***Introduction***

Schwannomas and meningiomas are two types of tumors affecting the central nervous system (CNS; the brain and spinal cord) which are generally considered as benign (non-cancerous) but in many cases require complex, risky surgery for removal. They account for approximately 8% and 37% of primary CNS tumors with 1.90 per 100,000 and 8.05 per 100,000 annual incidence, respectively. Observation, surgery, and radiation therapy are the mainstay of treatment for these tumors, as there is no effective chemotherapy or other medical treatment known at this time. Surgery can be curative if complete excision is possible without risking neurological damage; however, in many cases this is not possible. In addition, although radiation can be a suitable first-line therapy for schwannomas and an adjunct to surgery in certain meningiomas, there is variable response to radiation between different tumors, and we do not have adequate means of predicting which tumors will not respond to the treatment. The need for effective medical treatments is clearest in patients with the genetic disorder Neurofibromatosis Type 2 (NF2), a tumor-forming syndrome in which patients form multiple schwannomas and meningiomas and eventually succumb to their tumors.

Efforts have been made to use genomics, transcriptomics, and proteomics to identify possible drug targets in schwannomas and meningiomas, but have yet to prove successful. Metabolomics is a fairly young ‘-omics’ field based on identifying and quantifying the small molecules present in cells and tissues using mass spectrometry or nuclear magnetic resonance. In essence, metabolism reflects the downstream effects of all the complex molecular activity measured using other ‘-omics’ approaches (DNA, RNA, proteins). I have been working in the lab of PI Marlan Hansen (Department of Otolaryngology at UIHC) and in collaboration with Eric Taylor and the UI Metabolomics Core Facility to utilize metabolomic analysis of human schwannomas and meningiomas and the effects of radiation on patient-derived xenografts from these tumors. We hope to use these approaches to identify targetable metabolic vulnerabilities in the tumors that could be exploited for future clinical benefit.

**Project Idea 1:**

Ijare *et at*. Glutamine anaplerosis is required for amino acid biosynthesis in human meningiomas. Neuro Oncol. 2022 Apr 1;24(4):556-568. doi: 10.1093/neuonc/noab219. PMID: 34515312.

I would plan to reproduce Figure 1C and/or 2a using my own data, and doing so in a semi-automated fashion to make the data analysis reproducible, efficient, and less susceptible to accidental typographical errors. