Improving our understanding of papillary renal cell carcinoma with integrative genomic analysis

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Abstract: Papillary renal cell carcinoma (pRCC) is a heterogeneous and incompletely understood histologic subtype of kidney cancer. Recently, authors from The Cancer Genome Atlas Research Network performed a comprehensive molecular characterization of pRCC. Using multiple analytic methods, they identified 4 subgroups of pRCC with varied genotypic anomalies and probabilities of overall survival. This analysis elucidated the differences between type 1 and type 2 pRCC. Furthermore, type 2 pRCC was found to be heterogeneous itself, with at least 3 subtypes with distinct molecular features. This improved characterization and insight about potential driver mutations and altered pathways may lead to the development of more targeted agents and better patient stratification in clinical trials for pRCC.

Keywords: Kidney cancer; renal cell carcinoma (RCC); clear cell; papillary; non-clear cell; genomic

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Introduction

The past decade has been one of marked transformation in the management of renal cell carcinoma (RCC). The dawn of the targeted therapy era in 2006, with the introduction of sunitinib and sorafenib, fundamentally altered the management of advanced RCC (1). Numerous drugs were subsequently approved that target either the VEGF/VEGFR or mTOR pathways (2-9) (*Table 1*). Unfortunately, despite the rapid expansion of our therapeutic armamentarium, RCC continues to remain a major cause of cancer morbidity and mortality. In the United States, RCC was the seventh and tenth most common malignancy among men and women, respectively, with an estimated new 62,700 cases and 14,240 deaths in 2016 alone (11).

Papillary RCC (pRCC) accounts for approximately 15% of all kidney tumors making it the second most common histologic type of RCC (12). Until now, our understanding of the genetics and molecular biology of RCC has been primarily focused on clear cell RCC. As a result, there are no pRCC-specific FDA-approved therapies available to kidney cancer patients (13). However, in a recent publication in the *New England Journal of Medicine*, authors from The Cancer Genome Atlas (TCGA) Research Network describe a significant step towards understanding the molecular nature of pRCC (14). Identification of relevant molecular pathways and the accurate classification of pRCC subtypes are necessary to optimize clinical trial design and speed the development of novel targeted therapies.

Comprehensive molecular characterization of papillary renal cell carcinoma

Previously published next-generation sequencing studies have identified several mutated genes associated with pRCC including: *MET*, *NF2*, *SETD2*, and Nrf2 pathway genes (15,16). However, these mutations were found in only ~10–15% of pRCC tumors in these studies (15,16). The investigators of The Cancer Genome Atlas Research Network, in an attempt to improve our understanding and classification of pRCC, performed comprehensive molecular analysis, including whole-exome sequencing, identification of copy number alterations (CNAs), micro- and messenger-

Table 1 FDA-approved targeted therapies for advanced renal cell carcinoma

Therapy	Target	Treatment line	Comparison arm	Primary endpoint
Axitinib (9)	VEGFR	Second-line	Sorafenib	PFS
Bevacizumab + IFN-α (AVOREN) (2)	VEGF	First-line	Placebo + IFN-α	OS
Bevacizumab + IFN-α (CALGB) (7)	VEGF	First-line	IFN-α	OS
Everolimus (5)	mTOR	VEGFR failure	Placebo	PFS
Pazopanib (8)	VEGFR	First-line or cytokine failure	Placebo	PFS
Sorafenib (3)	VEGFR	Cytokine failure	Placebo	OS
Sunitinib (6)	VEGFR	First-line	IFN-α	PFS
Temsirolimus (4)	mTOR	First-line	IFN-α	OS

IFN, interferon; mTOR, mammalian target of rapamycin; OS, overall survival; PFS, progression-free survival; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Modified with permission from Singer *et al.* Curr Opin Oncol 2011 (10).

RNA sequencing, protein expression and DNA methylation analysis of 161 primary pRCC tumors (14).

