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Emerging molecular classification in RCC: implications for drug development

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Abstract

In the past decade, progress has been made in the development of targeted therapies for advanced renal cell carcinoma. However, as multiple therapeutic choices become available for clinicians, we currently lack effective indicators to allow physicians to choose the best treatment option for specific patients. For approved targeted therapies, potential molecules that could indicate drug effectiveness in a specific tumor follow naturally from both the therapeutic mechanism and the previously elucidated tumor biology. However, in advanced RCC, the use of these molecules as biomarkers for treatment selection has shown equivocal results and requires further investigation. In addition to looking at specific molecular targets, subclassification of tumors based on their molecular characteristics may also allow stratification of patients based on therapeutic benefits, providing information for treatment selection. Furthermore, the continued development of such tumor classification schemes will hopefully uncover other molecular targets that warrant development as future RCC therapies. The use of molecular classification of patients' tumors for treatment selection will provide the opportunity to increase the effectiveness of currently available therapies for advanced RCC and to judiciously pursue promising options for future RCC therapies.

Keywords

Renal cell carcinoma; Von Hippel-Lindau; Hypoxia Inducible Factor; Gene expression profiling

Introduction

With the advent of targeted therapy for various cancer types, the importance of understanding the underlying molecular pathogenesis of tumors escalated, as identifying the specific molecular pathways altered in an individual patient's tumor allow for more effective treatment selection for patients. Many notable successes for such classification schemes enhancing predictions for tumor response to specific therapy are recognized in acute leukemias, breast cancer, and non-small-cell lung cancer. In acute leukemia, DNA microarray data discovered molecular distinctions which allowed for the a priori stratification of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), which opened the door for predicting cancer subtype classification and suggested the genetic heterogeneity of cancer types[1]. In the case of breast cancer, tumors which express HER2 have been shown to be uniquely sensitive to targeting with a neutralizing therapeutic antibody, traztuzumab[2]. In non-small-cell lung cancer, mutations in the epidermal growth factor receptor (EGFR) confer sensitivity to treatment with EGFR small molecular tyrosine kinase inhibitors, erlotinib and gefitinib[3–5]. With these impressive success stories, the

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exploration of targeted therapies for cancer treatment has exploded and, due to its prominent resistance to traditional chemotherapeutic agents, renal cell carcinoma (RCC) is a prime candidate for targeted therapy.

In the United States, the incidence of RCC is currently rising, with it being the 7th leading cause of cancer in men and the 8th in women[6]. Despite the high incidence of RCC, median life expectancy remains 10 to 26 months, depending on other clinical factors. Renal cell carcinomas are separated into three main histological subtypes, including clear cell, papillary, and chromophobe. These histological subtypes are very diverse diseases and, when analyzed with molecular techniques, they actually tend to segregate into groups with distinct molecular characteristics, most likely due to subtype-specific activation of molecular pathways which also contribute the histological appearance observed in those tumor cells. Discovering these underlying molecular pathways and others which are differentially activated within histological subtypes will allow for more thorough exploration of targeted therapies for RCC and promises to improve the effectiveness of targeted therapies through stratification of which tumors will respond. In this review, we will focus on the currently available therapy for RCC, potential biomarkers and molecular classification of these therapies, development of molecular classification schemes to direct future personalized therapeutic strategies, and examination of the importance of exploring genome wide changes in RCC to further elucidate key molecular pathways and future drug development.

Current Therapy for Renal Cell Carcinoma

As suggested in the above introduction, advanced renal cell carcinoma is typically resistant to chemotherapy and currently available treatment options are limited in terms of their efficacy and response rates. While our understanding of the genetic pathways involved in the pathogenesis of RCC has vastly expanded in the past decades, available treatment strategies do not target molecules directly involved in these pathways. Instead, current therapies act by either broadly activating the immune system or inhibiting molecules located far downstream of the actual molecular insult.

