Validation of quantitative imaging biomarkers with histopathological analyses

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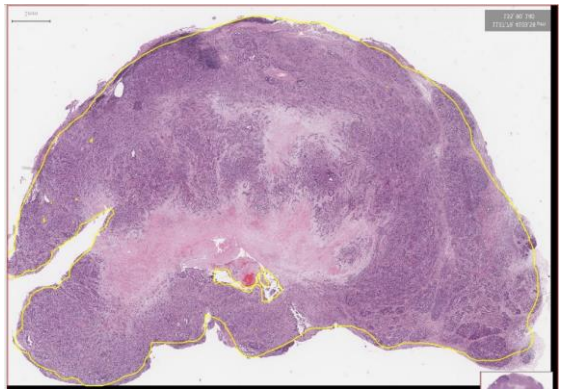
Introduction

- Tumor microenvironment plays key roles in pancreatic cancer initiation, progression, and treatment response.
- In a GEM model of PDA, we aim to validate imaging markers sensitive to tumor microenvironment by quantitative IHC analyses.

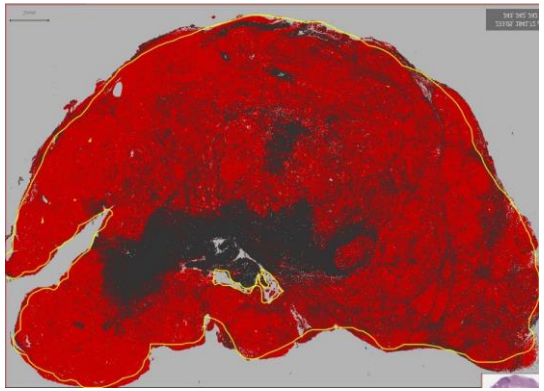
Methods

- KPC mice (*LSL-Kras$^{G12D/+}$;LSL-Trp53$^{R172H/+}$;Pdx-1-Cre*); A 1-2 mm thick fresh tumor slice was loaded in 10 mm vial filled with Fomblin oil.
- Ex vivo DW-MRI on 9.4 T vertical bore DirectDrive® MR system (Agilent) interfaced with 9-cm gradients; ten b-values from 0.64 - 4200 s/mm^{2,} FOV = 10 x 10 mm^2 , matrix =, 1 mm thick, acquisition time ~ 18 hours.
- After DW-MRI, the section was fixed in formalin and processed for HE, trichrome, sirus-red and reticulin staining. SHG was applied on the adjacent Paraffin section.
- IHC Analyses: performed in QuPath-0.2.0-m10. A classifier was built separately for necrosis, trichrome, sirus red and reticulin detection; Classifiers were built through annotation with training including live updates to assure specificity and sensitivity.
- Co-registration of HE and ex vivo ADC map was performed using affine transformation to account for HE image's geometric distortion and mutual information was applied to facilitate registration.

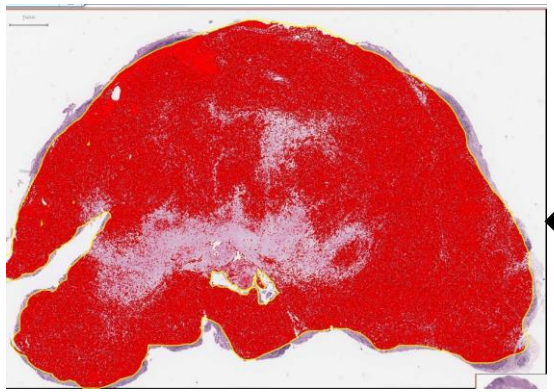
Areas of necrosis and Cell Index (# cells/unit area) and correlation with ADC values



