MP80-01: Radioproteomic analysis as a potential predictor of renal tumor histopathology

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Background

- Small renal masses (SRMs) are defined as renal tumors that measure ≤4cm in diameter. Standard of care is partial nephrectomy. However, 30.3% and 27.1% of ≤2 and 4cm SRMs, respectively, turn out to be benign tumors.
- Cancer specific survival (CCS) reported in ccRCC, papillary and chromophobe tumors who are surgically treated is 71%, 91% and 88%, respectively at 5 years. CSS at 20 years for ccRCC, papillary and chromophobe tumors is 52%, 83% and 81%, respectively.
- SRMs have demonstrated a growth rate of 0.22cm/year and a metastatic progression rate ranging from 1 to 6%.
- Alternative approaches including surveillance have been proposed for SRMs, predominantly in elderly and/or comorbid patients. There is currently a lack of understanding in the underlying biology within the tumor microenvironment.
- Renal biopsy is the method currently used to evaluate pre-operatively SRMs. However, the reported negative predictive value is 68%. Moreover, the rate of Clavien-Dindo ≥2 complications is 2%.
- Multiparametric MRI models offer a promising improvements to non-invasive diagnostics, currently they have shown suboptimal performance compared to renal biopsies.
- We consider that a radio-proteomic analysis may be an effective approach to identify those cases that will benefit of a more conservative approach and will provide biological information about the anti-tumor response.

Approach

- Blood (n=19) and blood-matched urine samples (n=11) were obtained from patients the same day of surgery at the time of admission to the hospital. Urine and blood samples were spun down (2000RPM, 10 min, 4C) to obtain serum and urine supernatant.
- We used the Olink Proteomics inflammation panel of 92 inflammation-markers. Proteins were detected through matched pair antibodies, coupled to unique, partially complementary oligonucleotides, and measured by qualitative real-time PCR. This DNA-coupled method provides specificity, excluding any antibody cross-reactivity.
- Multiparametric MRIs were done during the preoperative evaluation of each case in 13 patients from whom we collected matched blood and urine samples
- All patients underwent 1.5 T MRI scans. Conventional MRI sequences included axial gradient echo T1-weighted images, T2 weighted images with and without fat suppression.
- DWI were performed with 16 b-values for estimating IVIM diffusion parameters. For the dynamic contrast-enhanced sequences (DCE), intravenous administration of Gadobutrol (0.1 mmol/kg) was performed.
- ADC values were estimated as a mono-exponential fit over signal intensity values over all 9 b-values. IVIM analysis was performed on MATLAB platform (version R201b, MathWorks, Natick, MA, USA).
- Diffusion-coefficient (D), Perfusion-coefficient (D*) and Perfusion-fraction (f) were calculated in tumor-volume using an IVIM bio-exponential Bayesian fit. The analysis was performed by two observers in consensus.

References

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Results

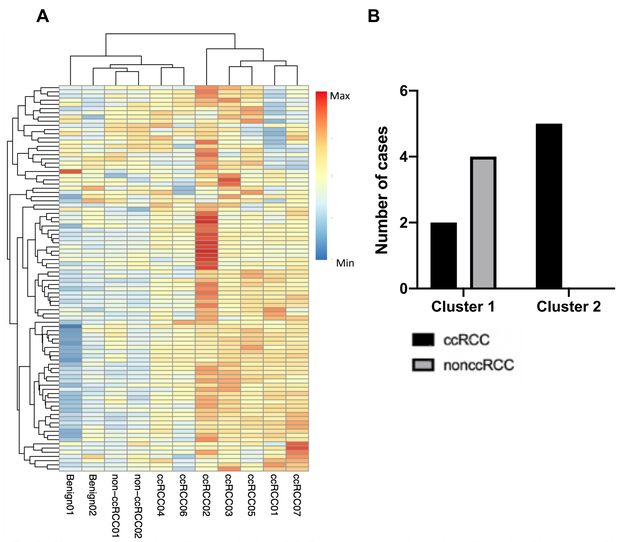

Fig.1. Unsupervised clustering analysis of inflammatory markers in urine. A) Clusters 1 (Non-Inflammatory) and 2 (Inflammatory) were defined on combined expression across 92 soluble analytes associated with inflammatory pathways. **B)** Bar graph showing number of ccRCC and non-ccRCC cases in clusters 1 and 2. One-sided Fisher's exact was performed displaying a statistically significant difference in the proportion of ccRCC and non-ccRCC cases between signatures ($p = 0.0455$)

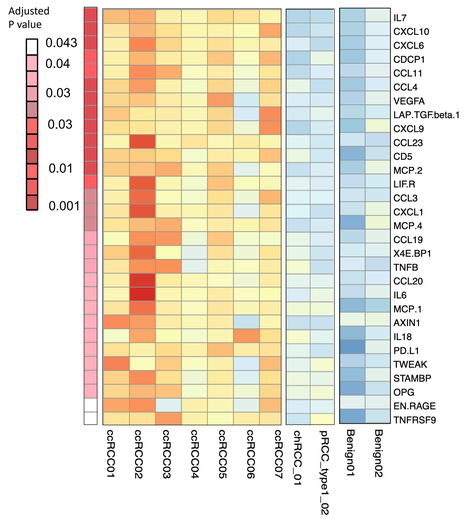

Fig.2. Differential protein expression analysis between described clusters 1 and 2 . We observed significant upregulation of inflammatory proteins in samples from ccRCC patients compared to non-ccRCC cases.

