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Validation of PAM expression in PNETs using Targeted Spatial Proteomics

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BACKGROUND

Pancreatic neuroendocrine tumors (PNETs) arise from pancreatic islets and are the second most common pancreatic cancer. Three clinical biomarkers, Synaptophysin (SYP), somatostatin receptor 2 (SSTR2), and Chromogranin-A (CGA), are currently used for diagnostic or theragnostic purposes. Due to the high degree of heterogeneity of marker expression across tumors, these current biomarkers do not adequately predict clinical outcomes, demonstrating the need for novel biomarker discovery. The protein Peptidylglycine α-amidating monooxygenase (PAM) is classically expressed in healthy neuroendocrine cells, and its expression has been shown to positively correlate with recurrence-free survival in NETs. However, its expression on a single-cell level is still uncharacterized in PNETs. In this pilot study, we examined how PNET biomarker positivity correlates with PAM expression using targeted spatial proteomics. Given its neuroendocrine functions, we hypothesize that PAM expression will be elevated in tumor cells positive for SYP and SSTR2.

METHODS

Our study screened for resected, sporadic, non-functional, grade 1/2 PNETS. This pilot analysis consists of twelve formalin-fixed paraffin-embedded tumor cores from eight patients, which were assembled into a tissue microarray (TMA). This TMA underwent multiplex immunofluorescence using the COMET platform, allowing for the simultaneous staining of all 12 samples for known PNET biomarkers (SYP, SSTR2, and CGA) as well as PAM. Image analysis was performed using a custom pipeline in Visiopharm and data analysis was performed in R. Tumor cells were defined by positive fluorescent signal for at least one of the three biomarkers: SYP, SSTR2, and CGA. Marker expression was measured as mean fluorescent intensity.

RESULTS

We performed a targeted proteomic analysis of PNETs at single-cell resolution in a pilot cohort of twelve specimens. We uncovered a remarkable degree of tumor heterogeneity in the expression of the 3 known PNET biomarkers (SYP, SSTR2, CGA) across patients. Samples had an average of 35,762 cells (range: 15,291-50,556) which on average contained 78.8% tumor cells (range: 49.5%-98.2%). PAM expression was compared between SSTR2(+)/SSTR2(-) and SYP(+)/SYP(-) tumor cell groups. PAM was found to have higher expression in both SSTR+ (1.35-fold; p=0.002) and SYP+ (1.9-fold; p=0.005) PNET cells.

CONCLUSIONS

We found that PAM expression is significantly elevated in PNET cells with higher SSTR2 or SYP expression using proteomic analysis at single cell resolution. Due to its inherent neuroendocrine properties, PAM is a potential marker of PNET differentiation. Future studies with expanded cohort size will expand upon defining tumor types to better understand the role of PAM, and if certain tumor subtypes predict clinical outcomes.

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