KIDNEY CANCER PATHOLOGY
IN THE NEW CONTEXT OF TARGETED THERAPY

Yves Allory[1,2], Stéphane Culine[1,3], Alexandre de la Taille[1,4]
[1], INSERM, U955, Team 7 "Translational research in genito-urinary oncogenesis", Créteil, France
[2] AP-HP, Department of Pathology, Henri Mondor Hospital, Créteil, France
[3] AP-HP, Department of Medical Oncology, Henri Mondor Hospital, Créteil, France
[4] AP-HP, Department of Urology, Henri Mondor Hospital, Créteil, France

Corresponding author:
Yves Allory
Department of Pathology
Henri Mondor Hospital
51, av. Mal de Lattre de Tassigny
94010 Créteil Cedex
Email : yves.allory@hmn.aphp.fr

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Abstract

Outcome in metastatic renal cancer remains poor with an overall survival at 5 years less than 10%. However, molecular pathology in kidney cancer has developed extensively in the few last years, providing basis for new systemic therapies, including anti-angiogenic drugs and mTOR inhibitors. The use of these targeted therapies in metastasis disease has improved the prognosis but still in a too limited range, with a lack of consistent predictive biomarkers. The multiple entities of renal tumors add complexity to the research of biomarkers and the design of clinical trials. This review aims to focus on pathways in renal cancer (VHL/HIF, mTOR, c-MYC, c-MET, immune response) in the respective tumor subtypes, accounting for the effects of targeted therapies, and providing the framework to search for relevant predictive biomarkers and propose new trials. This overview underscores that the pathways are often intermingled and common (at least partially) to the different tumor subtypes.

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 to 30% relapse after curative nephrectomy. The overall 5 year survival ranges from 85 % for patients with organ-confined disease, treated by partial or radical nephrectomy, to only 10% in patients with metastatic disease or relapse after nephrectomy (2). The renal cancer is not one entity but rather a collection of different types of tumors (the clear cell, papillary and chromophobe cell types being the most frequent), each derived from various parts of the nephron, with morphological and genetic features (*table 1*, WH0 2004 classification and emerging entities likely to be included in future WHO classification) (3). Molecular pathology in kidney cancer has developed extensively in the few last years, providing insights in underlying oncogenesis, with new basis for accurate classifications and more effective systemic therapy. However, in renal cancer, the use of targeted therapies in metastasis 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-2 (IL-2) and/or interferon-α (IFN-α), with durable response for less than 10% of patients. In years 2000s, a new paradigm has emerged in renal cancer with the use of effective targeted therapies, including anti-angiogenic agents (the anti-VEGF A antibody bevacizumab and VEGFR2 tyrosine kinase inhibitors sunitinib and sorafenib) and mammalian Targets Of Rapamycine (mTOR) inhibitors (temsirolimus and everolimus). Most of these drugs are used currently as first line treatment in metastatic disease (*table 2*, see current recommendations). A comparison of over-expressed genes in the three 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 appeared more limited for patients with papillary or chomophobe cell RCC (6). On the opposite, temsirolimus regimen seems to demonstrate a more significant effect on median survival for patients with non-clear cell RCC (including 75% papillary RCC), than for patients with clear cell RCC (7). Clinical trials are currently recruiting patients to assess precise effect of mTOR inhibitors on metastatic papillary RCC.

Meanwhile, the pathological classification of renal cancer has been extended significantly with the description of new entities based on histological and/or molecular criteria (*table 1*) (3). The diagnostic features of the new entities have been reviewed recently (8). Of note, some cases previously considered as clear cell carcinoma or papillary carcinoma should be diagnosed according to up-dated criteria, as carcinoma associated with translocation *TEF3* or *TFEB*, papillary carcinoma with clear cell, or carcinoma associated with acquired multicystic disease, for instance. A fraction of cases included in the clinical trials would be re-classified now in new entities, with a potential impact on the trial conclusions according to histological subtypes. Retrospective analysis of former trials on the basis of pathological re-examination

