Kidney Cancer Pathology in the New Context of Targeted Therapy

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Introduction

Renal cell carcinoma (RCC) accounts for 2–3% of all malignant diseases in adults [1]. The incidence of all stages of this cancer has increased over the last 20 years, contributing to an increasing mortality rate. Twenty to 30% present with metastasis and 20–30% relapse after curative nephrectomy. The overall 5-year survival ranges from 85% for patients with organ-confined disease treated with partial or radical nephrectomy to only 10% in patients with metastatic disease or relapse after nephrectomy [2]. Renal cancer is not one entity but rather a collection of different types of tumors (clear cell, papillary, and chromophobe cell types being the most frequent), each derived from various parts of the nephron, with morphological and genetic features (WHO 2004 classification and emerging entities likely to be included in future WHO classifications; table 1) [3]. Molecular pathology in kidney cancer has developed extensively in the few last years, providing insights into the underlying oncogenesis with a new basis for accurate classifications and more effective systemic therapy. However, in renal cancer, the use of targeted therapies in metastatic disease still lacks consistent predictive biomarkers [4]. This review...
Aims to focus on molecular profiles in renal cancer accounting for the effects of targeted therapies and substantiating the search for relevant predictive biomarkers.

**Classification and Clinical Trials**

Advanced disease is refractory to radiotherapy and known chemotherapies, and the only treatment available for metastatic disease has been, for a long time, immunotherapy based on interleukin (IL)-2 and/or interferon (IFN)-α, with a lasting response in less than 10% of patients. In the 2000s, a new paradigm has emerged in renal cancer with the use of effective targeted therapies, including antiangiogenic agents (anti-VEGF A antibody bevacizumab and VEGFR2 tyrosine kinase inhibitors sunitinib and sorafenib) and mammalian targets of rapamycin (mTOR) inhibitors (temsirolimus and everolimus). Most of these drugs are currently used as first-line treatment in metastatic disease (see current recommendations; table 2). A comparison of overexpressed genes in the 3 most frequent subtypes of renal cell carcinoma showed both common and specific sets of genes between clear cell, papillary, and chromophobe cell carcinomas, suggesting the potential importance of tumor subtyping when investigating biomarkers and targeted therapies [5]. Thus, the beneficial effects of VEGFR inhibitors sunitinib and sorafenib have been demonstrated for patients with clear cell RCC in distinct settings (table 2), and they appear more limited in patients with papillary or chromophobe RCC [6]. Conversely, the temsirolimus regimen seems to demonstrate a more significant effect on median survival in patients with non-clear cell RCC (including 75% of papillary RCC) than in patients with clear cell RCC [7]. Clinical trials are currently recruiting patients to assess the precise effect of mTOR inhibitors on metastatic papillary RCC.

Meanwhile, the pathological classification of renal cancer has been significantly extended with the description of new entities based on histological and/or molecu-
lar criteria (table 1) [3]. The diagnostic features of the new entities have been reviewed recently [8]. It is important to note that some cases previously considered to be clear cell carcinoma or papillary carcinoma should be diagnosed according to updated criteria, e.g. as carcinoma associated with translocation TEF3 or TFEB, papillary carcinoma with clear cell, or carcinoma associated with acquired multicyclic disease. A fraction of the cases included in the clinical trials would now be reclassified into new entities, with a potential impact on the trial conclusions according to histological subtypes. Retrospective analysis of former trials on the basis of pathological reexamination and new prospective trials should be performed to more precisely determine the relevance of the available therapies according to the different subtypes and prognosis groups of tumors. Overall, the context of intense research for consistent biomarkers predictive of treatment response underscores the interest in providing an accurate pathological diagnosis based on morphological, immunohistochemical, and genetic features [10].