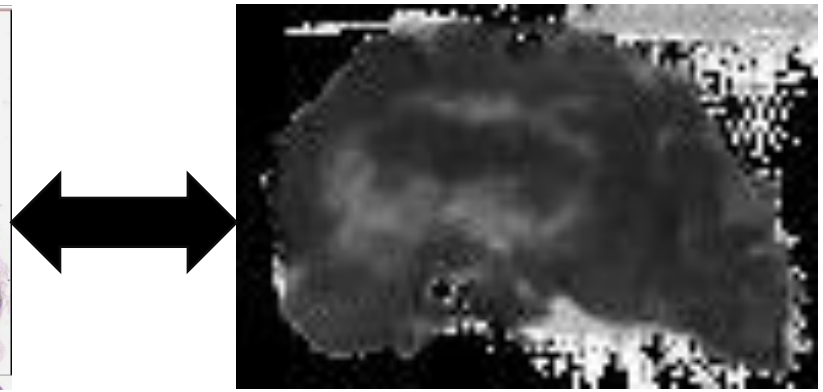
H&E



Necrosis (black), viable (red)



Cell detection (red)



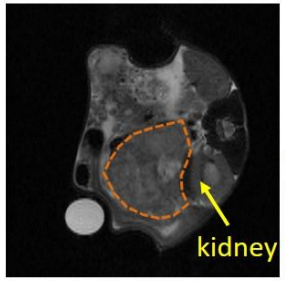
Necrosis = 33% (area) → Cell Index = 1.2

Viable = 77% (area) → Cell Index = 7.7

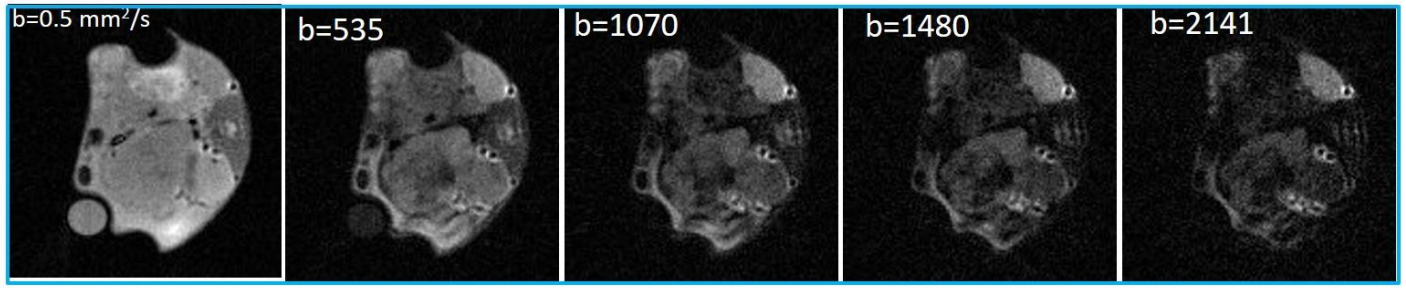
Low Cellularity areas corresponds to areas of bright ADC signal

Co-registration with ADC map from DW-MRI

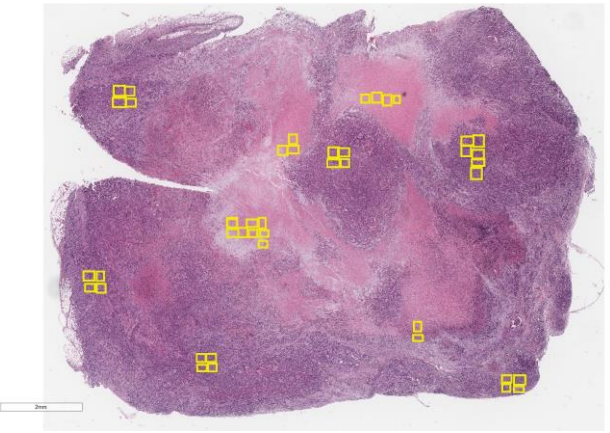
T2W-MRI



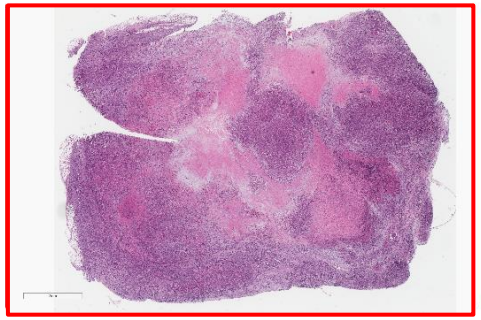
Radial spin-echo diffusion-weighted MR images



