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Laboratory-Kidney cancer

Comprehensive genomic profiling of metastatic collecting duct carcinoma, renal medullary carcinoma, and clear cell renal cell carcinoma

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Abstract

Introduction and Objective: Unlike clear cell renal cell carcinoma (CCRCC), collecting duct carcinoma (CDC) and renal medullary carcinoma (RMC) are rare tumors that progress rapidly and appear resistant to current systemic therapies. We queried comprehensive genomic profiling to uncover opportunities for targeted therapy and immunotherapy.

Material and Methods: DNA was extracted from 40 microns of formalin-fixed, paraffin-embedded specimen from relapsed, mCDC ($n = 46$), mRMC ($n = 24$), and refractory and metastatic (m) mCCRCC ($n = 626$). Comprehensive genomic profiling was performed, and Tumor mutational burden (TMB) and microsatellite instability (MSI) were calculated. We analyzed all classes of genomic alterations.

Results: mCDC had 1.7 versus 2.7 genomic alterations/tumor in mCCRCC (= 0.04). Mutations in *VHL* ($P < 0.0001$) and *TSC1* ($P = 0.04$) were more frequent in mCCRCC. *SMARCB1* ($P < 0.0001$), *NF2* ($P = 0.0007$), *RB1* ($P = 0.02$) and *RET* ($P = 0.0003$) alterations were more frequent in mCDC versus mCCRCC. No *VHL* alterations in mRMC and mCDC were identified. *SMARCB1* genomic alterations were significantly more frequent in mRMC than mCDC ($P = 0.0002$), but were the most common alterations in both subtypes. Mutations to *EGFR, RET, NF2*, and *TSC2* were more frequently identified in mCDC versus mRMC. The median TMB and MSI-High status was low with <1% of mCCRC, mCDC, and mRMC having ≥ 20 mut/Mb.

Conclusion: Genomic alteration patterns in mCDC and mRMC differ significantly from mCCRCC. Targeted therapies for mCDC and mRMC appear limited with rare opportunities to target alterations in receptor tyrosine kinase and MTOR pathways. Similarly, TMB and absence of MSI-High status in mCDC and mRMC suggest resistance to immunotherapies. © 2020 Published by Elsevier Inc.

Keywords: Genetics; Deep sequencing; Collecting duct RCC; Medullary RCC; Kidney cancer

1. Introduction

Epithelial malignancies arising in the kidney display diverse histology and molecular phenotypes, with clear cell renal carcinoma (CCRCC) being the most common subtype. While lower grade and localized CCRCC are generally cured with nephrectomy, CCRCC that progress or present with metastatic disease (mCCRCC) are associated with mixed outcomes. Some patients live many months to years after developing systemic disease and others pursue an aggressive clinical course with a rapid demise [1]. The renal cell carcinomas (RCC) that arise from the renal medulla include the collecting duct carcinoma (CDC) and

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the renal medullary carcinoma (RMC). CDC is an aggressive tumor that often invades the renal sinus and, on karyotyping, features characteristic gains at 13q and losses at 1p, 8p, 9p, and 16p [2]. Similar to CDC, RMC is also an aggressive form of RCC with shortened patient survival when compared with classic CCRCC [3, 4]. RMC is typically devoid of chromosomal losses or gains on karyotyping, but often features an inactivating *SMARCB1 (SWI/SNF)* genomic alteration. RMC is generally restricted to black patients, predominantly young men, and is clearly associated with sickle cell trait [3, 4]. In contrast with CCRCC, both CDC and RMC are associated with a rapid progression to metastatic disease (mCDC and mRMC) which is typically refractory to conventional chemotherapy and radiotherapy [5, 6]. The purpose of this comprehensive genomic profiling (CGP) study is to compare the molecular features of mCDC to separate cohorts of mRMC and mCCRCC, and uncover opportunities for targeted therapy and immunotherapy for these rare life-threatening malignancies.