Cytokine therapy

For metastatic renal cell carcinoma, immunotherapy options include high dose IL-2 and interferon. High dose IL-2 acts through the activation of cytotoxic T cells and growth of T helper cells, while interferon enhances MHC expression and antigen presentation. The use of high dose IL-2 remains an integral part of renal carcinoma therapy because of the small percentage who achieve complete responses and durable remissions with this therapy. However, this treatment is controversial due to the significant toxicities associated with its use, which include hypotension, thrombocytopenia, dyspnea, nephrotoxicity, and disorientation[7]. As a result of the significant toxicity profile of high dose IL-2, various strategies of low dose IL-2 and/or Interferon-alfa have been explored due to the decreased toxicity profile of these immunotherapy agents and potential to mimic the high dose IL-2 T cell stimulatory effects. These lower intensity therapies, however, compromise patient activity as in one comparative study in which high dose IL-2 displayed higher complete and overall response rates (8%, 25%) than a combined low dose therapy (2%, 12%)[7]. Similarly, a direct comparison of high and low dose IL-2 demonstrated response rates of 21% and 13% respectively[8]. Clearly the ability to achieve optimal drug exposure is important for attaining this responses; however, if tumor or host characteristics were available to reliably identify the subset of patients who have the greatest potential to tremendously benefit from these therapies on the basis of the underlying biology of their tumors, the enthusiasm for cytokine therapy would regain momentum. Such indicators have long been sought, and continue to be actively investigated. Due to the inconsistent response rates based on clinical criteria and substantial toxicities associated with IL-2, targeted therapies such as anti-VEGF pathway

and mTOR inhibitory agents have become the mainstays of treatment options for advanced RCC[9].

VEGF pathway directed therapy

In clear cell renal cell carcinoma, as many as 90% of cases contain mutations located in the von Hippel-Lindau tumor suppressor gene, which results in the stabilization of hypoxia inducible factors (HIF-1α and HIF-2α), a family of transcription factors that promote increased expression of hypoxia-inducible genes, including vascular endothelial growth factor (VEGF)[9]. Vascular endothelial growth factor activates tumor angiogenesis, while inhibiting dendritic cell maturation and tumor cell apoptosis[9], making it an attractive target for treatment of renal cell carcinoma, which produces abundant VEGF and is characteristically a vascular tumor. VEGF-targeted therapeutic options include VEGF neutralizing antibody and VEGF receptor tyrosine kinase inhibitors.

When compared to best supportive care in patients with advanced clear cell carcinoma, a first generation Raf kinase and VEGFR inhibitor, Sorafenib, displayed favorable results for the clinical utility of VEGF pathway-targeted therapy in patients who had previously received treatment with cytokine based therapy[10]. When Motzer, et al, compared Sunitinib, a multitargeted tyrosine kinase inhibitor of vascular endothelial and platelet derived growth factor receptors, to interferon-alfa as first line therapy in metastatic RCC, progression free survival was significantly longer in sunitinib (11 months) than in interferon-alfa (5 months) and, in their final analysis, objective response rate for sunitinib was 47% compared to 12% for interferon-alfa[11, 12]. Furthermore, sunitinib displayed a reduced toxicity profile, with patients reporting less treatment-related fatigue and better quality of life for sunitinib than for interferon-alfa[11]. Pazopanib, another VEGFR inhibitor that also inhibits platelet-derived growth factor receptor and c-Kit, significantly prolonged progression free survival when compared to placebo in a phase III study in treatment-naïve and cytokine pre-treated patients with advanced RCC (9.2 vs 4.2 months)[13]. Investigators also observed a significantly higher objective response rate of 30% with pazopanib compared to 3% with placebo, with no clinically important changes in quality of life reported for pazopanib treatment[13]. These studies have placed VEGFR directed therapy as a consistently effective treatment option for a substantial number of patients, even in largely unselected populations of clear cell histology tumors.

