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Method for Establishing and Characterizing Small Bowel Neuroendocrine Tumor Organoids

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Background: Small bowel neuroendocrine tumors (SBNETs) are rare cancers originating from enterochromaffin cells of the gut. Research in this field has been limited by the lack of patient-derived SBNET cell lines, which are slow growing and difficult to propagate. The few cell lines that have been established are not readily available and may lose NET cell characteristics after continued propagation in culture. Generating new cell lines requires significant time (years) given the indolent nature of well-differentiated SBNETs and necessity of many enrichment steps to eliminate the rapidly dividing cancer-associated fibroblasts.

Methods: To overcome these challenges, we developed a protocol to culture SBNET cells from surgically removed tumors as organoids in Matrigel with enriched DMEM/F12 medium or stem cell medium. Cultured organoids were tested for NET markers (Chromogranin A, Synaptophysin, and SSTR2) by immunofluorescent microscopy, immunohistochemistry, and gene and protein expression level quantification.

RESULTS: We successfully generated viable SBNET organoid cultures from 8 of 9 tumors, which we have been able to maintain in culture, and verified sustained expression of NET markers and low Ki67 positivity (Table). Phenotypic analysis of organoids showed that they form spheroid or ellipsoid structures and measure between 20 to 100 µm in diameter. Larger organoids bleb off to the surrounding milieu and form new organoids. For proper expansion, SBNET organoids require new medium every week and splitting every 2 to 4 weeks. They are slow growing and double in surface area every 14 days.

CONCLUSION: We established an in vitro strategy to grow and propagate cells from patient SBNETs as organoids that maintain NET marker expression. They grow slowly but can be expanded for testing new therapeutics. In addition, they have the potential to establish much needed mouse models of well-differentiated SBNETs for biological investigations and drug testing in vivo.