and new prospective trials should be performed to precise 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 for a treatment response underscores the interest to provide 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 settled 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 cytogenetic changes in sporadic forms support the distinction of the different subtypes of renal tumor identified historically on morphological examinations (*table 1*). A few key genes have been identified, in particular with the investigation of hereditary kidney cancer syndromes who rare clinical entities (2-3% of all renal cancer cases), but offer valuable insights into the pathogenesis of kidney cancer through identification of the underlying genetic mechanisms common to hereditary and sporadic forms of disease. Four major hereditary forms of renal cancers 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% cases), underscoring its pivotal role in this tumor subtype (11)(12). According to this, the 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 has shed light on the clear cell RCC subtype, showing that sporadic clear cell RCC without evidence of bi-allelic VHL inactivation fell into two groups, one group with genomic profiles that are much more similar to tumors with bi-allelic inactivation of VHL, and the other 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 under-expressed 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 appear also to be activated pathways both 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 HIF pathways

The loss of VHL (resulting from inactivation of both alleles) is a critical event in the pathogenesis in 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 polyubiquitylation 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 heterodimere with HIF1β and translocate into the nucleus to regulate specific targets through binding to the hypoxia-responsive elements (HREs) 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 who 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 produce 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, PDK4*), pH control (*CAIX* and *CAXII*), invasion/metastasis (*MMP1, SDF, CXCR4, c-Met*), proliferation and survival (*TGFα*) (18). This gene program activation accounts for the prominent angiogenesis observed in clear cell carcinoma and the effects of targeted therapy directed at 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, both with an effect in untreated metastatic RCC patients (median progression free survival 11 months) and in cytokine refractory metastatic clear cell RCC patients (median progression free survival 8.8 months) (21)(22). Sorafenib, another small kinase inhibitor, displays an 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 (23)(1).

The interest of available anti-angiogenic therapy in the adjuvant setting for tumors at risk of progression after curative nephrectomy is under investigation. Moreover, almost all kidney cancer patients treated by VEGF inhibitors experiment disease progression, and further strategies should include attempts to identify new gene/pathway addiction created in cells defective for VHL protein function, and to inhibit compensatory mechanism that promote tumor survival in the setting of VEGF pathway blockade. Interestingly, the HIF-independent effects of VHL loss remains poorly understood, but could involve the activation of NFκB pathway promoting survival, in particular with the removed inhibition of the NFκB agonist Card9 (24). A recent study pointed also at VHL loss consequences in mitotic spindle disorientation and promotion of genetic instability (25).

Another current issue is the validation of tumor biomarkers predictive of response to anti-angiogenic therapy. Recent studies have proposed clinical (time from diagnosis to VEGF-targeted therapy < 2 years, two or more metastatic sites, ECOG PS>0) and biological (neutrophils > 4.5 K/µL, platelets count > 300 K/µL, abnormal corrected plasmatic calcium level, LDH > 1.5 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 pre-therapeutic tumor samples, but their expression fails to predict a therapeutic response for patients submitted to anti-angiogenic 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 at initiation or during the first weeks of systemic treatment have been proposed also 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 the *VHL* gene status (inactivated by mutation or methylation *versus* wild type), complex results has been reported. Choueiri et al. have 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 (26). Also, the VHL gene status could be relevant for patients treated by sorafenib and bevacizumab, and not for patients treated by sunitinib, and new tested inhibitors axitinib or pazopanib (4)(27). These differences could underlie non-VHL related antitumor effects for sunitinib, axitinib and pazopanib, or be explained by a variable drug sensitivity of 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 anti-angiogenic therapeutic response.
As already mentioned, the clinical effects of anti-angiogenic drugs for patients with papillary RCC seem to be limited (6). Of note, the fumarate hydratase activity (which is decreased significantly in papillary RCC) is related in part to 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 (Hypoxia-inducible factor) by preventing proteasomal degradation (28)(29)(30). Elevated HIF drives transcription of key components of the glycolytic pathway, including GLUT1 and lactate dehydrogenase (LDH), inducing a 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, probably HIF independent, and involving in particular the DNA damage response (31).