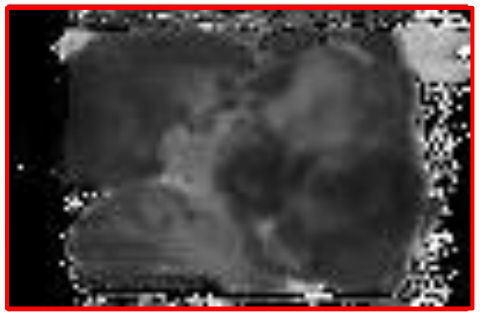
Set of ROIs defined on HE



HE stained section

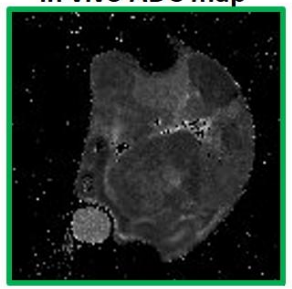


ex vivo ADC map

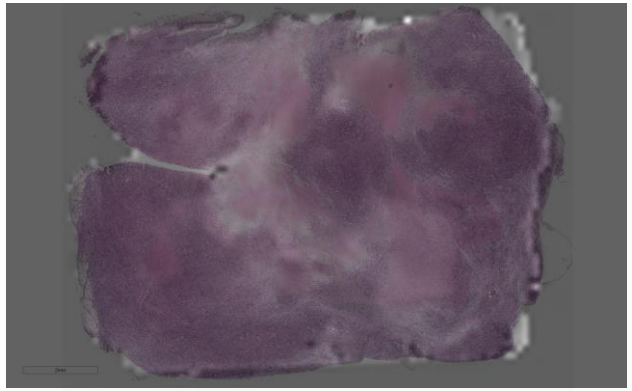


ex vivo DW-MRI of fresh tumor slice

in vivo ADC map

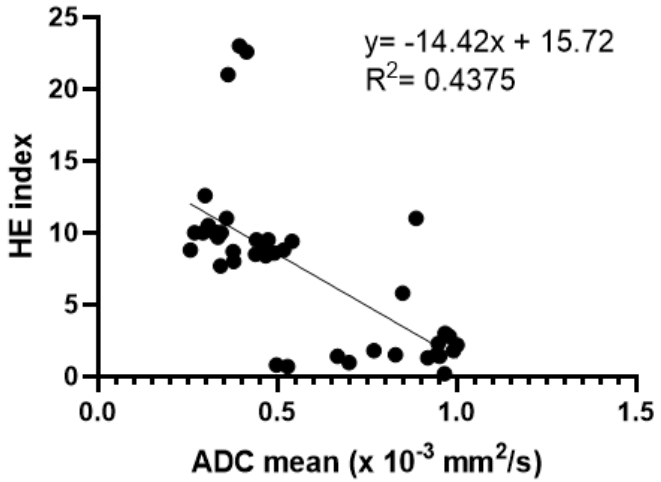


Transform HE and ADC map to the same space



Superimposed HE and ADC map

Compare the HE index and ADC metric from the same ROIs



Inverse correlation between HE index (cell density) and the Mean and Max of ADC.

Discussion & Conclusion

- **Staining & analysis protocols for evaluating tumor stroma (e.g., collagen content) and cellularity are established.**
- **ADC map produced from *Ex vivo* DW-MRI facilitates comparisons of ADC metrics with HE (cell density) index.**
- **An inverse correlation between the HE index and ADC metrics was confirmed.**
- **Multi-tissue block approach for co-registration with in vivo MRI is being evaluated.**

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