2. Methods

DNA was extracted from 40 microns of formalin-fixed, paraffin-embedded samples from 46 cases of mCDC, 24 cases of mRMC, and 626 cases of mCCRCC. CGP was performed on hybridization-captured, adaptor ligation-based libraries for up to 315 cancer-related genes. CGP was performed in a Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited laboratory (Foundation Medicine, Cambridge, MA). Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817). The pathologic diagnosis of each case was confirmed on routine hematoxylin and eosin stained slides and all samples forwarded for DNA extraction contained a minimum of 20% tumor nuclear area, compared with benign nuclear area. In brief, ≥ 50 ng DNAs was extracted from 40 microns of tumor samples in formalin-fixed, paraffin-embedded tissue blocks. The samples were assayed by CGP using adaptor-ligation and hybrid capture performed for all coding exons from 287 (version 1) to 315 (version 2) cancer related genes plus select introns from 19 (version 1) to 28 (version 2) genes frequently rearranged in cancer. Sequencing of captured libraries was performed using the Illumina HiSeq technology to a mean exon coverage depth of >500X, and resultant sequences were analyzed for all classes of genomic alterations including short variant alterations (base substitutions, insertions and deletions), copy number alterations (focal amplifications and homozygous deletions), and select gene fusions/rearrangements, as previously described [7]. Germline variants documented in the dbSNP database (dbSNP142; http://www.ncbi.nlm.nih.gov/SNP/), with 2‘ or more counts in the ExAC database (http://exac.broadinstitute.org/), or recurrent variants of unknown significance that were predicted by an internally developed algorithm (28) to be germline were removed, with the exception of known driver germline events (e.g., documented hereditary *BRCA1/2* and deleterious *TP53* mutations). Known confirmed somatic alterations deposited in the Catalog of Somatic Mutations in Cancer were highlighted as biologically significant [8]. All inactivating events (i.e., truncations and deletions) in known tumor suppressor genes were also called as significant. To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a ≥5% mutant allele frequency, indels with a ≥10% mutant allele frequency with ≥99% accuracy, and fusions occurring within baited introns/exons with >99% sensitivity [9]. Tumor mutational burden (TMB) was determined on 1.1 megabases (Mb) of sequenced DNA for each case based on the number of somatic base substitution or indel alterations per Mb after filtering to remove known somatic and deleterious mutations as previously described [10].

3. Results

3.1. mCDC versus mRMC

As shown in Table 1, the mCDC patients were significantly older and more frequently male than the 24 patients with mRMC. When the information was available from the patient records, sickle cell trait was identified in 10% of mCDC but was uniformly (100%) associated with mRMC ($P < 0.0001$). All (100%) of mCDC and mRMC in this comparative analysis were clinically advanced Stage III and IV tumors. Similar to the mCCRCC and mCDC cohorts, all mRMC cases were intermediate (Grade 3) or high grade (Grade 4). Also similar to mCDC, sequencing of the mRMC tumors revealed a low genomic alterations/tumor frequency and there were no *VHL* alterations. *SMARCB1* genomic alterations were significantly more frequent in mRMC than mCDC ($P = 0.0002$), but the most common alteration in both tumor types (Table 1, Fig. 1A and 1B). Potential therapy to kinases (*EGFR*, *RET*) and MTOR pathway targets (*NF2*, *TSC2*) were more frequently identified in mCDC versus mRMC (Table 1, Fig. 1A and 1B). At 1.8 mut/Mb, the median TMB was low for both subtypes with no (0%) mRMC cases showing ≥20 mut/Mb. Similar to mCDC, none (0%) of the mRMC cases featured MSI-high status.