In addition to targeting the VEGF receptor, the humanized anti-VEGF monoclonal antibody, Bevacizumab, was shown to significantly lengthen progression free survival when combined with interferon-alfa as compared to interferon-alpha alone as first line treatment in metastatic renal cell carcinoma[9]. Therefore, these studies display that VEGF-targeted therapy provides enhances efficacy over immunotherapy options for advanced renal cell carcinoma and validates the premise of molecular targets increasing cancer treatment efficacy.

mTOR directed therapy

Therapeutic targeting of the mammalian target of rapamycin (mTOR) pathway, which promotes cell proliferation and motility, has also proven effective in the treatment of advanced RCC[14]. Despite the fact that the prevalence of mTOR pathway activation remains to fully be evaluated in RCC, treatment of advanced RCC with temsirolimus, a currently approved mTOR inhibitor, displays prolonged overall survival compared with interferon as first line therapy in poor risk patients of all histologic subtypes[15]. Another mTOR inhibitor, everolimus, improved progression free survival when compared with placebo in patients who experienced disease progression while on VEGFR targeted therapy, although the impact on overall survival is not known[16]. Therefore, while the exact mechanism of action

for mTOR therapy in RCC remains unknown, mTOR inhibitors have proven to be an effective treatment option for patients with advanced disease[14].

Clinical features to predict response to targeted therapy

Traditionally, pre-treatment clinical factors have been used to stratify patients to different risk classifications in metastatic renal cell carcinoma, and these risk assessments have proved somewhat useful for guiding therapeutic selection using evidence-based medicine practices. These factors currently include performance status, disease-free interval, number of metastatic sites, and laboratory values, such as serum corrected calcium, hemoglobin, lactate dehydrogenase, and platelet and neutrophil counts. These clinical features are applied in the preferred MSKCC risk stratification model to stratify patients with metastatic RCC into three different overall survival groups for treatment originally with immunotherapy, and more recently in selecting VEGFR versus mTOR directed therapies[17]. Specifically, patients classified as poor risk should receive temsirolimus, while good and intermediate risk patients are most effectively treated with sunitinib first line therapy[18]. As well, host features independent of the tumor may imply the greater or lesser degrees of drug activity. For VEGF targeted therapies, hypertension, a side effect caused by the therapy's mechanism of action, also serves as a clinical indicator of VEGF signaling inhibition and warrants further exploration of whether the ambulatory blood pressure can be used to optimize therapy or track tumor response to VEGF-targeted therapy[19].

As expanded understanding of molecular tumor characteristics and more advanced clinical diagnostics are developed, prolonged progression free survival and enhanced response rates are being observed. However, despite the advances in treatment of advanced renal cell carcinoma, the enhanced responses provided by these various targeted therapies remain modest and leave room for both treatment selection to optimize treatment efficiency and for therapeutic development. As such, molecular classification schema for advanced RCC also must evolve to allow for optimal treatment selection for tumors.

Molecular Classification, benefit for use of current therapy

In addition to new therapies, the ability to choose the most effective treatment for individual tumors will also increase the efficiency of first line therapy for advanced RCC. By investigating the activated molecular components of a specific tumor, appropriate therapy attacking those molecules can be utilized, providing a clear rationale for treatment selection. With the recent proliferation of targeted therapies for RCC, intensive work is underway to provide molecular biomarkers of response to these therapies, primarily anti-VEGF therapies and mTOR inhibitors. The various indicator molecules being investigated for these therapies are selected based on the mechanism of the drug against the RCC tumor biology. Since *VHL* mutation is observed in more than 70% of sporadic ccRCC, the exquisite single agent activity of VEGF-targeted therapies is attributed to loss of the pVHL E3 ubiquitin ligase complex, resulting in increased HIF-α expression and upregulation of the hypoxia inducible genes, including VEGF[9]. As such, molecular indicators that would logically predict response to VEGF-therapy are *VHL* mutation status, HIF-α expression level, and VEGF expression level.

Despite the high prevalence of *VHL* mutations in RCC, *VHL* mutation status did not clearly affect the objective response rate of tumors treated with VEGF-targeted therapies[20, 21]. In these studies, *VHL* mutation status was classified as a change in amino acid sequence, a truncated protein, or *VHL* or promoter methylation, and, when analysis included all types of these *VHL* mutations, no affect on response rate to anti-VEGF therapies was observed[20, 21]. Interestingly, Rini et al observed an increased median TTP in tumors with evidence of *VHL* methylation or *VHL* mutation that was predicted to shift the reading frame compared to

tumors with no change in *VHL* (13.3 vs. 7.4 months)[20]. Upon further classification of *VHL* mutation status, Choueiri et al. found that mutations predicted to cause loss of pVHL function had a significantly increased response rate compared with wild-type *VHL* (52% vs. 31%), suggesting that further exploration of the specific *VHL* mutation status may be warranted[21].