Of these tumors, 75 were classified as papillary type 1 and 60 as type 2. As expected, the type 1 tumors were more likely to be lower grade than type 2 tumors. Analysis of CNAs resulted in the identification of three patterns: predominantly type 1 tumors with frequent gain of chromosomes 7 and 17; type 2 tumors with few CNAs; and type 2 tumors with aneuploidy, including frequent loss of chromosome 9p (14). Whole-exome sequencing identified 11 significantly mutated genes, including previously identified genes such as *MET*, *SETD2*, *NF2* and *BAP1*, among others. These mutations, many of which are part of known cancer-associated pathways, were present in a higher percentage of tumors than was reported by previous studies.

The majority of type 1 pRCC tumors (81%) had gains of chromosome 7 or altered *MET* status (mutation, gene fusion or splice variant of *MET*) (14). While these findings support the hypothesis of *MET* as a driver mutation in type 1 pRCC, it cannot be concluded from this evidence alone. Further supporting this theory, however, is the finding that levels of *MET* mRNA expression were significantly higher in type 1 tumors than type 2 tumors (14).

CDKN2A alterations were found in 21 tumors (13%) and included 25% of type 2 tumors (14). These alterations included focal loss of 9p21, mutation, or promotor hypermethylation of CDKN2A (14). Additionally, increased expression of miR-10b-5p was correlated with decreased expression of CDKN2A (14). CDKN2A altered tumors were found, on univariate analysis, to be associated with lower overall survival when compared to tumors without CDKN2A alterations (14).

A novel CpG island methylator phenotype (CIMP) was identified in nine tumors, all of which also had hypermethylation of the CDKN2A promoter (14). Eight out of 9 of these tumors were papillary type 2. CIMP-associated tumors, like FH-deficient tumors in hereditary leiomyomatosis and renal cell cancer (HLRCC), were noted to have worse survival and gene expression changes consistent with a Warburg-like shift to glycolysis-dependent metabolism (17).

A cluster-of-clusters analysis was performed using the various data types to identify pRCC subgroups (14). Four subgroups were identified (C1, C2a, C2b, and C2c) and were associated with progressively worse overall survival. C1 included primarily papillary type 1 tumors, while C2a and C2b included primarily papillary type 2. Subgroup C2c included only type 2 pRCC with CIMP-associated tumors, which had the lowest overall survival (14).

This analysis, which elucidated the complexity of pRCC and the heterogeneity of type 2 pRCC specifically, has significant implications for the design of future clinical trials and the development of targeted therapies for pRCC.

Therapies for papillary renal cell carcinoma

While all the pivotal trials leading to the approval of targeted therapies for RCC have focused on clear cell histology thus far, recent studies have investigated the optimal treatment regimens in non-clear cell RCC. Sunitinib was tested in pRCC in the SUPAP trial and was found to be active in both type 1 (median OS 17.8 mo) and type 2 (median OS 12.4 mo) pRCC (18). The RAPTOR trial evaluated everolimus as monotherapy in pRCC and

found that it was beneficial, with a median OS of 21 months and a similar difference between type 1 (median OS 28 mo) and type 2 (median OS 20 mo) (19). ASPEN (20) and ESPN (21) are two recently published phase 2 trials comparing sunitinib and everolimus as first line therapy in patients with metastatic non-clear cell RCC. Of note, there were significant differences in the trial populations—the ESPN trial included sarcomatoid clear cell RCC and 39.7% pRCC whereas ASPEN did not allow any clear cell RCC and 66% of subjects had pRCC. The ESPN trial was not able to show superiority of everolimus over sunitinib while the ASPEN trial concluded that sunitinib improved progression-free survival when compared to everolimus for non-clear cell RCC. Both trials, however, are limited by the significant heterogeneity of the non-clear cell RCC groups they studied and noted the need for improved patient stratification by molecular and genetic characteristics.