### Genetic Changes in Sporadic and Hereditary Renal Cancers

The genetic studies in sporadic and hereditary forms of renal cancer have created a relevant framework to integrate renal cancer pathology in the era of targeted therapy, providing a rationale for the treatments and suggesting potential predictive biomarkers. The recurrent cyto-genetic changes in sporadic forms support the distinction of the different subtypes of renal tumors identified historically on morphological examinations (table 1). A few key genes have been identified, in particular with the in-
vestigation of hereditary kidney cancer syndromes which are rare clinical entities (2–3% of all renal cancer cases), but they offer valuable insights into the pathogenesis of kidney cancer through identification of the underlying genetic mechanisms common to hereditary and sporadic forms of the disease. Four major hereditary forms of renal cancer have been related to the following genes: Von Hippel Lindau (VHL), Hepatocyte Growth Factor Receptor (c-MET), Fumarate hydratase (FH), and Folliculin (FCLN) (table 3). Among them, the tumor suppressor gene VHL (3p25) is frequently inactivated by deletion, mutation, or promoter methylation in sporadic forms of clear cell carcinoma (up to 86% of cases), underscoring its pivotal role in this tumor subtype [11, 12]. According to this, global gene expression analysis in clear cell RCC shows frequent inactive VHL and active hypoxia and VEGF pathways [13]. Interestingly, a recent genome-wide analysis of copy number changes and gene expression profiles shed light on the clear cell RCC subtype, showing that sporadic clear cell RCC without evidence of biallelic VHL inactivation falls into 2 groups, i.e. one group with genomic profiles that are much more similar to tumors with biallelic inactivation of VHL, and another group with genomic profiles highly dissimilar to the majority of clear cell RCC [14].

The oncogene c-MET (7q31) is frequently gained and occasionally mutated (13%) in sporadic papillary RCC (type 1). For the tumor suppressor gene FH, no mutations in sporadic RCC have been detected, but the FH pathway is frequently underexpressed in papillary RCC (types 1 and 2) [13]. Inactivating mutations of the gene FLCN have been detected in sporadic chromophobe cell RCC (11%), suggesting a tumor suppressor role, at least for the chromophobe-subtype oncogenesis [15]. AKT-mTOR and c-myc also appear to be activated pathways in fractions of clear cell and high-grade papillary RCC [13]. These signaling pathway alterations, whether specific or not for the different tumor subtypes, provide prime targets for systemic therapy in advanced disease.

VHL and Hypoxia-Inducible Factor Pathways

The loss of VHL (resulting from the inactivation of both alleles) is a critical event in the pathogenesis of most clear cell RCC [12]. The consequences include effects on the hypoxia-inducible factor (HIF) and HIF-independent effects. HIF is a heterodimeric transcriptional factor associating HIF1α (or HIF2α) with the partner HIF1β. The VHL gene product is a component of an E3 ubiquitin ligase complex that targets HIF1/2α subunits for polyubiquitinylation and proteasomal degradation [16]. This process is dependent on the hydroxylation of conserved proline residues on the α subunits of HIF1/2α in the presence of oxygen. When oxygen levels are low, or VHL is inactivated, HIF1α or HIF2α accumulate, form a heterodimer with HIF1β, and translocate into the nucleus to regulate specific targets through binding to the hypoxia-responsive elements located in the promoter/enhancer regions of hypoxia-inducible genes [17]. HIF1α or HIF2α share significant homology and regulate partially overlapping repertoires of hypoxia-inducible target genes but may have distinct effects on RCC cell growth [18, 19]. HIF3α is a third HIFα which probably acts as a dominant negative inhibiting the effects of HIF1α and HIF2α. According to in vitro and in vivo models, stabilization of HIF2α, but not HIF1α, is the critical oncogenic event in the development of clear cell RCC, and clear cell carcinoma produces either HIF1α and HIF2α or HIF2α alone. HIF-responsive gene products include genes involved in angiogenesis (VEGF, PDGF, SDF, CXCR4, TGFβ, and CTGF), glucose uptake and metabolism (HK2 and PDK4), pH control (CAIX and CAXII), invasion/metastasis (MMP1, SDF, CXCR4, and c-Met), proliferation, and survival (TGFRα) [18]. This gene-program activation accounts for the prominent angiogenesis observed in clear cell carcinoma and the effects of targeted therapy directed against VEGF or VEGFR2 (the main VEGF receptor expressed in clear cell RCC, also called KDR). Bevacizumab is a recombinant human monoclonal antibody able to bind and neutralize VEGF, resulting in decreased angiogenesis [20]. Sunitinib is a small tyrosine kinase inhibitor of VEGFR2, PDGFR-B, FLT-3, and c-KIT and has an effect on both untreated metastatic RCC patients (median progression-free survival, 11 months) and cytokine refractory metastatic clear cell RCC patients (median progression-free survival, 9 months) [21, 22]. Sorafenib, another small kinase inhibitor, displays activity against VEGFR2, VEGFR3, PDGFR-B, FLT-3, c-KIT, and RAF-1, assumed to account for the prolongation of progression-free survival observed both in previously untreated and cytokine refractory metastatic clear cell RCC patients [1, 23].