mTOR pathway

The PI3K-AKT-mTOR cascade appears to be another pivotal pathway in clear cell, but also non clear cell RCC. Upon the binding of ligands on membrane growth factor and/or cytokine receptors, the phospho-inositide 3 kinase generates PIP3 and activates AKT. PTEN is a phosphatase that promotes the generation of PIP2 from PIP3, regulating negatively the cascade. The phosphorylated AKT activates the 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 feed-back loop on IRS1/IRS2 receptors upstream to PI3K. Of note, the targets of S6 include the factor HIF1α, explaining why HIF1α expression is dependent on the mammalian target of rapamycin (mTOR) and sensitive to rapamycin or rapalogues such as temsirolimus or everolimus. This effect could account at least partially for the activity of mTOR inhibitors in kidney cancer. Phase III trials has 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 sorafenib and/or sunitinib become ineffective, both in clear cell and non clear cell RCC (7)(33)(34)(35). Furthermore, as the 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. have studied the activated status of mTOR pathway, using phospho-S6 as a marker this activation. Phospho-S6 was associated with tumor stage, grade, and disease specific survival in patients with localized or metastatic disease (36). A small retrospective analysis has suggested that high expression of phospho-AKT or phospho-S6 could be associated with 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. The 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 ability to activate AKT through phosphorylation. There are some evidences that HIF1α expression is dependent on both mTORC1 and mTORC2, and 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 explanation for a resistance to mTORC1 inhibitors. Furthermore, the action of mTORC1 inhibitors on S6 results in loss of feed-back inhibition and AKT phosphorylation through the mTORC2 (32). These considerations underscore the importance of targeting mTORC2 (inhibitors targeting both mTORC1 and mTORC2 are under investigation), and probably to combine treatment with new inhibitors of IRS1/IRS2 or PI3K.
The mTOR inhibitors could be of interest to treat metastatic chromophobe cell carcinoma, but the data are still limited (7). Of interest, mouse models deficient for the *FLCN* gene have been developed, developing oncocytic cysts and renal tumors, and mimicking the Birt-Hogg-Dubé (BHD) syndrome which predisposes subjects to develop renal carcinoma of nearly all subtypes (the chromophobe cell RCC subtype being the most frequent in BHD nevertheless) (40)(41)(42)(43). The tumor suppressor role for FLCN was demonstrated, but contradictory results regarding the role of FLCN in PI3K-AKT-mTOR pathway have been described, with the mTOR target phospho-S6 being increased or decreased upon 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 significantly observed in up to 20 % of clear cell RCC either by genome wide or specific FISH analysis, and correlated with concomitant over-expression, suggesting its involvement in renal oncogenesis (14)(44). Moreover, pathways analysis and experiments in cell lines support the activation of c-MYC pathway, resulting in cell cycle promotion (13). Recently, a study has demonstrated elegantly that HIFα effects on c-myc could distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma : the fraction of VHL-deficient clear cell RCC with co-expressed HIF1α and HIF2α could activate the AKT/mTOR and ERK/MAPK pathways and be likely to respond to anti-angiogenic and mTOR inhibitors, the fraction of VHL-deficient clear cell RCC with HIF2α expressed alone could promote the myc transcriptional activity, with higher rates of cell proliferation and tumor growth (45). The authors suggest that this molecular stratification according to HIF1α/HIF2α expression could provide a framework for sub-classifying tumors for targeted therapy. The pertinence of these two subtypes of clear cell RCC in regards of therapeutic response to mTOR inhibitors or anti-angiogenic remains to be tested. Furthermore, high grade papillary renal cell carcinoma (type 2) has been shown to be associated also with c-MYC signature (46). This signature was correlated with gain of chromosome 8q and over-expression of c-MYC located in 8q24. Overall, these observations raise the potential interest of future therapy targeting the c-MYC pathway in a fraction of clear cell and high grade papillary RCC, using 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 hereditary papillary renal cell carcinoma kindred and in tumors from patients with sporadic type 1 papillary renal cell carcinoma. 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 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 the sporadic papillary RCC. Most of the 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 renal cell carcinomas are associated with favorable outcome, but a recent study reports on metastatic type 1 papillary RCC with outcome even worse than for metastatic type 2 RCC (48). Even unusual, such metastatic type 1 papillary RCC are good candidate to be treated by 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 under current investigations in clinical trials. The c-MET receptor could belong to the "dependence receptor" family, and the 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, suggesting the interest to target c-MET pathway also in clear cell RCC (51). A cooperation between FH and c-MET in transformation and tumorigenesis was demonstrated also in a cell line model, underscoring how the pathways can interplay and the potential interest of combined targeted therapy (52).

