Generating neuroendocrine tumor models to build a disease specific dependency map

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Background

At the Broad Cancer Cell Line Factory (CCLF) we aim to generate novel cell models to increase disease representation and accelerate cancer research. With our collaborators at the Dana-Farber Cancer Institute and MD Anderson Cancer Center, we have collected 33 pancreatic (PNET) and small-intestinal (SINET) neuroendocrine tumor patient samples for model generation. Here, we have developed a robust strategy for the generation of PNET and SINET spheroid cell models. Our strategy combines high-throughput empirical rich media screening, 3D culturing systems, and a curated selection of supplemental growth factors. We have successfully maintained NET cultures above passage 5 and up to a T-75 culture vessel. We have identified classical chromosome 18 deletions, overexpression of secreted chromogranin A, and other NET markers such as SSTR2, VMAT1/2, and synaptophysin. Our design for for NET model derivation provides a new opportunity to derive shareable NET 3D models that could be applied for genetic and drug vulnerability profiling.

CCLF HYRBID strategy to improve model generation success rates

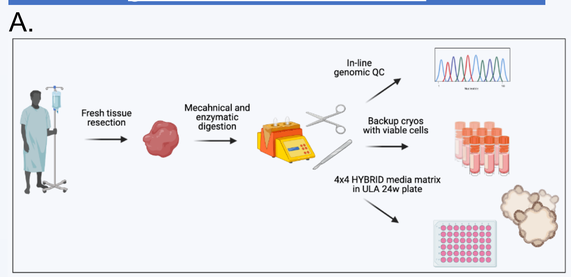

Figure 1. The CCLF has build a patient-partnered pipeline for industry-scale model generation. (A) Patient tissue is collected from all over the US through partnered institutions for model generation. Tissue is processed, initiated as suspension cultures in our HYBRID media matrix, sent for in-line genomic QC, and extra material is saved in storage for future re-initiation attempts. (B) Our comprehensive genomic QC panel targets 400 known cancer genes for mutations and copy number variations. We apply this panel to match the fidelity of the generated cell model with the original patient tumor. (C) Suspension cultures are formed as dissociated single cells aggerate together to form 3D spheroid structures.

Maintenance of PNET and SINET spheroid cultures over multiple passages

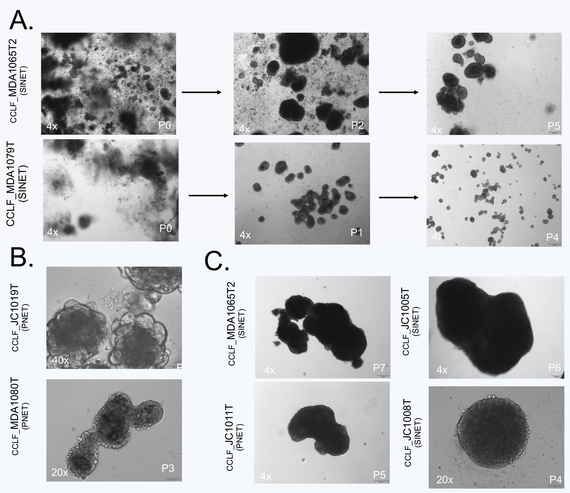

Figure 2. NET spheroid cultures demonstrate similar morphology and clustering behavior across passages. (A) Significant debris and single cells are present at 4x during tissue initiation which either die off or form clusters as the culture is expanded. (B) Spheroid cluster formation clearly visible at 40x and 20x magnification. (C) Late stage (>p3) cultures remain viable but begin to form tightly compact and dense spheroids which slows down growth rates, likely heterogenous clusters of NET cells and NET-associated fibroblasts.

Comprehensive cancer marker sequencing panel detects hallmark NET aberrations

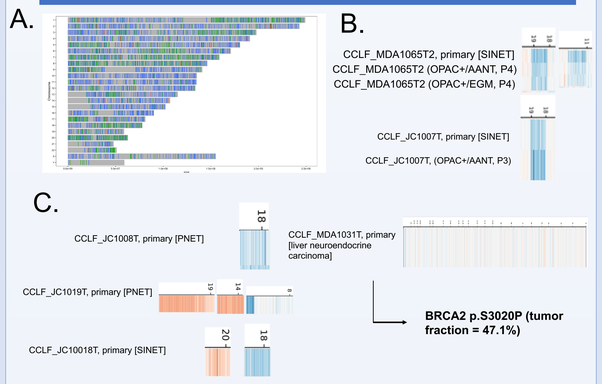

Figure 3. Genomic credentialing reveals high tumor purity matching original patient tissue in 3D cultures. (A) Our comprehensive cancer-specific sequencing panel covers 400 full exon genes, CNV callings across all chromsomes, MSI interpretation, and 88 fingerprinting SNPs. (B) Our panel detects high initial purity by SNVs and CNV loss/gain over a diverse range of chromosomes. (C) Sequenced early passage cultures (p3-p5) maintain CNVs in the original tumor tissue, such as hallmark chromosome 18 and 11 loss.