Available antiangiogenic therapy in the adjuvant setting for tumors at risk of progression after curative nephrectomy is under investigation. Moreover, almost all kidney cancer patients treated with VEGF inhibitors experience disease progression, and further strategies should include attempts to identify a new gene/pathway addiction created in cells defective for VHL protein func-
tion as well as to inhibit compensatory mechanisms that promote tumor survival in the setting of VEGF pathway blockade. Interestingly, the HIF-independent effects of VHL loss remain poorly understood but could involve activation of the NFκB pathway promoting survival, in particular with the removed inhibition of the NFκB agonist Card9 [24]. A recent study also pointed at the consequences of VHL loss in mitotic spindle disorientation and the promotion of genetic instability [25].

Another current issue is the validation of tumor biomarkers predictive of the response to antiangiogenic therapy. Recent studies have proposed clinical (time from diagnosis to VEGF-targeted therapy < 2 years, 2 or more metastatic sites, and ECOG PS > 0) and biological (neutrophils > 4.5 × 10^3/μl, platelet count > 300 × 10^3/μl, abnormal corrected plasmatic calcium level, and lactate dehydrogenase (LDH) > 1.5 times the upper limit of normal) criteria that should be tuned by tumor molecular features [4]. The molecules HIF1α, VEGF, VEGFR2, CAIX, all involved in the signaling cascade expression, have been tested in pretherapy tumor samples, but their expression has failed to predict a therapeutic response in patients submitted to antiangiogenic treatment [4]. Only the high HIF2α expression (assessed by Western blot) has been reported to be associated with sunitinib response in a small cohort of 43 patients, and the plasmatic levels of soluble forms of VEGFR2 and VEGFR3 upon initiation or during the first weeks of systemic treatment have also been proposed to be predictive of therapeutic response [4]. These results should be confirmed by further studies, and the current clinical trials aim to identify and/or validate such predictive biomarkers. Regarding VHL gene status (inactive by mutation or methylation vs. wild type), complex results have been reported. Choueiri et al. [26] found no association between VHL status and response rates or median progression-free survival, but the presence of ‘loss-of-function’ mutations was an independent factor associated with improved response. Moreover, VHL gene status could be relevant for patients treated with sunitinib and bevacizumab and not for patients treated with sunitinib and the new tested inhibitors axitinib or pazopanib [4, 27]. These differences could underlie non-VHL-related antitumor effects of sunitinib, axitinib, and pazopanib, or they could be explained by a variable drug sensitivity of the VHL/HIF/VEGF pathway in VHL wild-type RCC. Overall, these data support the need for further studies investigating the relationships between VHL gene status and the antiangiogenic therapeutic response.

As has already been mentioned, the clinical effects of antiangiogenic drugs in patients with papillary RCC seem to be limited [6]. It is important to note that fumarate hydratase activity (which is decreased significantly in papillary RCC) is related in part to the HIF pathway; FH inhibition leads to elevated intracellular fumarate which in turn acts as a competitive inhibitor of HPH (HIF prolyl hydroxylase), thereby causing stabilization of HIF by preventing proteasomal degradation [28–30]. An elevated HIF drives the transcription of key components of the glycolytic pathway, including GLUT1 and LDH, inducing the Warburg effect (the tendency of cancer cells to rely on glycolysis as their energy source). However, there are probably other tumor suppressor roles of FH that are probably HIF independent and involve, in particular, the DNA damage response [31].