Immune response

Immunotherapy aims to elicit an anti-tumor immune response resulting in significant disease remission. The most consistent antitumor activity has been reported with interferon α (IFN-α) and interleukin-2 (IL-2). The superiority of sunibitib, 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 interleukin-2 have demonstrated a durable response in 7-8% patients, supporting the use of this cytokine therapy for some patients with metastatic RCC (53)(54). Of note, IL-2 is the only therapy for kidney cancer that can produce a remission of disease that lasts after treatment is completed. According to published data, immunotherapy should be restricted to patients with metastatic RCC, good risk and clear cell subtype. Moreover, in clear cell RCC, the additional predictive features of better response to high-dose IL-2 could be an alveolar pattern > 50%, no granular of papillary features, and an 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 good-risk and high-CAIX expression versus less than 5% for patients in the poor-risk group with low CAIX expression (55). A current high-dose IL-2 trial is investigating currently its efficacy according to these predictive features to validate prospectively the selection criteria, and to identify the patients susceptible to benefit the most of immunotherapy. A recent study based on proteomic approach attempted to identify new biomarker in the immunotherapy setting (56).

Conclusion

During the ten last years, the intense research in renal cancer area has provided a huge amount of new molecular knowledge, providing rationale for targeted therapy in metastatic disease. Though no tissue biomarker can be recommended currently to predict therapeutic response, CAIX expression for high dose IL-2 immunotherapy, *VHL* gene status and HIF2α expression for anti-angiogenic drugs, and phosphorylated protein S6 expression for mTOR inhibitor use, are the leading candidates under investigation. Besides 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 turmors has been precised both on morphological and 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 to use the same drugs for different diseases. Future studies will investigate combination and sequential therapy, mechanisms of resistance, and their effects in adjuvant or neo-adjuvant settings.

References

1. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. Lancet. 2009 ;373:1119-1132.

2. Motzer RJ, Bander NH, Nanus DM. Renal-cell carcinoma. N. Engl. J. Med. 1996;335:865-875.

3. Eble, John, Sauter, Guido, Epstein, Jonathan, Seesterheen, Isabelle, éditeurs. Dans: World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon: IARC Press; 2004.

4. Atkins MB, Choueiri TK, Cho D, Regan M, Signoretti S. Treatment selection for patients with metastatic renal cell carcinoma. Cancer. 2009 ;115:2327-2333.

5. Jones J, Libermann TA. Genomics of renal cell cancer: the biology behind and the therapy ahead. Clin. Cancer Res. 2007 ;13:685s-692s.

6. Choueiri TK, Plantade A, Elson P, Negrier S, Ravaud A, Oudard S, et al. Efficacy of sunitinib and sorafenib in metastatic papillary and chromophobe renal cell carcinoma. J. Clin. Oncol. 2008 ;26:127-131.

7. Dutcher JP, de Souza P, McDermott D, Figlin RA, Berkenblit A, Thiele A, et al. Effect of temsirolimus versus interferon-alpha on outcome of patients with advanced renal cell carcinoma of different tumor histologies. Med. Oncol. 2009;26:202-209.

