Label-free phenotyping of duodenal neuroendocrine tumors using tissue autofluorescence microscopy and digital spatial profiling

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Background

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are a heterogenous group of diseases with complicated treatment and management decisions. For example, patients with Multiple Endocrine Neoplasia Type 1 (MEN1)-associated gastrinomas present with more aggressive tumors and poorer outcomes.[1] Recent work has shown that –omic technologies can phenotype GEP-NETs to accurately reflect important clinical parameters such as tumor aggressiveness and metastatic potential.[2] Unfortunately, technologies to acquire -omic signatures are technically complex, destructive to the sample, and expensive. These barriers impose severe limitations on the clinical utility of these technologies.

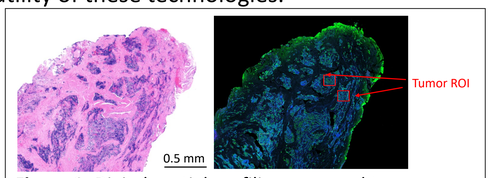


Figure 1: Digital spatial profiling was used to assess protein expression for regions corresponding to tumor, glands, and epithelium in 12 GEP-NET patient samples.

A method to phenotype tumors at the point of care that is label-free, reproducible, and non-destructive could significantly impact our ability to treat and manage GEP-NETs. Optical imaging, for example autofluorescence microscopy, represents a promising avenue that has many ideal characteristics for point-of-care applications – it is minimally-invasive, nondestructive, spatially-resolved, sensitive to many endogenous biomarkers, and rapid.[3] We aimed to assess the potential of autofluorescence microscopy for label-free tumor phenotyping.

Objective

Our work aims to demonstrate the use of tissue autofluorescence microscopy as a method of label-free tumor phenotyping for GEP-NETs. We test this by collecting imaging signatures for patient samples of GEP-NETs as well as conducting digital spatial profiling to stratify tumors by tumor type (MEN1 vs. non-MEN1).

Methods

We conducted Nanostring Digital Spatial Profiling (DSP) to evaluate the expression of 40 neural and immune-related proteins in surgically resected duodenal gastrinomas (n=12).[4]

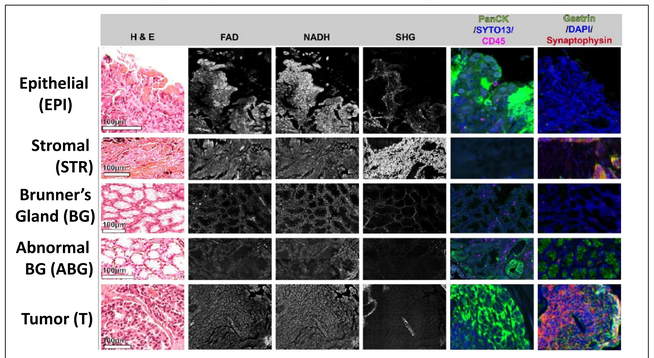
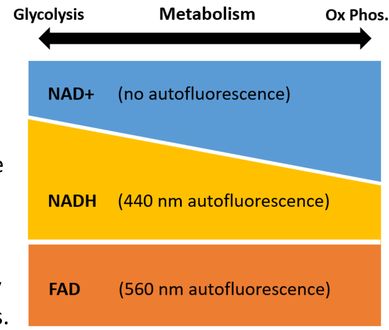


Figure 2: ROIs for different tissue types were selected and classified using H&E and IHC staining for guidance and registered to DSP ROIs.

We then performed tissue autofluorescence microscopy on serial tissue sections using a tunable multiphoton microscope with five excitation and emission wavelength bands to target fluorophores that are commonly differentially regulated in cancer including lipofuscin, collagen, NADH, FAD and Porphyrin. The optical redox ratio (NADH / FAD) was calculated as a measure of metabolism.[5]

A total of 183 regions of interest were examined between tumors, adjacent normal and abnormal-appearing epithelium, and the surrounding stroma. The two datasets were co-registered and results for both DSP and imaging signatures were stratified by tissue type and MEN1 status.