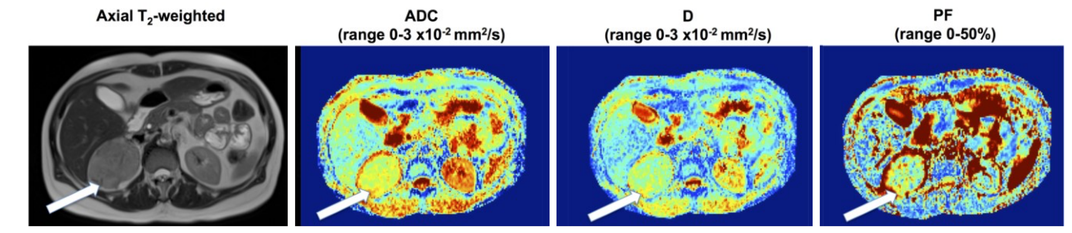

Fig.3. Representative mpMRI from a patient with a SMRs. Diffusion coefficient (D) and perfusion fraction (PF) were obtained by using Intravoxel Incoherent Motion (IVIM) model in Diffusion-weighted Imaging (DWI) sequences derived from MRIs where apparent diffusion coefficient (ADC) was estimated. IVIM allows a subset analysis on the diffusion of water represented by ADC revealing the contribution of water diffusion and perfusion to water motion.

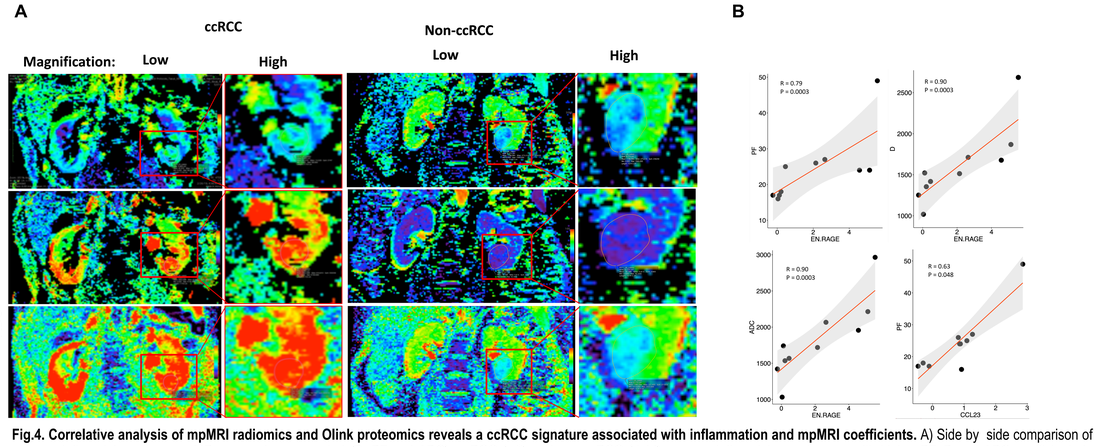

Fig.4. Correlative analysis of mpMRI radiomics and Olink proteomics reveals a ccRCC signature associated with inflammation and mpMRI coefficients. A) Side by side comparison of representative pictures from mpMRI parameters evaluated (D, PF and ADC). ccRCC (left) and non-ccRCC (right) representative pictures of cases with higher PF, D and ADC coefficients in ccRCC compared to non-ccRCC. B) Scatterplots from spearman correlation analysis on differentially expressed proteins between clusters 1 and 2 and mpMRI coefficients (D, Dstar, PF and ADC). A significant positive correlation was found between EN.RAGE and ADC, PF and D. PF also had a significant strong correlation with CCL23.

Discussion

- Unsupervised clustering analysis from Olink proteomics data in urine identified inflammatory signatures in patients with SRMs that distinguished ccRCC vs non-ccRCC tumors
- Differential expression analysis between these signatures showed significant differences on 30 inflammatory markers in urine that was not observed in plasma.
- We hypothesize that the early stage of SRMs produce a local inflammatory response in the kidney, but it does not induce a strong systemic immune response.
- Urine from ccRCC patients showed a significant increase in concentration of inflammatory biomarkers in comparison to non-ccRCC patients.
- We observed strong positive correlations between mpMRI parameters and analytes from Olink Proteomics that showed significant differential expression between ccRCC and non-ccRCC patients such as EN.RAGE and CCL23
- EN.RAGE as well as CCL23 are involved in promoting the recruitment, expansion and activation of innate and adaptive immune cells.
- Collectively, the data may suggest broad effects on the early immune landscape of SRMs that can distinguish ccRCC tumors as effectively as renal biopsies.
- Detailed analysis of the inflammatory milieu during early stage ccRCC may identify novel targets for immunotherapies for more advanced stages of disease
- Our results need to be tested and validated in a larger cohort. Future studies should include proteomic and transcriptomic assessment directly on tumor tissue where matched samples of urine and plasma are available.
- We consider that this approach will allow to identify prognostic and diagnostic biomarkers that predict 1) Malignant tumors that may require surgical therapy. 2) benign or indolent tumors that could be treated less aggressively, and 3) profile the tumor microenvironment of SRMs

Conclusion

- We reported 2 protein clusters that distinguish histological subtypes between ccRCC vs non-ccRCC.
- We have also described a mpMRI protocol that correlates with expression of pro-inflammatory biomarkers in urine and may reflect changes in the tumor microenvironment without the need of renal biopsies. These findings need further validation with tumor tissue and a larger cohort of patients
- While we studied inflammatory-markers in serum, our analyses did not reveal any significant clusters that could distinguish between histological subtypes.
- This approach may provide a novel, non-invasive strategy to identify prognostic and diagnostic biomarkers that predict 1) Malignant tumors that may require surgical therapy. 2) benign or indolent tumors that could be treated less aggressively, and 3) profile the tumor microenvironment of SRMs

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