8. Srigley JR, Delahunt B. Uncommon and recently described renal carcinomas. Mod. Pathol. 2009;22 Suppl 2:S2-S23.

9. Cytogenetic and molecular tumor profiling for type 1 and type 2 papillary renal cell carcinoma. Klatte T, Pantuck AJ, Said JW, Seligon DB, Rao NP, LaRochelle JC, Shuch B, Zisman A, Kabbinavar F. Clin Cancer Res. 2009;15:1162-1169.

10. Cheng L, Zhang S, MacLennan GT, Lopez-Beltran A, Montironi R. Molecular and cytogenetic insights into the pathogenesis, classification, differential diagnosis, and prognosis of renal epithelial neoplasms. Hum. Pathol. 2009 ;40:10-29.

11. Young AC, Craven RA, Cohen D, Taylor C, Booth C, Harnden P, et al. Analysis of VHL Gene Alterations and their Relationship to Clinical Parameters in Sporadic Conventional Renal Cell Carcinoma. Clin Cancer Res. 2009 ;15:7582-7592.

12. Kaelin WG. The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. Clin. Cancer Res. 2007 ;13:680s-684s.

13. Furge KA, Tan MH, Dykema K, Kort E, Stadler W, Yao X, et al. Identification of deregulated oncogenic pathways in renal cell carcinoma: an integrated oncogenomic approach based on gene expression profiling. Oncogene. 2007 ;26:1346-1350.

14. Beroukhim R, Brunet J, Di Napoli A, Mertz KD, Seeley A, Pires MM, et al. Patterns of gene expression and copy-number alterations in von-hippel lindau disease-associated and sporadic clear cell carcinoma of the kidney. Cancer Res. 2009 ;69:4674-4681.

15. Gad S, Lefèvre SH, Khoo SK, Giraud S, Vieillefond A, Vasiliu V, et al. Mutations in BHD and TP53 genes, but not in HNF1beta gene, in a large series of sporadic chromophobe renal cell carcinoma. Br. J. Cancer. 2007 ;96:336-340.

16. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature. 1999 ;399:271-275.

17. Maxwell PH. The HIF pathway in cancer. Semin. Cell Dev. Biol. 2005;16:523-530.

18. Smaldone MC, Maranchie JK. Clinical implications of hypoxia inducible factor in renal cell carcinoma. Urol. Oncol. 2009;27:238-245.

19. Kaelin WG. Treatment of kidney cancer: insights provided by the VHL tumor-suppressor protein. Cancer. 2009 ;115:2262-2272.

20. Yang JC, Haworth L, Sherry RM, Hwu P, Schwartzentruber DJ, Topalian SL, et al. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. N. Engl. J. Med. 2003 ;349:427-434.

21. Motzer RJ, Michaelson MD, Rosenberg J, Bukowski RM, Curti BD, George DJ, et al. Sunitinib efficacy against advanced renal cell carcinoma. J. Urol. 2007;178:1883-1887.

22. Motzer RJ, Hutson TE, Tomczak P, Michaelson MD, Bukowski RM, Rixe O, et al. Sunitinib versus interferon alfa in metastatic renal-cell carcinoma. N. Engl. J. Med. 2007;356:115-124.

23. Escudier B, Eisen T, Stadler WM, Szczylik C, Oudard S, Staehler M, et al. Sorafenib for treatment of renal cell carcinoma: Final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial. J. Clin. Oncol. 2009;27:3312-3318.

24. Yang H, Minamishima YA, Yan Q, Schlisio S, Ebert BL, Zhang X, et al. pVHL acts as an adaptor to promote the inhibitory phosphorylation of the NF-kappaB agonist Card9 by CK2. Mol. Cell. 2007 ;28:15-27.

25. Thoma CR, Toso A, Gutbrodt KL, Reggi SP, Frew IJ, Schraml P, et al. VHL loss causes spindle misorientation and chromosome instability. Nat. Cell Biol. 2009;11:994-1001.

26. Choueiri TK, Vaziri SAJ, Jaeger E, Elson P, Wood L, Bhalla IP, et al. von Hippel-Lindau gene status and response to vascular endothelial growth factor targeted therapy for metastatic clear cell renal cell carcinoma. J. Urol. 2008;180:860-865.