3.2. mCDC versus mCCRCC

As seen in Table 2, 70% ($n = 32$) of the 46 mCDC patient group were male with a median age of 55 years (range 28 −90 years). Similarly, 72% of the 626 mCCRCC patient cases were male with a median age of 59 years. All (100%) of the mCDC and mCCRCC were clinically advanced Stage IV tumors at the time of sequencing with the primary CDC used for CGP in 70% of cases and a metastasis biopsy was sequenced in 30%. All (100%) of the mCDC were

Table 1
Comparative clinical and genomic features of metastatic collecting duct carcinoma (mCDC) and metastatic renal medullary carcinoma (mRMC)

Genomic Alteration	mCDC (n=46 cases)	mRMC (n=24 Cases)	Significance[a] p-value
Median age (years)	55	29	$P < 0.0001$
Gender	70% male	46% male	NS
Sickle Trait status when known	10%	100%	$P < 0.0001$
Mean Alteration/tumor	1.6	1.8	NS
VHL	0%	0%	NS
SMARCB1	19%	67%	$P = 0.0002$
NF2	14%	8%	NS
FBXW7	8%	8%	NS
CDKN2A	8%	12%	NS
EGFR	5%	0%	NS
RET	5%	0%	NS
TP53	5%	17%	NS
TSC2	3%	0%	NS
TMB Median (mut/Mb)	1.8	1.8	NS
TMB ≥ 10 mut/Mb	5%	0%	NS
TMB ≥ 20 mut/Mb	0%	0%	NS
MSI-High	0%	0%	NS

MSI = microsatellite instability; TMB = tumor mutational burden.
[a] Significance defined as *P*-value < 0.05.

Table 2
Comparative clinical and genomic features of metastatic collecting duct carcinoma (mCDC) and metastatic clear cell renal cell carcinoma (mCCRCC)

Genomic Alteration	mCDC ($n = 46$ cases)	mCCRCC ($n = 626$ Cases)	Significance[a] *P*-value
Median age (years)	55	59	NS
Gender	70% male	72% male	NS
Mean alteration/tumor	1.6	2.7	0.04
VHL	0%	78%	<0.0001
SMARCB1	19%	1%	<0.0001
NF2	14%	2%	0.0007
RB1	5%	1%	0.02
EGFR	5%	3%	NS
RET	5%	0%	0.0003
TP53	5%	12%	NS
TSC1	0%	8%	0.04
TSC2	3%	1%	NS
TMB Median (mut/Mb)	1.8	2.7	NS
TMB ≥ 10 mut/Mb	5%	< 1%	NS
TMB ≥ 20 mut/Mb	0%	< 1%	NS
MSI-High	0%	< 1%	NS

MSI = microsatellite instability; TMB = tumor mutational burden.
[a] Significance defined as *P*-value < 0.05.