mTOR Pathway

The PI3K-AKT-mTOR cascade appears to be another pivotal pathway in clear cell RCC but also in non-clear cell RCC. Upon the binding of ligands on membrane growth factor and/or cytokine receptors, the phosphoinositide 3 kinase generates PIP3 and activates AKT. PTEN is a phosphatase that promotes the generation of PIP2 from PIP3, negatively regulating the cascade. The phosphorylated AKT activates mTOR complex 1 (mTORC1) through inhibition of TSC1/TSC2, and mTORC1 activates protein synthesis through phosphorylation of key regulators such as the P70 S6 kinase (S6) [32]. Activated phosphorylated S6 (phospho-S6) exerts a negative feedback loop on IRS1/IRS2 receptors upstream to PI3K. It is important to note that the targets of S6 include factor HIF1α; this explains why HIF1α expression is dependent on mTOR and sensitive to rapamycin or rapalogues such as temsirolimus and everolimus. This effect could account at least partially for the activity of mTOR inhibitors in kidney cancer. Phase III trials have shown that temsirolimus improves overall survival in patients with advanced RCC and poor prognostic features, and everolimus improves progression-free survival in patients for which sunitinib and/or sunitinib has become ineffective, in both clear cell and non-clear cell RCC [7, 33–35]. Furthermore, as signaling downstream to VEGFR involves the PI3K-AKT-mTOR pathway, the mTOR inhibitors might theoretically affect both tumor cells and tumor-associated endothelial cells. Pantuck et al. [36] studied the activated status of the mTOR pathway using phospho-S6 as a marker for this activation. Phospho-S6 was associated with tumor stage, grade, and disease-specific survival in patients with localized or meta-
static disease. A small retrospective analysis has suggested that high expression of phospho-AKT or phospho-S6 could be associated with the response to temsirolimus [37]. The value of these biomarkers and other candidates within the PI3K-AKT-mTOR pathway must be validated in larger retrospective and prospective studies. PTEN expression does not seem to have any predictive value in that context [38].

Besides mTORC1, mTORC2 is another mTOR complex in the pathway with the ability to activate AKT through phosphorylation. There is some evidence that HIF1α expression is dependent on both mTORC1 and mTORC2 and that HIF2α expression is dependent only on mTORC2 [39]. As temsirolimus and everolimus are only active on mTORC1, HIF2α is not targeted by these therapies, providing an explanation for the resistance to mTORC1 inhibitors. Furthermore, the action of mTORC1 inhibitors on S6 results in the loss of feedback inhibition and AKT phosphorylation through mTORC2 [32]. These considerations underscore the importance of targeting mTORC2 (inhibitors targeting both mTORC1 and mTORC2 are under investigation) and probably of combining treatment with new inhibitors of IRS1/IRS2 or PI3K.

The mTOR inhibitors could be of interest for treating metastatic chromophobe cell carcinoma, but the data are still limited [7]. It is interesting that mouse models deficient for the FLCN gene have been generated, developing oncocytic cysts and renal tumors and mimicking Birt-Hogg-Dubé (BHD) syndrome which predisposes subjects to develop renal carcinomas of nearly all subtypes (the chromophobe cell RCC subtype being the most frequent in BHD nevertheless) [40–43]. The tumor suppressor role of FLCN has been demonstrated, but contradictory results regarding the role of FLCN in the PI3K-AKT-mTOR pathway have been described, with the mTOR target phospho-S6 being increased or decreased depending on the context and/or the model. Additional studies are mandatory before considering that inhibitors of both mTORC1 and mTORC2 might be effective as potential therapeutic agents for BHD-associated kidney cancer.

**Myc Pathway**

A c-MYC gain (8q24) has been observed in up to 20% of clear cell RCC via either genome-wide or specific FISH analysis, and it has been correlated with concomitant overexpression suggesting its involvement in renal oncogenesis [14, 44]. Moreover, pathway analysis and experiments in cell lines support activation of the c-MYC pathway, resulting in cell cycle promotion [13]. Recently, a study elegantly demonstrated that HIF1α effects on c-myc could distinguish 2 subtypes of sporadic VHL-deficient clear cell renal carcinoma: the fraction of VHL-deficient clear cell RCC with coexpressed HIF1α and HIF2α could activate the AKT/mTOR and ERK/MAPK pathways and be likely to respond to antiangiogenic and mTOR inhibitors, and the fraction of VHL-deficient clear cell RCC with HIF2α expressed alone could promote myc transcriptional activity, with higher rates of cell proliferation and tumor growth [45]. The authors suggest that this molecular stratification according to the HIF1α/HIF2α expression could provide a framework for subclassifying tumors for targeted therapy. The pertinence of these 2 subtypes of clear cell RCC with regard to the therapeutic response to mTOR inhibitors or antiangiogenics remains to be tested. Furthermore, high-grade papillary renal cell carcinoma (type 2) has also been shown to be associated with the c-MYC signature [46]. This signature was correlated with the gain of chromosome 8q and overexpression of c-MYC located in 8q24. Overall, these observations raise a potential interest in future therapy targeting the c-MYC pathway in a fraction of clear cell and high-grade papillary RCC using the MYC inhibitor or siRNA strategy, for instance.