27. Rini BI, Jaeger E, Weinberg V, Sein N, Chew K, Fong K, et al. Clinical response to therapy targeted at vascular endothelial growth factor in metastatic renal cell carcinoma: impact of patient characteristics and Von Hippel-Lindau gene status. BJU Int. 2006;98:756-762.

28. Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung Y, et al. HIF

overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell. 2005;8:143-153.

29. Sudarshan S, Sourbier C, Kong H, Block K, Valera Romero VA, Yang Y, et al. Fumarate hydratase deficiency in renal cancer induces glycolytic addiction and hypoxia-inducible transcription factor 1alpha stabilization by glucose-dependent generation of reactive oxygen species. Mol. Cell. Biol. 2009;29:4080-4090.

30. Pollard PJ, Brière JJ, Alam NA, Barwell J, Barclay E, Wortham NC, et al. Accumulation of Krebs cycle intermediates and over-expression of HIF1alpha in tumours which result from germline FH and SDH mutations. Hum. Mol. Genet. 2005;14:2231-2239.

31. Yogev O, Yogev O, Singer E, Shaulian E, Goldberg M, Fox TD, et al. Fumarase: a mitochondrial metabolic enzyme and a cytosolic/nuclear component of the DNA damage response. PLoS Biol. 2010;8:e1000328.

32. Hudes GR. Targeting mTOR in renal cell carcinoma. Cancer. 2009;115:2313-2320.

33. Motzer RJ, Escudier B, Oudard S, Hutson TE, Porta C, Bracarda S, et al. Efficacy of everolimus in advanced renal cell carcinoma: a double-blind, randomised, placebo-controlled phase III trial. Lancet. 2008;372:449-456.

34. Motzer RJ, Hudes GR, Curti BD, McDermott DF, Escudier BJ, Negrier S, et al. Phase I/II trial of temsirolimus combined with interferon alfa for advanced renal cell carcinoma. J. Clin. Oncol. 2007;25:3958-3964.

35. Hudes G, Carducci M, Tomczak P, Dutcher J, Figlin R, Kapoor A, et al. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. N. Engl. J. Med. 2007 Mai;356:2271-2281.

36. Pantuck AJ, Seligson DB, Klatte T, Yu H, Leppert JT, Moore L, et al. Prognostic relevance of the mTOR pathway in renal cell carcinoma: implications for molecular patient selection for targeted therapy. Cancer. 2007 ;109:2257-2267.

37. Cho D, Signoretti S, Dabora S, Regan M, Seeley A, Mariotti M, et al. Potential histologic and molecular predictors of response to temsirolimus in patients with advanced renal cell carcinoma. Clin Genitourin Cancer. 2007;5:379-385.

38. Figlin RA, de Souza P, McDermott D, Dutcher JP, Berkenblit A, Thiele A, et al. Analysis of PTEN and HIF-1alpha and correlation with efficacy in patients with advanced renal cell carcinoma treated with temsirolimus versus interferon-alpha. Cancer. 2009;115:3651-3660.

39. Toschi A, Lee E, Gadir N, Ohh M, Foster DA. Differential dependence of hypoxia-inducible factors 1 alpha and 2 alpha on mTORC1 and mTORC2. J. Biol. Chem. 2008;283:34495-34499.

40. Chen J, Futami K, Petillo D, Peng J, Wang P, Knol J, et al. Deficiency of FLCN in mouse kidney led to development of polycystic kidneys and renal neoplasia. PLoS ONE. 2008;3:e3581.

41. Hartman TR, Nicolas E, Klein-Szanto A, Al-Saleem T, Cash TP, Simon MC, et al. The role of the Birt-Hogg-Dubé protein in mTOR activation and renal tumorigenesis. Oncogene. 2009;28:1594-1604.

42. Hasumi Y, Baba M, Ajima R, Hasumi H, Valera VA, Klein ME, et al. Homozygous loss of BHD causes early embryonic lethality and kidney tumor development with activation of mTORC1 and mTORC2. Proc. Natl. Acad. Sci. U.S.A. 2009;106:18722-18727.