The modest effects of *VHL* mutations on tumor response to VEGF-targeted therapy can be attributed to many factors. First, the authors of both studies did not fully explore the extent of loss of heterozygosity (LOH) in the RCC tumor samples. While 85–98% of clear cell RCC with somatic mutation of *VHL* also exhibit loss of the other *VHL* allele, not all tumors with a *VHL* mutation necessarily experienced LOH and could possibly retain a normal *VHL* allele and, therefore, some functional pVHL[22]. In addition to further exploring the *VHL* mutation status, elucidation of the downstream effects of the mutations in *VHL* on VEGF expression remains a complicated task due to both the diverse implications of various mutations and the complicated nature of tumor biology. Indeed, the effects of *VHL* mutations on protein activity remain difficult to classify and even the point mutation R167Q, recognized to cause ccRCC, pheochromocytoma, and hemangioblastoma in the familial von-Hippel Lindau syndrome, retains HIF-α ubiquitylation activity in vitro[23]. In 40% of the mutations analyzed by Rini et al, only 1 or 2 amino acids were altered, potentially allowing the pVHL E3 ubiquitin ligase complex to retain some residual activity in these tumors[20]. *VHL* promoter methylation status also could have ambiguous effects on *VHL* expression, potentially allowing residual activity of pVHL. Finally, an elevated response rate to VEGF-targeted therapy could be observed in tumors without *VHL* mutations if these tumors exhibit *VHL*-independent activation of the HIF pathway, allowing the targeted therapy to still act on increased VEGF expression. Therefore, since *VHL* mutation status did not precisely associate with tumor response to VEGF-targeted therapy, downstream molecules more closely related to VEGF activation should be explored as potential predictors of response to VEGF-targeted therapy.

Directly downstream of pVHL, HIF-α is the next logical molecule to explore as a potential biomarker of response to anti-VEGF therapy. In fact, in contrast to *VHL* mutations, increased baseline levels of HIF-1α or HIF-2α were associated with an increased objective response rate of advanced RCC to sunitinib[24]. Despite the seeming potential of using HIF-α as predictor of response, many factors contribute to the complexity of HIF-α expression, complicating its predictive value. First, multiple forms of HIF-α exist, with HIF-1α and HIF-2α most commonly implicated in the development of RCC, as nicely summarized in a recent review[25]. While both HIF-1α and HIF-2α are induced by hypoxia and can be overexpressed in RCC, they are not functionally redundant, acting in both overlapping and distinct pathways. Proliferation signatures are preferentially induced by HIF-2α, while gluconeogenesis and profiles consisted with apoptosis are frequently induced by HIF-1α [25]. In RCC samples, HIF-1α and HIF-2α are also differentially expressed, with HIF-2α typically being considered more oncogenic than HIF-1α [25]. Therefore, when analyzing the clinical utility of HIF-α as a predictor of response to targeted therapy, the expression of both HIF-1α and HIF-2α must be measured independently, and, in situations where they are both expressed, it may be difficult to tease out which isoform is affecting the response. Further complicating the use of HIF-α in treatment selection, increased expression of HIF-α in RCC tumor samples can result from HIF-α stabilization either through *VHL* inactivation or hypoxia-mediated mechanisms, especially in a highly proliferating tumor environment. While the mechanism of increased HIF-α expression might not necessarily matter for VEGF-targeted treatment as long as the result is increased VEGF expression in vivo, the potential exists that, during nephrectomy, a hypoxic environment ensues, allowing increased HIF-α expression as an artifact of the surgical procedure and not a feature of the tumor biology. Since the HIF-α expression levels are typically analyzed by immunohistochemical

analysis of tumor samples following nephrectomy, hypoxia-induced expression during surgery could change the HIF-α expression levels from the baseline tumor values in vivo. However, despite these complexities, HIF-α expression levels have been associated with distinctions in the staging properties of advanced RCC and, as the directly upstream step to VEGF over-expression, provide a promising option for further exploration as a predictive biomarker.

