Comprehensive profiling of renal medullary and collecting duct carcinomas

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Abstract #572

Background: Renal medullary carcinoma (RMC) is an aggressive malignancy affecting predominantly young African Americans with sickle cell trait (SCT) or disease (SCD), while a pathologically similar collecting duct carcinoma (CDC) affects patients without sickle cell trait. Clinical responses to chemotherapy and IL-2 in RMC/CDC are poor and novel therapies are needed.
Design: 9 patients with RMC (ages 13-58 y. o., all male) and 15 patients (ages 26-74 y. o., M:F=13:2) with collecting duct carcinoma (CDC) were studied. Expression of PD-L1 was evaluated with 2 monoclonal antibodies (SP142 and SP263) and tumor infiltrating lymphocytes (TIL) were evaluated for PD1 expression (MRQ-22 antibody) using immunohistochemistry (IHC). Additional studies included ALK protein expression (D5F3 antibody), gene translocation (break apart FISH), next generation sequencing (NGS), and microsatellite instability (MSI).
Results: Cancer cell PD-L1 expression above the threshold (≥2+, ≥5%) was seen in 7/9 RMC and 5/13 CDC cases. Concordance between 2 PD-L1 antibodies was 94.4%. PD-1+ TIL were absent in 6/18 cases and variably present in 12/18 cases (from 1 to >15 TIL/40x power field). No MSI was detected in any of the cases tested (0/6). No case expressed ALK protein, but one case of CDC showed *ALK* gene re-arrangement. Mutations were identified in *SMARCB1, FH, TP53 (3x), ATM, BRCA2, CHEK2 (2x), NF2 (3x), SETD2,* and *CDKN2A*. No mutations in *VHL* or *KDR* were detected. One patient with RMC (and SCT) achieved complete clinical remission after treatment with bevacizumab plus paclitaxel.
Conclusion: RMC and CDC strongly express PD-L1 in the majority of cases (12/22), suggesting that these patients may benefit from targeting the PD-L1/PD1 interaction. The absence of MSI in these cancers indicates a different mechanism of PD-L1 upregulation from colorectal carcinomas. Consistent with our previous study that showed frequent activation of (pseudo)hypoxia-induced pathways in RMCs (Human Pathology 2011;42:1979), we describe a case of RMC successfully treated with anti-VEGF therapy.

Methods

Formalin-fixed paraffin-embedded (FFPE) tissues from 24 patients with either RMC (n=9) or CDC (n=15) were investigated for the expression of PD-1 (TIL) and PD-L1 (neoplastic cells) using automated immunohistochemical methods at a CAP/CLIA-certified lab (Caris Life Sciences, Phoenix, AZ). Antibodies, vendors, and corresponding thresholds were as follows:

Biomarker	Antibody (Vendor)	Threshold
PD-1	MRQ-22 (Ventana)	> 1 TILs/hpf*
PD-L1	SP142 (Ventana)	2+ or 3+ intensity in ≥5% of malignant cells
	SP263 (Ventana)	

** TILs/hpf = tumor infiltrating lymphocytes per high powered field*

Other potentially theranostic biomarkers were evaluated by immunohistochemistry (IHC), fluorescence or chromogenic in situ hybridization (FISH or CISH), and/or microsatellite stability (MSI) testing. Next generation sequencing (NGS) was performed when enough tissue was available.

Results

Tumor	Median Age (Age Range)	Gender (Male:Female)	Ethnicity*
RMC	27 (13-58)	9:0	AA (7/9), non-AA (2/9)
CDC	61 (26-74)	13:2	NA

**AA – African American NA – Not Available*

Table 1 – Demographic information on RMC and CDC specimens. Details on the specimens are shown above. Most specimens analyzed were from male patients, with African-Americans comprising the majority of confirmed RMC (77.8%, 7/9). Four of the AA profiled had the sickle cell trait, two had documented sickle cell disease, and one had an unknown history of either disease.

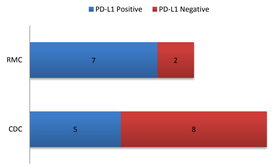

Figure 1 – PD-L1 by IHC in RMC and CDC using SP142 antibody. Shown on the left are the number of patients staining positive (blue) and negative (red). Both renal cell carcinomas exhibited staining, with 77.8% (7/9) in RMC and 38.5% (5/13) in CDC.

Specimen	SP142 Result	SP263 Result
RMC#1	Positive (2+/70%)	Positive (2+/50%)
RMC#2	Positive (2-3+/70%)	Positive (2+/70%)
RMC#3	Negative (2+/1%)	Negative (2+/1%)
RMC#4	Positive (2-3+/100%)	Positive (2-3+/100%)
RMC#5	Negative (2+/2%)	Negative (2+/2%)
RMC#6	Positive (3+/20%)	Positive (2+/10%)
RMC#7	Positive *(3+/90%)	Negative (0/100%)
CDC#1	Negative (0/100%)	Negative (0/100%)
CDC#2	Negative (<1%)	Negative (<1%)
CDC#3	Negative (<1%)	Negative (<1%)
CDC#4	Negative (0/100%)	Negative (0/100%)
CDC#5	Positive (3+/80%)	Positive (3+, 80%)
CDC#6	Negative (0/100%)	Negative (0/100%)
CDC#7	Negative (0/100%)	Negative (0/100%)
CDC#8	Positive (2+/5%)	Positive (3+/30%)
CDC#9	Positive (3+/100%)	Positive (3+/100%)
CDC#10	Positive (2+/5%)	Positive (2+/60%)
CDC#11	Negative (2+/<1%)	Negative (2+/<1%)

Table 2 - Concordance between two PD-L1 IHC antibody clones. Eighteen RMC and CDC specimens were also stained with the SP263 antibody for comparison with the SP142 antibody. Scores are shown in the figure on the left. Scoring was either positive or negative using the aforementioned threshold. Staining intensity was either 0 (absent), 1+ (weak), 2+ (fair), or 3+ (strong). Percent values corresponds to percent tumor membrane staining. A concordance analysis showed 94.4% (17/18) agreement. * The sole discordant case RMC#7 had un-characteristic cytoplasmic staining using SP142 antibody.