43. Hudon V, Sabourin S, Dydensborg AB, Kottis V, Ghazi A, Paquet M, et al. Renal tumour suppressor function of the Birt-Hogg-Dubé syndrome gene product folliculin. J. Med. Genet. 2010;47:182-189.

44. Yamaguchi S, Yoshihiro S, Matsuyama H, Nagao K, Fukunaga K, Matsumoto H, et al. The allelic loss of chromosome 3p25 with c-myc gain is related to the development of clear-cell renal cell carcinoma. Clin. Genet. 2003;63:184-191.

45. Gordan JD, Lal P, Dondeti VR, Letrero R, Parekh KN, Oquendo CE, et al. HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. Cancer Cell. 2008;14:435-446.

46. Furge KA, Chen J, Koeman J, Swiatek P, Dykema K, Lucin K, et al. Detection of DNA copy number changes and oncogenic signaling abnormalities from gene expression data reveals MYC activation in high-grade papillary renal cell carcinoma. Cancer Res. 2007;67:3171-3176.

47. Schmidt LS, Nickerson ML, Angeloni D, Glenn GM, Walther MM, Albert PS, et al. Early onset hereditary papillary renal carcinoma: germline missense mutations in the tyrosine kinase domain of the met proto-oncogene. J. Urol. 2004;172:1256-1261.

48. Klatte T, Pantuck AJ, Said JW, Seligson DB, Rao NP, LaRochelle JC, et al. Cytogenetic and molecular tumor profiling for type 1 and type 2 papillary renal cell carcinoma. Clin. Cancer Res. 2009;15:1162-1169.

49. Bellon SF, Kaplan-Lefko P, Yang Y, Zhang Y, Moriguchi J, Rex K, et al. c-Met inhibitors with novel binding mode show activity against several hereditary papillary renal cell carcinoma-related mutations. J. Biol. Chem. 2008;283:2675-2683.

50. Goldschneider D, Mehlen P. Dependence receptors: a new paradigm in cell signaling and cancer therapy. Oncogene. 2010;29:1865-1882.

51. Bommi-Reddy A, Almeciga I, Sawyer J, Geisen C, Li W, Harlow E, et al. Kinase requirements in human cells: III. Altered kinase requirements in VHL-/- cancer cells detected in a pilot synthetic lethal screen. Proc. Natl. Acad. Sci. U.S.A. 2008 ;105:16484-16489.

52. Costa B, Dettori D, Lorenzato A, Bardella C, Coltella N, Martino C, et al. Fumarase tumor suppressor gene and MET oncogene cooperate in upholding transformation and tumorigenesis. FASEB J [Internet]. DOI: 10.1096/fj.09-146928.

53. Fisher RI, Rosenberg SA, Fyfe G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. Cancer J Sci Am. 2000 ;6:S55-57.

54. McDermott DF, Regan MM, Clark JI, Flaherty LE, Weiss GR, Logan TF, et al. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. J. Clin. Oncol. 2005;23:133-141.

55. Atkins M, Regan M, McDermott D, Mier J, Stanbridge E, Youmans A, et al. Carbonic anhydrase IX expression predicts outcome of interleukin 2 therapy for renal cancer. Clin. Cancer Res. 2005;11:3714-3721.

56. Jones J, Otu HH, Grall F, Spentzos D, Can H, Aivado M, et al. Proteomic identification of interleukin-2 therapy response in metastatic renal cell cancer. J. Urol. 2008 ;179:730-736.

57. Motzer RJ, Mazumdar M, Bacik J, Berg W, Amsterdam A, Ferrara J. Survival and prognostic stratification of 670 patients with advanced renal cell carcinoma. J. Clin. Oncol. 1999;17:2530-2540.

58. Mekhail TM, Abou-Jawde RM, Boumerhi G, Malhi S, Wood L, Elson P, et al. Validation and extension of the Memorial Sloan-Kettering prognostic factors model for survival in patients with previously untreated metastatic renal cell carcinoma. J. Clin. Oncol. 2005;23:832-841.

