Undergraduate Research Seminar
Wednesday, April 1st, 2015 5:30 p.m.
Leigh 309

Marc Ferrell

"RNA Splicing Event Analysis by Junction Alignment to Full-Length Sequences"

In the effort to develop more accurate methods of transcriptome sequencing and analysis, we have combined the benefits of full-length transcript sequencing with exon and intron junction predicting software to develop a new method of transcriptome analysis. Most molecular biologists study transcriptomics by conducting short-read sequencing and aligning these reads to a reference genome. To avoid systematic coverage bias caused by direct genome alignment—which persists even with longer reads—we have made use of a comprehensive database of exon-exon and exon-intron junctions. Courtesy of the McIntyre group, this database includes sequences of junctions between all consecutive combinations of exons and introns in the mm9 mouse genome assembly. We have aligned these junction sequences to full-length transcripts of mouse (*mus musculus)* neural stem cells and oligodendrocytes. Contrary to the conclusions of short-read RNA-seq, we have found that intron retention is underrepresented in these cell types. Furthermore, enrichment analysis of our data suggests that exon skipping is far more common in differentially expressed genes, while alternatively expressed genes are not enriched with a specific event type. This pilot study has allowed us to develop a workflow for this analysis, and we plan to repeat the experiment with larger sample sizes in the near future.

Danielle McDougall

"Liquid Chromatography and Mass Spectrometry Method Development for Global Lipidomic Analysis"

The objective of this research was to optimize global lipidomics methods using liquid chromatography (LC) and mass spectrometry (MS). American Red Cross blood plasma was used for optimization. Lipids were extracted from the sample using the Matyash lipid extraction method. This allowed for isolation of small, non-polar molecules. Using Dionex Ultimate 3000 UHPLC with the Q-Exactive mass spectrometer, the samples were analysed for a global lipidomic analysis. The LC gradient was optimized for separation of co-eluting lipid classes and to ensure the best detection. LC columns from different vendors were compared on factors such as peak shape, peak capacity, and reproducibility. The comparison of C18 columns from Thermo, Waters and Supelco showed that the Waters column demonstrated the best peak shapes and highest peak capacity. The optimized LC gradient held 20% B at the start for 1 minute before gradually increasing to 98% B over 16 minutes. The MS method alternated scans between full scan and all- ion fragmentation scan mode. Tandem mass spectrometry was used as needed for ID confirmation. This provided all the information needed to match the fragmentation patterns to the parent ions. The method developed has demonstrated to be robust for global lipidomics of blood plasma.

Christopher Louviere

"Application of a combinatorial natural product therapy for the treatment of high-grade gliomas"

Drug resistance developed from an application of chemotherapy at a maximum tolerated dose is the most commonly experienced outcome in cancer therapy. Based on the Warburg Effect and previous research from the oncology community documenting the anti-cancer potential of specific nutraceutical compounds, the purpose of this research was to develop a treatment that enables both horizontal and vertical targeting of high-grade glioma tumors (GBM). The treatment (termed CA) uses a combination of two distinct approaches. The first approach involves the simultaneous application of three nutraceutical products (NPs)- curcumin, epigallocatechin-3-gallate, and sulforaphane- which have previously demonstrated anti-cancer properties and have well-documented safety profiles. Each NP was previously shown to inhibit the expansion of glioma cells, with the combination of all three compounds producing a synergistic effect. The second aspect includes limiting carbohydrate intake in test subjects, thereby hindering the glucose metabolism of and impeding energy production in cancer cells. Our hypothesis was: if tested *in vitro* using both subcutaneous and intracranial xenograft models, the application of CA would produce a statistically significant increase in lifespan of NOD/SCID test mice with GBM. To investigate this hypothesis in a subcutaneous model, GBM cell suspensions were injected into the right flanks of the test mice, and subjects were sorted into their respective treatment and control groups once tumor palpations were observed. Tumor sizes and weights were measured three times per week, and test animals were sacrificed, and tissue harvested, once tumors had reached endpoint volume. In the intracranial model, GBM cell suspensions were transplanted laterally of each animal's Bregma point, and test subjects were given their respective treatments five days post-transplant. Weights and body scores were assessed three times per week, and animals were sacrificed, and tissue harvested, once significant neurological decline was observed. The results show CA increases the lifespan of test subjects in a statistically significant manner. CA has also illustrated synergistic effects when combined with the chemotherapeutic standard of care for high-grade glioma, temozolomide (TMZ), and has demonstrated the ability to resensitize TMZ-resistant cells to the chemotherapy treatment. Collectively, the data suggests CA is a viable non-toxic and affordable antineoplastic treatment for high-grade glioma tumors.