Tumor	Positive
RMC	57.1% (4/7)
CDC	72.7% (8/11)

Table 3 – PD-1 by IHC in RMC, CDC. Overall, a high percentage of RMC and CDC displayed PD-1 expression, a biomarker for T-cell tumor infiltrating lymphocytes (TILs).

Results (continued)

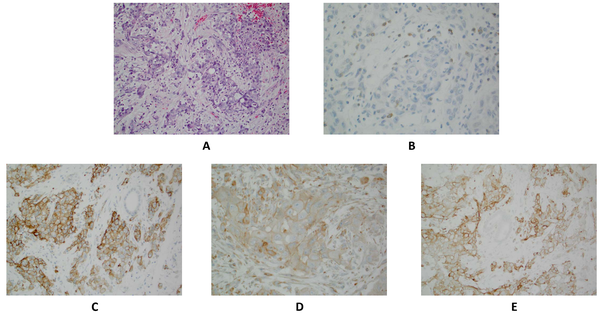

Figure 2 – Renal medullary carcinoma (RMC) Immunohistochemical images. Shown above are images of a patient with RMC including H&E (A), PD-1 at 20X (B), PD-L1 SP142 at 20X (C), PD-L1 SP142 at 40X (D), and PD-L1 SP263 at 20X (E).

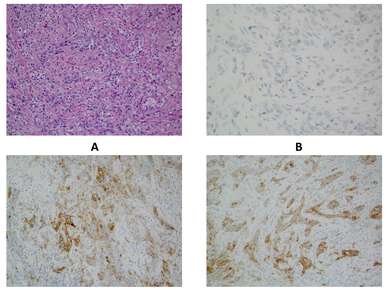

Figure 3 – Collecting duct carcinoma (CDC) Immuno-histochemical images. Shown to the left are images of a patient with collecting duct carcinoma including H&E (A), PD-1 at 20X (B), PD-L1 SP142 at 20X (C), and PD-L1 SP263 at 20X (D).

Test/Methodology	Number Tested	Results
MSI (Fragment Analysis)	6	0.0% (0/6)
ALK rearrangement (FISH)	7	14.3% (1/7)
ALK rearrangement (IHC)	11	0.0% (0/11)
MET amplification (CISH)	6	0.0% (0/6)
PTEN loss (IHC)	9	22.2% (2/9)

Table 4 – Multiplex testing outside of Next Generation Sequencing (NGS). Additional tests on the cohort are shown above. MSI (microsatellite instability) was not detected. Although no ALK rearrangement was detected by IHC, ALK rearrangement was identified by FISH in one CDC specimen.

Results (continued)

RMC and CDC Pathogenic and Presumed Pathogenic Mutations by NGS
BRCA2, CDKN2A, CHEK2, FH, SMARCB1, TP53
RMC and CDC Variant of Unknown Significance Mutations by NGS
APC, ATM, BRCA1, BRCA2, CDKN2A, CSF1R, ERBB2 (HER2), KIT, KDR, NOTCH1, RET, SRC, TP53

Table 5 – Analysis of RMC and CDC using Next Generation Sequencing. Pathogenic (i.e. tumorigenic), presumed pathogenic, and variant of unknown significance results are shown.

Update on case report of a patient with renal medullary carcinoma successfully treated with anti-VEGF therapy (*Lipkin 2015*).

A 27 year-old AA male with metastatic renal medullary carcinoma (diagnosed in July 2013) was treated with induction PCG (paclitaxel + cisplatin + gemcitabine) following an earlier left radical nephrectomy. Given the tumor's rarity and no standard of care, genomic profiling was requested, which showed PTEN deficiency and low protein expression of RRM1, implying a potential benefit to mTOR inhibitors and gemcitabine, respectively. Patient was then administered everolimus (in December 2013) and maintained complete remission (CR). Secondary to elevated ALT and AST and, later, PD in June 2014, everolimus was discontinued. Since this time, the patient has been treated with gemcitabine and, later, paclitaxel plus bevacizumab. On a recent CT scan in December 2015, the patient continued to show no identifiable disease.

Conclusions

- PD-1 and PD-L1 expression detected in both RMC and CDC implies potential utility of immune checkpoint inhibitors in these diseases.
- A multiplatform approach can identify various potential targets in these orphan diseases.
- Patients with RMC and sickle cell disease may benefit from anti-angiogenic therapy despite lack of VHL or KDR mutations.
- Studies with larger cohorts and treatment outcomes are warranted in these rare malignancies.

References

1. Topalian, SL, DM Pardoll, *et al.* (2015). "Immune checkpoint blockade: a common denominator approach to cancer therapy". *Cancer Cell*. 27:450-461.
1. Lipkin, J.S., M. Joshi, et al. (2015). "Therapeutic approach guided by genetic alteration use of MTOR inhibitor in renal medullary carcinoma with loss of PTEN expression". Cancer Biol Ther. 16(1):28-33.
2. Pal, S.K., J.S. Ross, et al. (2015). "Characterization of clinical cases of collecting duct carcinoma of the kidney assessed by comprehensive genomic profiling". Eur Urol. doi:10.1016/j.eururo.2015.06.019.