Generating neuroendocrine tumor models to build a disease specific dependency map

Although there were a few of established neuroendocrine tumor (NET) cell lines, these models unfortunately provided a limited scope in studying NETs due to disease heterogeneity and model fidelity. To accelerate NET therapeutic discovery, we are developing laboratory tumor cell models that could be applied for functional genomic studies and shared with the NET research community. Here, we created an efficient acquisition platform to acquire small intestinal and pancreatic NET (SINET and PNET) living tissue samples and showed the proof-of-concept to derive long-term 3 dimensional (3D) NET cell models from patients samples using a novel model derivation approach. The generation of such models will enable us to systematically profile all possible NET vulnerabilities and identify potential therapeutic targets. Additionally, our ultimate goal is to create a public resource by sharing these established cancer cell models through third party distributors and genomic credentialing data through the Dependency Map (DepMap) portal.

To be able to ensure the later growing cell model fidelity, we firstly used a comprehensive cancer detecting sequencing panel (400 major cancer genes and chromosomal arms level regions included) in receiving samples to identify somatic driver events. We have mainly observed copy number variations consistent with hallmark genomic signatures of neuroendocrine tumors, such as chromosome 18 deletions in SINETs. Secondly, unlike other research groups' model derivation methods, at the Broad Cancer Cell Line Factory (CCLF), we have taken an empirical rich media screening strategy combined with 3D culturing systems, namely HYBRID technology, to allow up to 64 media conditions in one given tumor patient sample. The HYBRID approach greatly improves the overall success rate of model derivation in many rare and pediatric cancers from 0-3% to 43%. With several media matrix design iterations, we narrowed down 4 nutrient rich media that reflect some of the necessary growth factors for *ex vivo* growth of NET cells. These media were chosen to enrich tumor cell populations and to limit contamination by fibroblastic cell outgrowth from the original tissue. According to publication and our collaborator expertise, we have included a selection of supplemental growth factors such as FGF-9, FGF-1, PDGFBB, Heparin, and MEM non-essential amino acids into the HYBRID design. Finally, all tissue sample initiations are performed in a 3D culturing system using low-adherent high-throughput vessels to allow cells to form spherical structure. Several spheroids derived from 6 patient samples (1 liver, 3 small intestine, 2 pancreas) by the HYBRID technology are currently growing promisingly without normal cell or fibroblast contamination. After 3 passages, we genetically verified these alpha growing models compared with its paired tumor tissues. We understand it is crucial to have a further characterization to ensure the model fidelity; therefore, few classicNET specific markers were applied in passed genomic QC models, such as the presence of secreted chromogranin A.

Our model derivation strategy has proven successful at maintaining NET cell cultures from the pancreas and small intestine beyond passage 5 and up to a T-75 culture vessel. We have developed these methods from a cohort of 11 patient samples which were verified as harboring NET associated point mutations or copy number alterations. From this cohort, 8 samples were successfully propagated to ≥ passage 3 (72%) as suspension cultures. All 8 samples were sequenced to validate the presence of NET specific mutations and 6 passed our quality control (75%), yielding an overall success rate of 54%. Spheroid NET cultures have a doubling time between 1-3 weeks which is in the average range for other disease 3D cancer organoid/spheroid models. Although many cultures are still growing, two of the SINET models have reached passage 7. This SINET model maintained classical chromosome 18 deletions that were observed in the paired tumor tissue. In the NET biomarker analyses, the preliminary data shows that these SINET models express Chromogranin A and serotonin/5-HT strongly. We are also able to identify expression of important NET and lineage specific markers such as VMAT-1, SSTR2, and synaptophysin by immunofluorescent staining of spheroids and single cell FACS analysis. Overall, the HYBRID media matrix design for NET model derivation provides a

new opportunity to derive SINET 3D models that could be applied for genetics and drug vulnerability profilings.

The ability to propagate cell cultures for a long-term provides an exciting opportunity to study NET biology. Our next steps are to continue expanding the SINET models and optimizing the NET model derivation protocol that includes improving the cell doubling time while maintaining accurate tumor biology. As we also grow our cohort size, we expect to not only increase the number of NET models but also add more diversity of genotypes that was important for clinical treatment actions. Eventually, once NET spheroid models can be robustly propagated, we plan to test the genome wide level CRISPR screening and small molecular compound screening towards building the NET specific dependency map.