Brain Tumour Foundation of Canada – Summer Studentship Report for Summer 1

Karl Narvacan

Project: Evaluation of nCounter technology in copy number variation analysis for the classification, diagnosis and predictive prognosis of diffuse gliomas

Introduction

The purpose of the study was to develop and evaluate a new analytical method in performing copy number variation (CNV) analysis through the novel nCounter technology to aid in the classification, therapeutic management and prognosis of diffuse brain tumours.

The first phase of the project was obtaining tissue samples, preparing them for analyses, followed by processing with nanoString nCounter technology. This required myself to shadow and be part of the laboratory team in Molecular Pathology under Dr Izevbaye, where Fluorescence In-Situ Hybridization (FISH) is performed on tissue samples. Being part of the diagnostics lab allowed me to learn about the technology that is used in FISH, and learn how to contrast the process with the nCounter technology in looking at copy number variations in specific genomic loci.

For reference, the nCounter gene expression system counts individual copies of up to 800 different regions in the human genome in a single step using a Reporter Probe, which is similar to a 'color-coded barcode' that allows for reading multiple genes of interest per sample while allowing for a much higher resolution in CNV analysis.

In my work in the summer, qualitative analyses of our group found several advantages of nCounter over FISH, including the lack of need for enzymatic reactions, decreased bias in microscopy, high sensitivity coupled with high multi-gene analysis capability, lower detection limits, and a digital readout. Furthermore, nCounter is able to provide a robust readout from as little as 300 nanograms of starting material and is able to analyze up to 72 samples per day unattended, as compared to several days for a single sample analyzed via FISH. As of late, we had obtained 48 samples that had been processed by both FISH and nCounter technology by the molecular pathology laboratory. These samples have to be analyzed in the second part of my summer studentship.

My first task was to learn how to navigate the nCounter software works in processing the data. This required reviewing online documentation and videos to learn the software and apply it to our data in acquiring our results. After some trial runs in acquiring CNVs from the nCounter-processed subjects, I was able to apply the process to obtaining all the copy numbers for the relevant genomic loci *ATRX*, *IDH*, 1p/19q co-deletion and *MGMT*. Statistical analyses are underway to process the data to determine any significant copy number variations, in order to correlate them with the diagnosis and prognosis of the subject.

Future directions include reviewing clinical charts of the subjects involved in the study and tabulating the CNV results vis-à-vis their diagnosis to determine whether there is any correlation between the alternations in CNVs and the diagnosis/type of tumor and prognosis of the subject. Presentation is expected at the end of the second summer at the DRiVE Days conference within the Department of Laboratory Medicine & Pathology, University of Alberta.