As expected for VEGF-targeted therapy in advanced RCC, VEGF expression levels act as both a potential pharmacodynamic biomarker and as a very attractive indicator of response to treatment. The practical application of VEGF expression as a predictive indicator of response is apparent since increased VEGF expression could make tumors more susceptible to VEGF inhibition. Furthermore, by directly measuring VEGF levels, you eliminate the guesswork of the downstream effects of *VHL* mutation or HIF-α expression on the therapeutic target. Serum VEGF expression also serves as a very pragmatic measurement since it is easily attained from patients and can be repetitively measured over the treatment course.

In determining the utility of VEGF expression as a pharmacodynamic biomarker, two separate studies found that VEGF serum baseline levels significantly predicted progression free survival[26, 27]. Porta et al reported that patients with a baseline serum VEGF level greater than 707 pg/mL as determined by ELISA (normal range: 0–707 pg/mL) had a relative risk of 2.14 of disease progression, which proved to be significantly different from those below this threshold value [26]. Similarly, when Rini et al investigated the utility in measuring different forms of soluble VEGF (VEGF-A and VEGF-C) and soluble VEGF receptor (sVEGFR)-3, they observed that lower serum baseline levels of sVEGFR-3 and VEGF-C are associated with longer progression free survival[27]. However, VEGF-A levels are not associated with progression free survival[27]. Moreover, it remains uncertain how the circulating levels of VEGF relate to the unique biology of an individual tumor.

To investigate the potential use of VEGF expression as a treatment response indicator, Rini et al. also analyzed the effect of sunitinib on serum VEGF levels. After measuring plasma VEGF levels on days 1, 14, and 28 of cycle 1 and days 1 and 28 of cycles 2–4, they observed that sVEGFR-3 and VEGF-C decreased during therapy[27]. Also, despite no correlation between baseline levels and progression free survival for VEGF-A, plasma VEGF-A levels significantly increased during treatment with sunitinib and decreased back to near-baseline levels by the end of the off-treatment periods [27]. The observation that these VEGF proteins react differently to VEGF-targeted therapy potentially suggest different regulatory mechanisms and both of the recognized changes should be investigated to determine if a certain level of change of either protein correlates with different therapeutic response rates[27]. One caveat is that these results were potentially complicated by previous therapy with bevacizumab, which Rini, et al, recognized to affect the changes in VEGF-A and sVEGFR-3 levels in a time-dependent manner since last bevacizumab exposure[27]. This association could potentially alter the observed changes in serum VEGF levels over the course of treatment and further exploration of VEGF as an indicator of treatment response should take this effect into account. As such, to evaluate the use of VEGF expression as an indicator of response to targeted therapies, change in serum VEGF-A and VEGF-C levels over the treatment course must be further explored, potentially in patients without previous exposure to VEGF-targeted therapy.

Similar to the VEGF-targeted therapies, potential pharmacodynamic biomarkers for response to the mTOR inhibitors include important components of the specific signaling pathway. Upstream of mTOR, growth factors bind and activate a receptor tyrosine kinase in the cellular membrane, which activates the PI3K/Akt pathway, resulting in increased levels

of mTOR through inhibition of TSC2[28]. The initial steps of this cascade may be negatively regulated by PTEN, a frequently mutated tumor suppressor in many cancers. Once mTOR is activated, downstream molecules, including phospho-S6 and eIF4E, regulate protein synthesis and cell growth[28]. Interestingly, mTOR activation in RCC tumors results in increased HIF-1α expression, which subsequently increases carbonic anhydrase IX expression[28]. To elucidate which molecules in this pathway could potentially serve as treatment indicators, Cho, et al, selected specific components of this pathway to analyze in RCC tumors, including carbonic anhydrase IX, phospho-Akt, phospho-S6 ribosomal protein, and PTEN expression levels and *VHL* mutation status[28].