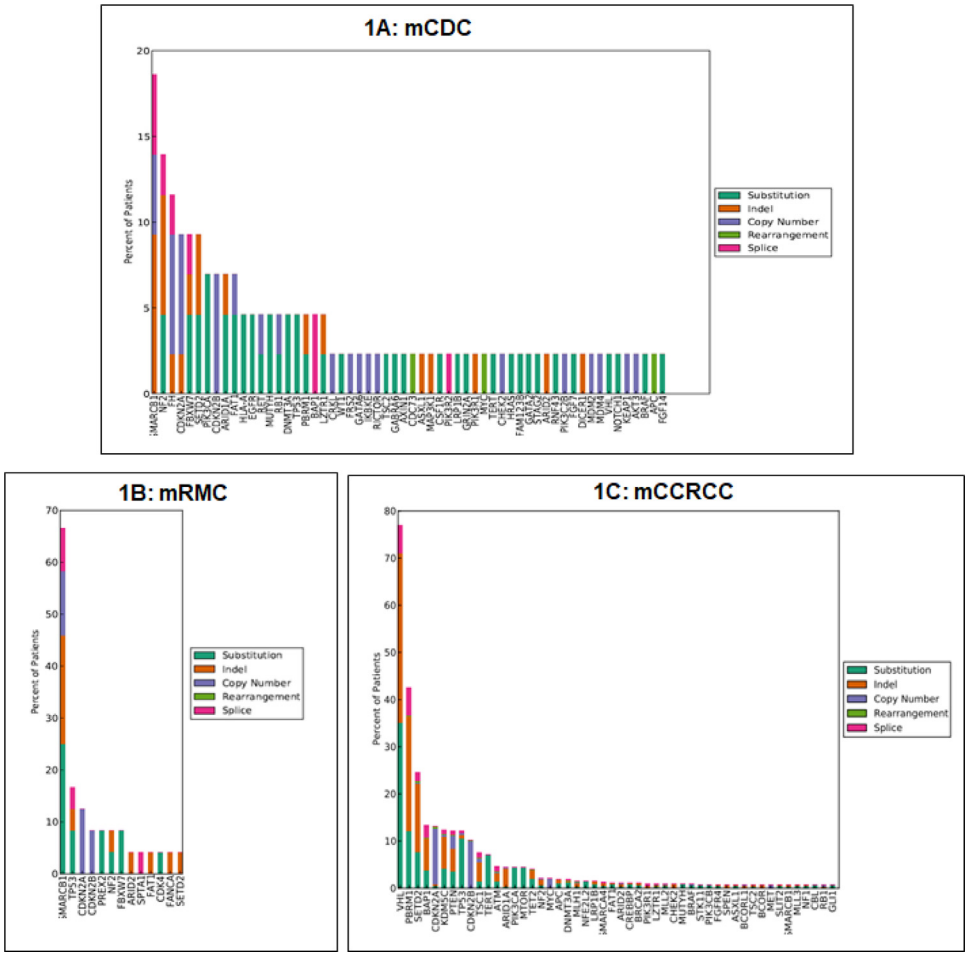

Figure 1. LongTail Plots of genomic alterations in metastatic collecting duct carcinoma (mCDC) (1A), metastatic renal medullary carcinoma (mRMC) (1B), metastatic clear cell renal cell carcinoma (mCCRCC) (1C).

intermediate (Grade 3) or high grade (Grade 4). mCDC cases had a lower genomic alteration/tumor frequency of 1.8 compared to 2.7 in mCCRCC ($P = 0.04$) (Table 2). Genomic alterations in the *VHL* ($P < 0.0001$) and *TSC1* ($P = 0.04$) genes were more frequent in mCCRCC versus *SMARCB1* ($P < 0.0001$), *NF2* ($P = 0.0007$), *RB1* ($P = 0.02$), and *RET* ($P = 0.0003$) in mCDC (Table 2, Fig. 1A and 1**C**). No significant difference in alterations to *TP53*, *EGFR* and *TSC2* were noted. The median TMB was low for both tumor types (1.8 mutations/Mb in mCDC and 2.7 mutations/Mb in mCCRCC) with very low frequencies of tumors with ≥20 mut/Mb (0% for mCDC and <1% for mCCRCC). No (0%) mCDC cases had MSI-high status versus < 1% in mCCRCC.

4. Discussion

In this study, both mCDC and mRMC show significant differences in genomic alterations versus mCCRCC patients. The mCCRCC are predominantly driven by an inactivating alteration in the VHL gene, a finding not frequently identified in mCDC or mRMC. The mCCRCC cohort has a higher alteration/tumor frequency than mCDC or mRMC. Compared to mCDC, mCCRCC, and mRMC have alterations in potential kinase targets such as *EGFR* and *RET*.

Drugs targeting the MTOR pathways have been approved for the treatment of RCC since 2009 [11, 12]. In the current study, potential biomarkers predictive of MTOR inhibitor efficacy were identified in all 3 tumor types (8% *TSC1* in mCCRCC, 14% *NF2*, and 3% *TSC2* in mCDC and 8% *NF2* in mRMC). Based on strong clinical evidence from multiple case reports and extensive preclinical evidence, *NF2* inactivation may predict sensitivity to mTOR inhibitors, including approved agents such as everolimus and temsirolimus [13, 14]. *NF2* inactivation may predict sensitivity to MEK inhibitors, such as approved agents trametinib and cobimetinib.

