Single Cell Sequencing

Over the past year, the Nicholas Navin PhD and the Navin lab have made two major discoveries in breast cancer research with potential for clinical impact. Your funding has supported the development of a new technology called **arc-well**, which uses nanowells to perform single cell DNA sequencing on archival tissue samples that are routinely stored as histology slides in pathology departments as FFPE (formalin-fixed paramorhaldehyde) materials. This method opens up many new avenues of investigation into clinical slide samples that have been stored around the world in cancer hospitals and often have long-term clinical outcome data available (in contrast to tissue samples collected prospectively).

We used arc-well to analyze breast cancer tissues from early-stage disease patients called **DCIS (ductal-carcinoma-in-situ)** with matched recurrence samples (invasive breast cancer) that often occur years to decades later in women. DCIS is a major clinical problem, since only about 5-10% of women that have DCIS detected by routine mammography ever progress to invasive disease, yet most women are treated aggressively to prevent progression thereby leading to much overtreatment and surgical procedures in women that would never progress to invasive breast cancers. Our single cell data showed that specific subpopulations of tumor cells remained dormant for years to decades and then abruptly re-expanded to form the invasive tumors that presented a major risk to the women. These data answered a question of whether the late-stage invasive breast cancers were related to early disease that is often detected during routine mammography. This study was submitted to the prestigious journal *Nature a*nd is currently under revisions.

Our second study used single cell RNA sequencing methods to study **triple-negative breast cancer** to understand variable responses to chemotherapy, which is the standard of care. Although chemotherapy is an old form of treatment, about 50% of TNBC patients respond very well (complete responders) and therefore this remains the standard of care. In the 50% of patients that do not respond, they often progress to metastatic disease, therapy resistance and death within 1-2 years. In this study we used single cell RNA-seq technologies to study 130 women at the pre-treatment time point and identified many gene signatures in the tumor cells and the microenvironment (vascular cells, immune cells, connective cells) that are associated with poor chemotherapy response.

FUTURE FUNDING:

Using this data, we plan to develop a diagnostic assay that can be used to determine which 50% of women with TNBC should receive chemotherapy and have complete response, and also determine which 50% of women should seek alternative treatment options, sparing them the severe side-effects of chemotherapy they currently experience. This data will be submitted to another prestigious journal (*Nature Medicine*) before the end of the year and we plan to work closely with our clinical colleagues in diagnostics to develop a predictive assay.