Detectable levels of synaptophysin expression in genomically verified tumor samples transitioned to 2D

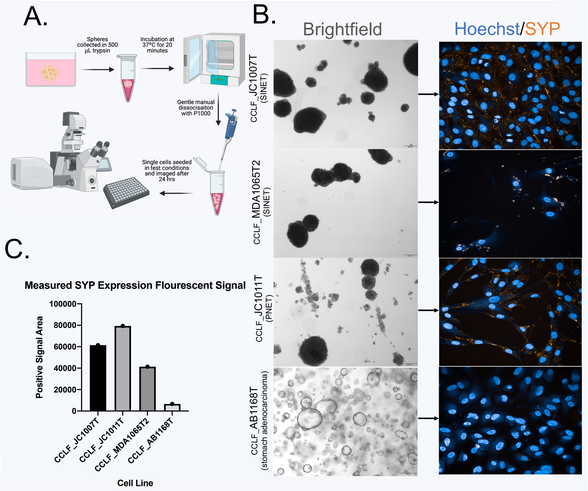

Figure 4. Synaptophysin expression detected in NET cell lines using fluorescent imaging. (A) Spheroid clusters are dissociated into single cells after incubating for 20 minutes with trypsin at 37˚C and mechanical breakup with a P1000 pipette and then seeded in a 96w plate as 2D cultures for imaging. (B) Cells were allowed to recover for 24hrs and then stained with Hoechst 33342, mouse anti-synaptophysin (clone SY38), and goat anti-mouse-Alexa594. Imaging performed at 20x magnification. Imaging performed using the Operetta CLS analysis system. 3D stomach adenocarcinoma cell line included as negative control. (C) Quantified positive signal area across n = 20 fields in duplicate wells.

A high throughput strategy to overcome slow 3D NET cell line proliferation

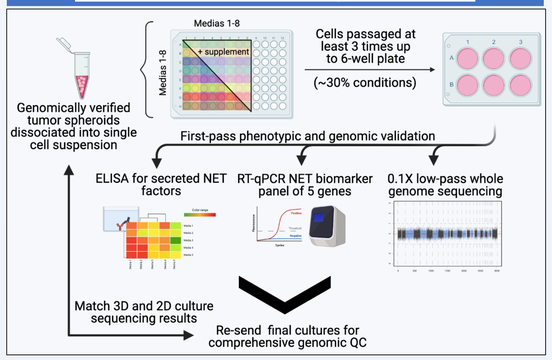

Figure 5. A high throughput, 2D transition pipeline to expand NET spheroids. Stagnant 3D cultures are dissociated into single cells and seeded into an 8x8 HYBRID media matrix for expansion. Cultures undergo phenotypic QC for NET markers (RNA, protein) and hallmark mutations (DNA) to confirm the presence of NET cells. Culture conditions which pass QC are sent for more comprehensive sequencing. Results are compared to the original spheroid culture to verify the culture as tumor.

RT-qPCR measures RNA expression of 5 NET biomarkers in 2D cultures

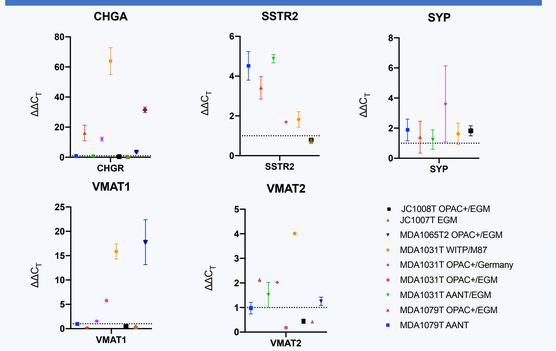

Figure 6. RT-qPCR reveals variable overexpression of key NET biomarkers in different cell lines, media conditions. Gene expression for 5 key biomarkers (synaptophysin, chromogranin A, somatostatin receptor 2, and vascular monoamine transporter 1/2) as compared to GAPDH expression and a normal human fibroblast line.

Chromogranin A as a proximal readout for tumor purity during HYBRID screening

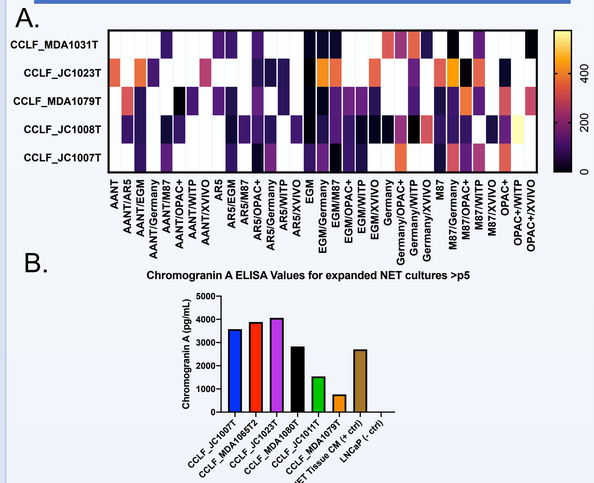

Figure 7. Chromogranin A detection in condition media as a proxy for predicting tumor fraction. (A) Chromogranin A ELISA is performed on dissociated spheroids re-expanded in 2D with our HYBRID approach after >3 passages. Supernatant is 3 mL conditioned media from confluent 6-well. Concentration is in pg/mL. (B) 2D cell lines expanded further to T-75 vessel in top media condition demonstrate detectable, variable chromogranin A expression.

Conclusion and Future Directions

We have developed a robust strategy to grow PNET and SI 3D cell cultures from fresh tissue while maintaining defining genotypic and phenotypic NET characteristics. Our next steps will continue investigating methods to further expand our cell lines so we may share these models with the NET research community and begin performing whole-genome CRISPR screening and drug repurposing screens to create a NET specific dependency map.