This study compares genomic features of mCDC and mRMC. While all mRMC cases were associated with sickle cell trait, sickle cell trait was also identified in 10% of mCDC patients. Although a disease defining association for mRMC, positive sickle cell trait status has been previously reported in mCDC [9]. Routine histology and immunohistochemistry have delineated clear pathologic differences between CDC and RMC, yet similar anatomic locations and high-grade patterns create overlap that can influence the precision of the pathologic diagnosis [1, 9, 15, 16]. The high frequencies of genomic alterations in the *SMARCB1* gene in mCDC (19%) and mRMC (67%) confirm genomic overlap. The SMARCB1 protein, also known as INI-1, is completely lost in a variety of tumor types, including atypical teratoid rhabdoid tumors, epithelioid sarcomas, schwannomas, synovial sarcomas and RMC [17-19]. *SMARCB1/INI1* functions as a tumor suppressor gene and is a core subunit of the ATP−dependent SWI/SNF chromatin-remodeling complex [19]. Attempts to target *SMARCB1/INI1* with targeting agents is currently in early stage clinical development [19, 17, 18]. Therapeutic approaches include the EZH2 inhibitor EPZ-6438 (E7438), hedgehog inhibitors, Cdk inhibitors, and/or Aurora kinase inhibitors [20-22].

Numerous studies have confirmed the aggressive and conventional therapy-resistant nature of mCDC and mRMC whether treated by cytotoxic chemotherapy or nontargeted multikinase inhibitors approved for RCC treatment such as sorafinib and sunitinib [23-25]. Given the approved indication for immune checkpoint inhibitors (ICPI) for the treatment of mCCRCC, interest has for non-clear cell renal cancer [26, 24]. The efficacy of ICPI for mCCRCC is unusual in that standard biomarkers predictive of responses are essentially negative in RCC [27]. In the current study, both the TMB and MSI biomarkers included in the CGP assay failed to identify significant subsets of mCDC and mRMC that would likely benefit from ICPI treatments. However, given that this is also the case for mCCRCC, it is possible that both mCDC and mRMC will respond to ICPI without a significant neo-antigen production associated with MSI-High and high TMB status. Case reports have noted a response to ICPI for mCDC tumors with elevated programmed death-ligand 1 (PD-L1) expression [28, 29]. One group even reports a complete response to nivolumab and ipilimumab [30]. While this data is promising, clinical outcome studies are needed to confirm benefit from immunotherapies

There are several limitations of this study. First, despite being among the largest sequenced series in the literature, the mCDC and mRMC cohorts are still relatively small. Second, the sequencing was performed on the tissue samples submitted to the reference laboratory and it is possible that heterogeneity of tumor was not completely reflected in the tested aliquot. Nevertheless, the driver mutations seem to be present as truncal events even in branched tumoral evolution and would likely be identified in the sequencing of any part of the tumor. Third, while the panel of the 315 genes is quite comprehensive, it is possible that there may be other important genes that were simply not included in the testing panel, thus limiting our findings. Fourth, the PD-L1 biomarker for ICPI response was not tested. Despite assessments of TMB and MSI status, PD-L1 expression is a strong predictor for response to therapy. Lastly, the clinical benefit of these findings has yet to be determined. This study provides guidance on specific systemic therapies that may improve clinical outcomes among patient subgroups with significant therapeutic unmet needs. Future studies or trials implementing these therapies are needed to confirm the clinical relevance and benefit.

In summary, in addition to their histologic differences, the frequencies and types of genomic alterations seen in both mCDC and mRMC differ significantly from that seen in mCCRCC. The opportunities for biomarker driven targeted therapies for both mCDC and mRMC appear limited with only rare opportunities to target receptor tyrosine kinase and MTOR pathways for mCDC. Similarly, the

relatively low TMB and absence of MSI-High status in both mCDC and mRMC predict that these tumors may be resistant to immunotherapies. However, further studies assessing PD-L1 expression should be explored.