**c-MET Pathway**

Activating mutations in the tyrosine kinase domain of the c-MET gene (7q31) have been detected in the germ line of affected individuals in the hereditary papillary RCC (HRPC) kindred and in tumors from patients with sporadic type 1 papillary RCC. c-MET is the receptor for the hepatocyte growth factor (HGF) (7q21.1), and the HGF/c-MET signaling pathway is involved in proliferation, survival, cell growth, differentiation, and cell migration. The involvement of the HGF/c-MET pathway in papillary RCC oncogenesis is supported by the frequent trisomy of chromosome 7 observed in sporadic type 1 papillary RCC, but the modest rate of c-MET mutation (13%) could suggest that other major pathways are to be investigated in sporadic papillary RCC. Most inherited cases are low-grade tumors occurring rather in the 5th decade. However, an early-onset HPRC phenotype has been described, including metastasis progression [47]. Likewise, most sporadic type 1 papillary RCC are associated with a favorable outcome, but a recent study reported on metastatic type 1 papillary RCC with outcomes even worse than...
those for metastatic type 2 RCC [48]. Such metastatic type 1 papillary RCC are good candidates for treatment with drugs targeting the c-MET pathway according to different strategies, antagonism of ligand/receptor interaction, inhibition of tyrosine kinase catalytic activity, and blockade of receptor effector interactions [49]. Such options are currently being investigated in clinical trials. The c-MET receptor could belong to the ‘dependence receptor’ family, and blockade of the pathway is expected to promote apoptosis in tumor cells [50]. Furthermore, a recent screen detected c-MET as a kinase required for survival in VHL-defective renal cancer cells, prompting an interest in targeting the c-MET pathway also in clear cell RCC [51]. Cooperation between FH and c-MET in transformation and tumorigenesis was also demonstrated in a cell line model, underscoring the way pathways can interplay and the potential interest in combined targeted therapy [52].

Immune Response

Immunotherapy aims to elicit an antitumor immune response resulting in significant disease remission. The most consistent antitumor activity has been reported with IFN-α and IL-2. The superiority of sunitinib, temsirolimus, and bevacizumab plus IFN-α over IFN-α alone has limited the role of single-agent IFN-α. However, trials with high-dose intravenous bolus IL-2 have demonstrated a durable response in 7–8% patients, supporting the use of this cytokine therapy in some patients with metastatic RCC [53, 54]. It is important to note that IL-2 is the only therapy for kidney cancer that can produce a remission of the disease that lasts after the treatment has been completed. According to published data, immunotherapy should be restricted to patients with metastatic RCC, good risk, and a clear cell subtype. Moreover, in clear cell RCC, additional predictive features of a better response to high-dose IL-2 could be an alveolar pattern >50%, no granular or papillary features, and expression of carbonic anhydrase IX (CAIX) in immunohistochemistry in >85% of tumor cells [55]. Indeed, in one study the response rate was 59% for patients with a good risk and high CAIX expression versus less than 5% for patients in the poor-risk group with low CAIX expression [55]. A high-dose IL-2 trial is currently investigating its efficacy according to these predictive features to prospectively validate the selection criteria and to identify the patients likely to benefit the most from immunotherapy. A recent study based on a proteomic approach attempted to identify a new biomarker in the immunotherapy setting [56].

Conclusion

During the last 10 years, intense research in the renal cancer field has provided a huge amount of new molecular knowledge, supplying a rationale for targeted therapy in metastatic disease. Though no tissue biomarker can currently be recommended to predict therapeutic response, CAIX expression for high-dose IL-2 immunotherapy, VHL gene status, and HIF2α expression for antiangiogenic drugs, as well as phosphorylated protein S6 expression for mTOR inhibitor use, are the leading candidates under investigation. Besides the tissue analysis in progress, other useful biomarker studies include clinical features, functional imaging, and blood investigations. Parallel to the emergence of targeted therapy, the classification of renal tumors has been defined both on a morphological and on a molecular basis, appearing more complex than 10 years ago, and the design of future clinical trials should take into account this variety of tumor subtypes to provide the most relevant conclusions. Meanwhile, the pathways involved in renal cancer are amazingly intermingled and shared at least partly by the different tumors subtypes, suggesting common oncogenetic determinants and the possibility of using the same drugs for different diseases. Future studies will investigate combination and sequential therapy, mechanisms of resistance, and their effects in adjuvant or neoadjuvant settings.

References

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