Results

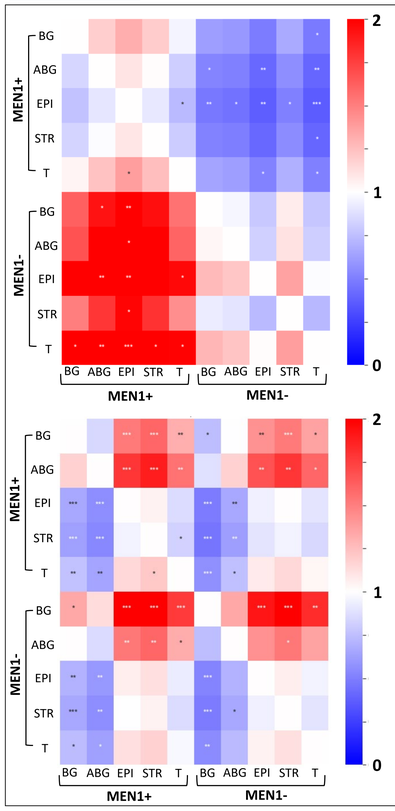


Figure 3: Heatmaps of optical redox ratio and lipofuscin-dominant autofluorescence channel. Each cell in the heatmap is represented as column divided by row. The results show significant increases in both redox ratio and lipofuscin autofluroescence for MEN1+ tissues compared to MEN1- counterparts. This suggests that there may be an increased level of glycolysis in MEN1+ tumors, suggesting higher metabolic rate. Lipofuscin is a byproduct of cellular senescence and these results suggest that MEN1+ tissues may experience a higher level of senescence. Ultimately, these two imaging markers could be promising for label-free tumor phenotyping by providing insight into the underlying biological processes. (* p<0.05), (** p<0.01), (*** p<0.001)

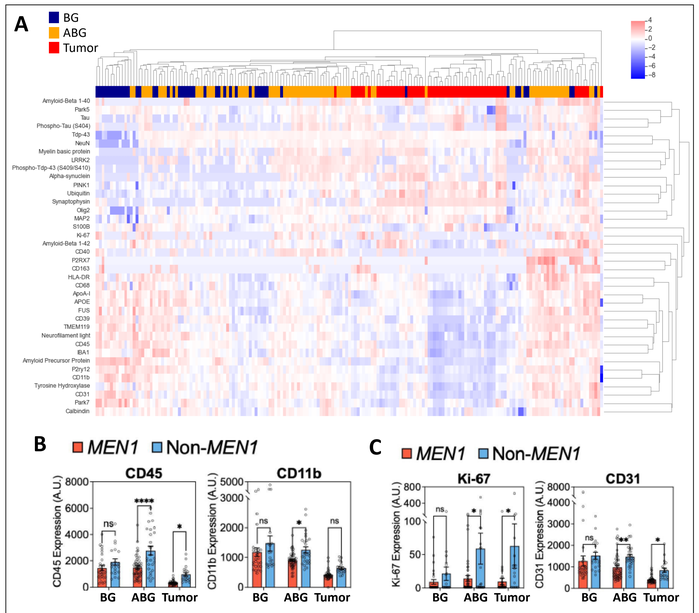


Figure 4: (**A**) Dendrogram of protein expression for all ROIs showing hierarchical clustering. (**B,C**) Expression of immune markers is significantly lower in MEN1+ tissues compared to MEN1- counterparts. Similarly, Ki-67 expression is significantly lower in MEN1+ tissues compared to MEN1- counterparts. Comparing to our imaging results, the reduction in immune presence may be tied to increased cell senescence, and the variations in Ki-67 could be tied to the metabolic variations measured by the redox ratio.

Conclusions

Analysis of the co-registered imaging and proteomic datasets demonstrates high correlation between our imaging markers, proteomic markers, and patient MEN1 status, suggesting that the technology could be used for point-of-care phenotyping. Our results show that our tissue autofluorescence markers, specifically NADH and Lipofuscin, are highly correlated with MEN1 status, suggesting metabolic alteration and a potential impact on senescence. Differences were also observed in the imaging marker for collagen, which could be related to the activation of cancer associated fibroblasts.

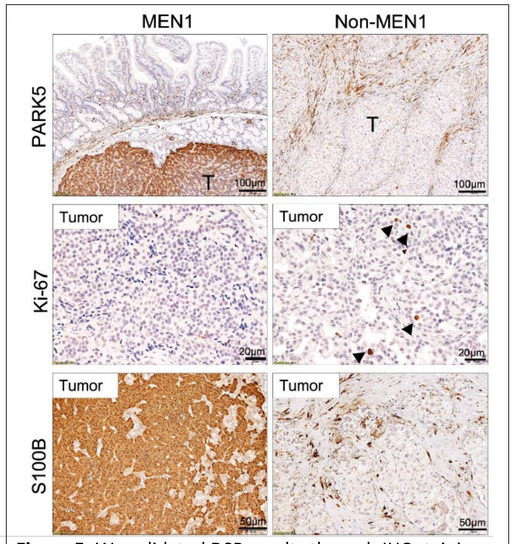


Figure 5: We validated DSP results through IHC staining to confirm relative abundances of protein markers. We show an increase in neuroglial markers for MEN1+.

Acknowledgements

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