Table. Renal cell carcinoma classification: WHO 2004 classification and emerging entities

Tumor type	**Main recurrent genetic changes**
WHO 2004	
Clear cell RCC	3p25 *VHL* , 3p21 *RASSF1A*, 3p14.2 *FHIT* : Deletion, mutation, methylation
Multilocular cystic RCC	3p25 *VHL* : Mutation
Papillary RCC	Trisomy 7, 17 ; gain 7q31 *c-MET* ; Y loss
Chromophobe RCC	1,2,6,10,13, 17, 21, Y Multiple chromosome loss
Collecting duct carcinoma	Monosomy 1, 6, 14, 15, 22 (based on a few cases)
Renal medullary carcinoma	No gain or loss on CGH (based on a few cases)
RCC associated with Xp11.2 translocation	Translocation *PSF-TFE3* t(X;1)(p11.2;p34), *PRCC-TFE3* t(X;1)(p11.2;q21), *CLTC-TFE3* t(X;17)(p11.2;q23), *ASPL-TFE3* t(X;17)(p11.2;q25), ? t(X ;3)(p11.2 ;q12), or *NonO-TFE3* inv(X)(p11.2;q12)
Post neuroblastoma RCC	To be precised
Mucinous tubular and spindle cell carcinoma	Multiple chromosome losses (based on a few cases)
RCC unclassified	Not relevant
Emerging entities	
RCC associated with 6p21 translocation	Translocation *Alpha-TFEB* t(6;1)(p21;q12)
Tubulocystic carcinoma	Trisomy 7, 17 ; Y loss
Acquired cystic disease-associated RCC	To be precised
Clear cell papillary RCC	To be precised
Thyroid-like follicular carcinoma	To be precised
Oncocytic papillary RCC	Trisomy 7, 17 ; Y loss (based on a few cases)
Leiomyomatous RCC	3p25 *VHL* , 3p14.2 *FHIT* : Deletion (based on a few cases)

Abbreviation: RCC, renal cell carcinoma; CGH, comparative genomic hybridization

Table 2. Targeted therapy in renal cancer: standard recommendations 2010

Setting	Tumor subtype[1]	Context[2]	1st choice (evidence of phase III)	Alternative
First-line therapy				
	CC	Good or intermediate risk	Sunitinib or Bevacizumab + interferon	High-dose IL-2
	CC	Poor risk	Temsirolimus	Sunitinib
	NCC (PAP)	All risks	New drugs tested in clinical trials	
Second-line therapy				
	CC	Prior cytokine	Sorafenib	Sunitinib
	CC	Prior VEGFR inhibitor	Everolimus	New drugs tested in clinical trials
	CC / NCC	Prior mTOR inhibitor	New drugs tested in clinical trials	New drugs tested in clinical trials

[1]Tumor subtype: CC, clear cell; NCC (PAP), non clear cell (in particular, papillary carcinoma); [2], risk according to MSKCC risk status (57)(58).

Table 3 Major hereditary forms of renal cancers

Syndrome	Chromosome location	gene	Associated renal tumor subtype
Von Hippel-Lindau	3p25	*VHL*	Clear cell RCC
Hereditary papillary RCC	7q31	*c-MET*	Papillary RCC (type I)
Hereditary leimyomatosis and RCC	1q42	*FH*	Papillary RCC (type II)
Birt-Hogg-Dubé	17p11	*FLCN*	Oncocytoma, chromophobe RCC, Hybrid oncocytic tumor, (less frequently, Clear cell or papillary RCC)
Familial RCC associated with constitutional chromosome 3 translocation	3p and 3q	*FHIT* and others?	Clear cell RCC

All these hereditary forms are associated with autosomal dominant inheritance. RCC, renal cell carcinoma. *VHL*, von hippel Lindau, *c-MET*, hepatocyte growth factor, *FH*, fumarate hydratase, FLCN, folliculin