In their small exploratory analysis of 20 tumors from a randomized phase II trial of temsirolimus treatment, Cho, et al, observed an association between clinical response to temsirolimus and higher phospho-S6 expression; 11 of 20 tumors were classified as having high expression[28]. Clinical response was defined as partial response, minor response, or stable disease over more than 4 cycles of therapy. Of the 20 tumors, 19 were acceptable for phospho-Akt expression analysis and investigators also observed a trend towards a positive association between phospho-Akt expression and a clinical response ($p=0.07$)[28]. No association was detected between objective response or clinical benefit and carbonic anhydrase IX expression or *VHL* mutational status. Additionally, despite the downregulation of *PTEN* gene expression in 20–30% of renal cell carcinoma and its regard as a negative prognostic factor for disease-specific survival in advanced disease, no clear association was seen between clinical response and *PTEN* expression[28].

Despite the small size of this study, the association between clinical response and higher phospho-S6 expression and the trend towards an association between clinical response and phospho-Akt expression warrant further exploration. In other studies of the mTOR pathway, phospho-S6 was observed to be increased in 59% of ccRCC[29] and in vitro analysis also suggests that PI3K/Akt activation may serve as a biomarker of mTOR response[30]. Therefore, due to previous supporting evidence and the reported study by Cho, et al, a larger trial analyzing the association between expression levels and clinical response to temsirolimus could help determine the utility of phospho-S6 and phospho-Akt expression as predictive biomarkers of response to mTOR inhibitors.

The investigation of the potential molecular predictors for response to current treatment options has uncovered many potential biomarkers warranting further investigation in larger trials. However, it is also important to recognize that the tumor biology of RCC has proven to be very complex and molecular pathways discussed above are rarely affected in isolation. Therefore, these biomarkers and pathways must be analyzed together in clinical practice when optimizing treatment selection since one tumor may have activation of multiple of the above pathways. Ideally, this analysis could be combined into a classification scheme which considers the various affected pathways to devise an optimal treatment strategy for an individual patient, perhaps even utilizing combination therapy of the targeted therapies. While no such clear molecular classification scheme has proven effective yet, we are beginning to uncover potentially effective ways of molecularly classifying patients for therapeutic selection with currently available agents.

Molecular Classification, potential to direct future therapeutic strategy

Conventional histologic classification schemes incorporate broad histological subtypes of clear cell, papillary, or chromophobe based on both morphological and immunophenotyping as well as reporting on general histological features such as Fuhrman grade, appearance of necrosis, and lymphovascular invasion. These features are subjective by definition, and while they can provide an immediate assessment of tumor tendencies for aggression, many

tumors display intermediate characteristics which are ineffective in assigning tumor specific risk of recurrence or death. As we suggested in the introduction, histologically similar tumors tend to naturally segregate into subtypes based on molecular features. It is logical to infer, then, that a priori assignments of tumors into molecular subclassifications may provide a more robust means of stratifying patients for therapeutic benefit. A number of classification strategies have been developed which display a new way of looking at renal cell carcinoma and use an expanded global analysis of activated tumor biology to stratify tumors by similar molecular features. By broadly analyzing activated downstream pathways, investigators rely less upon the status of a single biomarker and can synthesize molecular expression data reflecting the net effect of multiple deregulated pathways. Ultimately, such a strategy may prove to be superior for selecting the optimal targeted approach for distinct tumor classifications, increasing therapeutic effectiveness by applying them in the correct population. Additionally, these classifications may shed light on new molecules for targeted therapy development.