5. Conclusion

Relatively low TMB and absence of MSI-High status in both mCDC and mRMC predict that these tumors may be resistant to immunotherapies. Yet, genomic sequencing may offer some opportunities in targeting tyrosine kinase receptors and MTOR pathway.

References

[1] Hsieh JJ, Purdue MP, Signoretti S, et al. Renal cell carcinoma. Nat Rev Dis Primers 2017;3:17009. https://doi.org/10.1038/nrdp.2017.9.

[2] Dimopoulos MA, Logothetis CJ, Markowitz A, et al. Collecting duct carcinoma of the kidney. Br J Urol 1993;71(4):388–91. https://doi.org/10.1111/j.1464-410x.1993.tb15978.x.

[3] Davis CJ Jr., Mostofi FK, Sesterhenn IA. Renal medullary carcinoma. The seventh sickle cell nephropathy. Am J Surg Pathol 1995;19(1):1–11. https://doi.org/10.1097/00000478-199501000-00001.

[4] Shetty A, Matrana MR. Renal medullary carcinoma: a case report and brief review of the literature. Ochsner J 2014;14(2):270–5.

[5] Vera-Badillo FE, Templeton AJ, Duran I, et al. Systemic therapy for non-clear cell renal cell carcinomas: a systematic review and meta-analysis. Eur Urol 2015;67(4):740–9. https://doi.org/10.1016/j.eururo.2014.05.010.

[6] Abern MR, Tsivian M, Polascik TJ, et al. Characteristics and outcomes of tumors arising from the distal nephron. Urology 2012;80(1):140–6. https://doi.org/10.1016/j.urology.2012.03.034.

[7] Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotechnol 2013;31(11):1023–31. https://doi.org/10.1038/nbt.2696.

[8] Forbes SA, Beare D, Gunasekaran P, et al. COSMIC: exploring the world's knowledge of somatic mutations in human cancer. Nucleic Acids Res 2015;43(Database issue):D805–11. https://doi.org/10.1093/nar/gku1075.

[9] Yip D, Steer C, al-Nawab M, et al. Collecting duct carcinoma of the kidney associated with the sickle cell trait. Int J Clin Pract 2001;55(6):415–7.

[10] Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. Genome Med 2017;9(1):34. https://doi.org/10.1186/s13073-017-0424-2.

[11] Kwiatkowski DJ, Choueiri TK, Fay AP, et al. Mutations in TSC1, TSC2, and MTOR are associated with response to rapalogs in patients with metastatic renal cell carcinoma. Clin Cancer Res 2016;22(10):2445–52. https://doi.org/10.1158/1078-0432.Ccr-15-2631.

[12] Voss MH, Molina AM, Motzer RJ. mTOR inhibitors in advanced renal cell carcinoma. Hematol Oncol Clin North Am 2011;25(4):835–52. https://doi.org/10.1016/j.hoc.2011.04.008.

[13] Petrilli AM, Fernandez-Valle C. Role of Merlin/NF2 inactivation in tumor biology. Oncogene 2016;35(5):537–48. https://doi.org/10.1038/onc.2015.125.

[14] Tolcher AW, Bendell JC, Papadopoulos KP, et al. A phase IB trial of the oral MEK inhibitor trametinib (GSK1120212) in combination with everolimus in patients with advanced solid tumors. Anna Oncol 2015;26(1):58–64. https://doi.org/10.1093/annonc/mdu482.

[15] Gupta R, Billis A, Shah RB, et al. Carcinoma of the collecting ducts of Bellini and renal medullary carcinoma: clinicopathologic analysis of 52 cases of rare aggressive subtypes of renal cell carcinoma with a focus on their interrelationship. Am J Surg Pathol 2012;36(9):1265–78. https://doi.org/10.1097/PAS.0b013e3182635954.