Foretinib, a multikinase inhibitor with activity against MET and VEGF receptors, among others, was evaluated in a phase 2 trial of patients with pRCC (22). Overall response was noted in 13.5% of subjects and median progression free survival (PFS) was 9.3 mo, with median OS not reached (22). Importantly, the presence of a germline *MET* mutation, but not other types of MET pathway activation, was predictive of response (22). In the TCGA analysis, only 3 of 75 type 1 pRCC tumors were found to have germline *MET* mutations, confirming this as a rare entity in sporadic cases (14). However, 81% of patients had some form of altered *MET* status and thus, MET remains a promising target in type 1 pRCC. Following this study of foretinib, multiple active trials are evaluating MET-inhibition in pRCC. One arm of EORTC 90101 (NCT01524926 "CREATE") is evaluating crizotinib, an inhibitor of MET and ALK, in type 1 pRCC. Other small molecule MET inhibitors, INC280, tivantinib (ARQ-197), and AZD6094, are being investigated in active phase 2 trials (NCT02019693, NCT01688973, and NCT02127710, respectively).

An early prospective trial specific to pRCC evaluated single agent erlotinib, an EGFR tyrosine kinase inhibitor (TKI) (23). Erlotinib monotherapy had an overall response rate of 11% and was generally well tolerated in this trial (23). Combination therapy targeting VEGFR and EGFR using bevacizumab and erlotinib was shown to have activity in familial type 2 pRCC in a retrospective study of patients with hereditary leiomyomatosis and renal cell cancer (HLRCC) (24). Prospective evaluation of this approach is ongoing in a two-arm phase 2 trial (NCT01130519) enrolling patients with HLRCC as well as sporadic pRCC (25). Interim results from this study have demonstrated activity in both subsets of patients (26). Another ongoing phase I/II study (NCT02495103) is evaluating the multikinase inhibitor vandetanib (with activity against VEGFR, EGFR, and ABL1) in combination with metformin in patients with advanced HLRCC, succinate dehydrogenase (SDH) RCC, and sporadic pRCC. The TCGA analysis found that 11.2% of pRCC tumors had a loss of 1p36, which includes a negative regulator of EGFR and is associated with EGFR amplification (14). Patients with this anomaly may be more likely to benefit from EGFR-directed therapy with the addition of erlotinib to treatment regimens.

Future directions

The identification and characterization of the aberrant pathways and multiple subtypes of pRCC by the TCGA Research Network should lead to future trials that stratify subjects by the genetic characteristics of their tumors in order to identify and validate these potential prognostic and therapeutic biomarkers. For example, studies of EGFR-directed therapy may evaluate loss of 1p36 as a biomarker of improved response rates. Germline *MET* mutations have been associated with response to MET-directed TKI therapy (22) and future studies may evaluate the use of MET pathway activation as a predictive biomarker.

Nivolumab, a programmed death 1 (PD-1) checkpoint inhibitor, was approved in November 2015 by the FDA for second-line treatment of advanced clear cell RCC after a randomized phase III trial reported improved overall survival and fewer serious adverse events when compared to everolimus (22). Current and future research will evaluate checkpoint inhibition in neoadjuvant and adjuvant settings, as first-line therapy, and in combination therapy regimens for advanced disease. The use of checkpoint inhibition in pRCC is a promising area of investigation as 10% of pRCC tumors have been shown to express programmed death-ligand 1 (PD-L1) a rate similar to the expression in clear cell RCC tumors (27,28).

Conclusions

In this era of precision oncology, the ideal therapy is one that is targeted to the specific genetic and molecular abnormalities found in a patient's tumor. TCGA Research Network investigators have made significant progress towards that goal in their elucidation of the aberrant pathways present in pRCC and in refining the

categorization of papillary type 1 and 2 tumors. Papillary RCC is a heterogeneous disease and no systemic therapy has yet been recognized as the "gold standard" for patients with pRCC. With a better understanding of the underlying biology of this disease, future investigators will be able to identify more promising agents and design trials to include patients most likely to benefit from the proposed treatment. As the results of several pRCC trials have taught us, a one-size-fits-all strategy is not likely to result in good outcomes for our patients. Now, armed with information from this TCGA study, we can design smarter trials with better agents in order to find the best treatment for each patient every time.

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