The first classification scheme builds upon the known tumor biology of *VHL* mutation to stratify tumors and to investigate the resulting molecular pathways affected by differential HIF- 1α and HIF-2α expression[31]. In this analysis, Gordan, et al, stratified tumors based on their *VHL* mutation status and HIF-1α and HIF-2α expression patterns and observed three groups: ccRCCs with intact *VHL*, pVHL deficient ccRCCs expressing HIF-1α/HIF-2α (H1H2), and pVHL deficient ccRCCs expressing only HIF-2α (H2). Distinct molecular pathway activation was observed in an analysis of the different subgroups in which the numbers of tumors were balanced between subgroups. The subgroup with intact *VHL* and the H1H2 subgroup displaying enhanced Akt/mTOR and ERK/MAPK signaling and the H2 subgroup exhibiting increased levels of cMyc activity and Ki-67 overexpression, resulting in enhanced proliferation and resistance to replication stress[31]. Survival data was not available for these tumors. Since such remarkably different pathways are activated in this classification, it is predicted that subgroups would display varied responses to molecularly targeted therapies and such responses demand further exploration.

The use of gene expression profiling to classify clear cell RCC tumor subsets has been explored as a means to identify consistently distinct subclassifications. Several studies have persistently demonstrated that at least two stable subsets can be identified by hierarchical clustering, as recently reviewed[32]. One large study of 177 clear cell tumors examined differences in gene expression in relation to clinical features, identifying the existence of at least two subgroups with clear association with survival[33]. A classification scheme for clear cell RCC was recently developed to define two stable subsets based on a limited set of gene features, identified as ccA and ccB [34]. This classification schema, characterized through core molecular differences in the expression pattern using a novel computational strategy of pattern recognition, also displays independent association with survival when applied to the set of 177 tumors described above[34]. ccA tumors, associated with a better prognosis, overexpress a more pro-angiogenic profile, while ccB tumors are associated with a poorer prognosis and express a more immature and aggressive molecular phenotype of genes involved in epithelial to mesenchymal transition and cell cycle regulation[34]. Since these two subgroups of ccRCC express such vastly different molecular profiles, it naturally follows that such differences will result in them responding to differently molecularly targeted therapies. Therefore, the application of this classification scheme may have clinical utility as a predictor of response to targeted therapies and, as such, in the selection of optimal therapy. Furthermore, as these profiles and the core tumor biology driving their differences are more fully explored, key therapeutic targets for each subtype may be identified; further advancing targeted therapy for advanced RCC.

Finally, enhancing our understanding of the root (genetic) causes of kidney cancers is essential for building robust and rational targeting strategies. The lessons we have learned from targeted therapy in diseases such as breast and lung cancer have revealed that identifying and targeting tumor cell specific molecules provides the most effective methods of combating these cancers with successes measured in long-term survival. This advancement will depend on exhaustive examination of the genome and epigenome of renal cell carcinomas through systemic sequencing of the coding regions, copy number analysis, and genome wide expression arrays. Several recent studies have explored copy number variation and cancer exome sequencing using current generation technologies in clear cell RCC[35–38]. Encouragingly, trends are emerging which will enable future studies to focus on high impact genetic regions. Specifically, in addition to the loss of 3p well-associated with VHL loss, consistent losses of 4q, 6q, 8p, 9p and 14 q and gains of 1q, 5q, and 7 have been observed (Table 1). One group utilizing these methods to provide deep sequencing of over 3,000 known cancer-associated genes identified inactivating mutations in two genes that encode histone modification enzymes, *SETD2* and *JARID1C*[35]. The presence of mutations in histone modification enzymes could potentially seriously alter the gene expression in such tumor samples due to the importance of histone function in DNA condensation, which prevents transcription. In this analysis, they also saw *NF2* mutations in tumors with intact *VHL* and the involvement of other "probable cancer genes"[35]. The results of this study highlight the importance of broadening our exploration of RCC samples to genome-wide approaches since the mutations in histone modification enzymes would not have been found in a simple screen of molecules involved in the typical RCC pathogenesis pathways.

A second study that used genome-wide approaches to explore the molecular classification of ccRCC analyzed genome-wide changes of copy number variations and gene expression profiles in both VHL-disease associated and sporadic ccRCC tumors[36]. In their results for copy number variation, the group observed 14 areas of nonrandom copy-number change, with equal incidence of deletion and amplification. When determining the relevant genes in these peaks, Beroukhim, et al, identified *VHL, CDKN2A,* and *CDKN2B* as potential genes in two of the deletion peaks and *MYC* as the only gene in one of the amplification peaks[36]. For these and the remaining peaks, other candidate oncogenes were identified based on the peak location and genes known to be involved in tumorigenesis[36].