[16] Karakiewicz PI, Trinh QD, Rioux-Leclercq N, et al. Collecting duct renal cell carcinoma: a matched analysis of 41 cases. Eur Urol 2007;52(4):1140–5. https://doi.org/10.1016/j.eururo.2007.01.070.

[17] Agaimy A. The expanding family of SMARCB1(INI1)-deficient neoplasia: implications of phenotypic, biological, and molecular heterogeneity. Adv Anat Pathol 2014;21(6):394–410. https://doi.org/10.1097/pap.0000000000000038.

[18] Margol AS, Judkins AR. Pathology and diagnosis of SMARCB1-deficient tumors. Cancer Genet 2014;207(9):358–64. https://doi.org/10.1016/j.cancergen.2014.07.004.

[19] Kohashi K, Oda Y. Oncogenic roles of SMARCB1/INI1 and its deficient tumors. Cancer Sci 2017;108(4):547–52. https://doi.org/10.1111/cas.13173.

[20] Smith ME, Cimica V, Chinni S, et al. Therapeutically targeting cyclin D1 in primary tumors arising from loss of Ini1. PNAS 2011;108(1):319–24. https://doi.org/10.1073/pnas.0913297108.

[21] Lee S, Cimica V, Ramachandra N, et al. Aurora A is a repressed effector target of the chromatin remodeling protein INI1/hSNF5 required for rhabdoid tumor cell survival. Cancer Res 2011;71(9):3225–35. https://doi.org/10.1158/0008-5472.Can-10-2167.

[22] Staehler M, Haseke N, Schoppler G, et al. Carcinoma of the collecting ducts of Bellini of the kidney: adjuvant chemotherapy followed by multikinase-inhibition with sunitinib. Eur J Med Res 2008;13(11):531–5.

[23] Shah AY, Karam JA, Malouf GG, et al. Management and outcomes of patients with renal medullary carcinoma: a multicentre collaborative study. BJU Int 2017;120(6):782–92. https://doi.org/10.1111/bju.13705.

[24] Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med 2015;373(19):1803–13. https://doi.org/10.1056/NEJMoa1510665.

[25] Kroeger N, Xie W, Lee JL, et al. Metastatic non-clear cell renal cell carcinoma treated with targeted therapy agents: characterization of survival outcome and application of the International mRCC Database Consortium criteria. Cancer 2013;119(16):2999–3006. https://doi.org/10.1002/cncr.28151.

[26] Carlo MI, Chaim J, Patil S, et al. Genomic characterization of renal medullary carcinoma and treatment outcomes. Clin Genitourin Cancer 2017;15(6):e987–e94. https://doi.org/10.1016/j.clgc.2017.04.012.

[27] Maia MC, Yang ES, Agarwal N, et al. A step towards predicting checkpoint inhibitor response in kidney cancer. Lancet Oncol 2017;18(8):982–3. https://doi.org/10.1016/s1470-2045(17)30533-8.

[28] Mizutani K, Horie K, Nagai S et al. Response to nivolumab in metastatic collecting duct carcinoma expressing PD-L1: A case report. Molecular and clinical oncology. 2017;7(6):988-90. doi:10.3892/mco.2017.1449.

[29] Yasuoka S, Hamasaki T, Kuribayashi E, et al. Nivolumab therapy for metastatic collecting duct carcinoma after nephrectomy: A case report. Medicine (Baltimore) 2018;97(45). https://doi.org/10.1097/MD.0000000000013173:e13173-e.

[30] Watanabe K, Sugiyama T, Otsuka A, et al. Complete response to combination therapy with nivolumab and ipilimumab for metastatic collecting duct carcinoma of the kidney. International cancer conference journal 2020;9(1):32–5. https://doi.org/10.1007/s13691-019-00389-z.