The observation that RCC is not associated with a high somatic mutational burden but might express neoantigens and show a rich immune cell infiltrate has led to test IOs in different stages of the disease. CheckMate 025 was the first large phase III clinical trial comparing the PD-1 inhibitor nivolumab to everolimus in patients with locally advanced or metastatic RCC after having progressed on at least one line of VEGF-TKI pretreatment [19]. However, patients expressing PD-L1 (Programmed cell death 1 ligand) more than 1% ($n = 181$) showed a worse OS in both treatment arms, thus suggesting a prognostic not a predictive role of this biomarker in the second-line setting.

In the first-line treatment setting the combination of nivolumab plus ipilimumab (anti-CTLA-4) yielded improved clinical benefit compared to sunitinib in treatment-naıve patients with metastatic ccRCC (mccRCC) [20]. Intermediate- and high-risk patients according to IMDC risk score with a tumor expressing PD-L1 ≥1% showed a higher overall response rate (ORR) rate and longer PFS compared to sunitinib. Conversely, patients in the favorable risk group with PD-L1-negative tumors experienced a significant longer PFS and ORR with sunitinib treatment [20].

There are several molecular explanations for the lack of a strong association between PD-L1 expression of the tumor and response rate to IOs. There exists an intrinsic PDL-1 expression of tumors driven by oncogenic signals on the one hand and an extrinsic PD-L1 expression of tumors as a response to interferon gamma production of immune cells in the tumor microenvironment on the other hand. This is a possible explanation for differential effectivity of IOs in tumors with intrinsic versus extrinsic PD-L1 expression which is not reflected by PD-1 staining. In addition, the tumor microenvironment might be infiltrated by Treg cells and M2 polarized macrophages or myeloid derived suppressor cells hindering T effector activity [21, 22]. Therefore, T-effector/interferon (IFN)-gamma response-, angiogenesis-, and myeloid inflammatory-gene expression signatures have been suggested to predict response to VEGF-TKIs and IOs [23]. Survival and ORR advantages were maintained in all PD-L1 categories, but tumors with worst prognostic features like frameshift insertion and deletion showed a greater benefit to IOs [24–26]. Unfortunately, there is no correlation between high mutational load and MSKCC or IMDC prognostic criteria.

Furthermore, the implementation of PD-L1 as a biomarker is hampered by intratumoral heterogeneity of PD-L1 expression. Lopez et al. showed that a multisite tumor sampling strategy might be superior to the current tumor sampling protocols, since tumors can harbor regions with differential expression levels of PD-L1 [27]. PD-L1 levels are commonly assessed in primary tumors. However, there exists a numerically larger variance of PD-L1 expression within

the primary tumor as in metastatic lesions, which are the focus of systemic therapy [28, 29]. To establish PD-L1 expression as a more reliable predictive biomarker more accurate assessment of PD-L1 expression in metastatic lesions should be considered.

In summary, testing for PD-L1 expression is not recommended by current RCC guidelines for clinical decision-making.

Inflammatory markers in RCC

The prognostic role of tumor-infiltrating neutrophils, elevated blood neutrophils and elevated blood neutrophil-to-lymphocyte ratio (NLR) has been associated with poor clinical outcome in several tumor entities [30, 31]. Assessment of the degree of systemic inflammation prior to the treatment might help to predict treatment response. High NLR is associated with poor response to VEGF targeted treatment and immunotherapy and is of important value for treatment monitoring. NLR is an easily measurable and cost-effective parameter in addition to C-reactive protein (CRP) and are related to systemic inflammation. It has been shown that an early decline of an elevated NLR at week 6 during IO treatment is associated with significantly improved outcomes in mRCC patients [32].

Emerging prognostic biomarkers independent of histologic subtype

DNA methylation

DNA methylation in cancer may occur either prior to or following cell transformation through mutations [33]. Several lines of evidence suggest that most methylation changes occur in a programmed manner in subpopulations of tissue cells during normal aging, probably predisposing them to tumorigenesis. In the TCGA-RCC data set variable hypermethylation probes associated with 1532 genes were used leading to the identification of hypermethylated subgroups within ccRCC and pRCC histological subtypes samples. Increased DNA hypermethylation was associated with significantly poorer survival. This means that that DNA hypermethylation in tumors correlated with higher-stage of disease and poorer survival [5].

Metabolism

New tumor cell metabolism markers like ribose sugar or AMPK have been identified in the TCGA-RCC data set [34, 35]. Ribose sugar metabolism (RSM) gene signature was heterogeneously expressed in the different RCC subtypes. The RSM gene signature was highest in CIMP RCC and was significantly increased in type 2 pRCC [5]. Tumors with low ribose metabolism had a better prognosis compared to tumors with high ribose metabolism. These findings illustrate that tumor metabolism might be a new drug target in the future.

Compliance with ethical guidelines

Conflict of interest J. Terzic and T. Bauernhofer declare that they have no competing interests.

Ethical standards All human and animal studies have been approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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