This study also used the genomic profiles to classify ccRCC and found that sporadic tumors without biallelic *VHL* inactivation segregated into a group similar to tumors with biallelic *VHL* inactivation and a group very distinct from the majority of ccRCC. The presence of tumors with intact VHL, but a similar genomic profile to tumors with biallelic *VHL* inactivation potentially suggests a *VHL* independent activation of that pathway, which could potentially explain the results seen in the previously discussed study regarding *VHL* mutation status as a predictor for response to VEGF-targeted therapy. Interestingly, VHL disease-associated tumors were more similar to a subgroup of sporadic tumors, except very homogeneous to each other within that subgroup[36]. Once again, the merits of genome-wide approaches to the molecular pathogenesis of RCC are illustrated in this study and require further inquiry to truly advance the understanding and treatment of RCC.

Conclusions

Advances in targeted therapies for advanced renal cell carcinoma over the past decade are evident in the number of currently approved therapies and those under development. Such targeted therapies confer advantages in treatment, including decreased adverse events compared with immunotherapy and the potential for a more effective therapeutic response. However, despite the recent progress in developing targeted therapies and their utility, physicians are still limited in their ability to truly optimize treatment selection for patients

due to the lack of effective molecular classification of tumors. While exhaustive research is currently being explored for molecular classification based on currently available targeted therapies and known molecular pathways, molecules identified by this approach still demand further validation as pharmacodynamic biomarkers. Molecular classification schemes also may provide great clinical advantages for treatment strategies since they typically analyze underlying biology of RCC. Finally, despite clear association with mutation in *VHL* and associated pathways, RCC is genetically heterogeneous and requires genome-wide exploration to fully ambush the tumor from all possible targets. As such, broadening the knowledge of molecular pathways important in the pathogenesis of RCC will allow further classification of tumor subtypes and choice of optimal first line therapy for individual patients.

Future Directions

Further development of targeted therapies both for currently elucidated molecular pathways involved in the pathogenesis of RCC should continue to allow advances in the clinical response to such therapies. Furthermore, new studies identifying highly associated genetic or molecular features should be considered to guide therapeutic development and biology-stratified clinical trial design. This recognition of other relevant targets and targeting them will effectively enhance drug activity and effectiveness. For both current and future molecularly targeted therapies, classifying patients into molecular risk groups will enable appropriate use of drug treatment to ensure that the most effective therapy gets to the patients with the greatest likelihood to respond. Finally, enhanced understanding of the genetic, epigenetic, and molecular features which drive kidney cancer growth and progression is needed for continued advances in therapeutic development and optimization.

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Table 1

Genomic approaches to understanding clear cell RCC.

Study	Genomic strategy	Regional deletion	Regional Gain	Genes Identified
Dalgliesh, et al. 2010	SNP array 6.0 (Affymetrix) PCR based exon resequencing	3p 4 6q 8p 9p 14q	1q 2 5q 7 12	SETD2, JARID1C, UTX, NF2
Berhoukim, et al. 2009	SNP Sty I (250K) (Affymetrix)	1p 3p 4q 6q 8p 9p 14q	1q 2q 5q 7q 8q 12p 20q	CDKN2A CDKN2B MYC
Pei, et al. 2010	SNP array 6.0 (Affymetrix) Classical Cytogenetics	1p32.2-p33 3p 3p21.31-p22.1 3p24.3-p25.3 6q23.1-qter 14q	5q 5q34-qter 7	3;5 translocation
Chen, et al. 2009	SNP array (Illumina 317K)	3p 4q28.3-qter 6q23.3-q27 8p12-pter 9q32-qter 9p13.3-pter 10q22.3-qter 13 14q24.1-qter	5q 1q25.1-qter 5q32-ter 5q35.3 7 7p 7q21.13-qter 8q24.12-qter	NRG1