SDHB DEFICIENCY IN NEUROBLASTOMA
Scott Khuu, Monique Morrison (Rodney Stewart)
Department of Oncological Sciences

Neuroblastoma (NB) is the most common cancer of infants and arises in the neural crest-derived peripheral sympathetic nervous system. Despite advances in surgery, chemotherapy and radiation, the outcomes for patients with metastatic NB remains dismal. Identifying genes and genetic pathways that contribute to NB are required in order to design more rational therapies that selectively target NB cells while preserving normal surrounding cells. The 1p36 region of chromosome 1 is commonly deleted in aggressive MYCN-amplified NB, suggesting that one or more tumor suppressor genes in this region cooperates with MYCN to form NB. The SDHB gene is located at 1p36 and is a bona-fide tumor suppressor in paraganglioma and pheochromocytoma, cancers derived from the same neural crest precursors as NB. Thus, the project’s goal is to determine if loss of sdhb promotes NB either by itself or in combination with MYCN amplification.

Using Transcription Activator-Like Effector Nucleases (TALENs) technology, zebrafish embryos with potential sdhb mutations in exon 4 were generated. To identify mutants, we optimized different sets of PCR primers to determine which set gave identical melt curves on a HRMA analyzer. Three different primer combinations were tested at three different temperatures leading to one primer set/temperature combination with an optimized curve from the HRMA analysis. Challenges to finding primer sets may stem from the fact that one primer was located in intronic regions, which often contain single nucleotide polymorphisms (SNP’s) easily detected by HRMA. In a sample size of over 240 sdhb TALENs fish, less than 10% showed heteroduplex melt that was suggestive of potential mutations (See Figure 1). A fragment of DNA that included the region of the mutation was amplified by PCR, purified and submitted for Sanger sequencing to further verify mutations. Genomic DNA from the HRMA was also subcloned into the pGEM vector to provide a longer template for sequencing analysis. Sequencing results showed some mutations were deletions.

The identified mutants were crossed with each other in order to produce progeny with the same sdhb mutations for the monitoring of their growth and to see if tumors arise according to the hypothesis. This procedure still consisted the use of HRMA, but unlike the past, about 50% of the progeny exhibited the melting curve that was similar to the ones before. These fish were separated into tanks according to their date of birth and will be checked on periodically in the coming months for their tumors.

Further research remains to be conducted to fulfill the project’s objectives. While the fish are left to grow, the project will continue to identify more mutants and repeat the same process of crossing those mutants in order to produce progeny that can be monitored in the upcoming months for NB.
Figure 1. HRMA analysis of sdhb mutants. Column 1 (grey) shows the control, while column 2 (red) shows heteroduplex melt curves that indicates potential mutations.