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

Thomas Knapp[1], Suzann Duan[2], Juanita Merchant[2], Travis Sawyer[3].

[1]Biomedical Engineering, University of Arizona; [2]College of Medicine, University of Arizona; [3]College of Optical Sciences, University of Arizona.

BACKGROUND

Gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are an extremely 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. Recent work has shown that sequencing (transcriptomic, proteomic) can phenotype GEP-NETs to accurately reflect important clinical parameters such as tumor aggressiveness and metastatic potential. 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. 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. We aimed to assess the potential of autofluorescence microscopy for label-free tumor phenotyping.

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). 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. A total of 183 regions of interest were examined between tumors, adjacent normal and abnormal-appearing epithelium, and the surrounding stroma. Results for both DSP and imaging signatures were stratified by tissue type and MEN1 status. The two datasets were then co-registered and a correlation analysis was conducted between the imaging and proteomic markers.

RESULTS

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.

CONCLUSIONS

Tissue autofluorescence could be a valuable tool for point-of-care tumor phenotyping and for augmenting pathological analysis. Ultimately, this technology could be broadened to other types of NETs, with long-ranging potential for giving rise to a new class of "optical phenotyping" technologies.

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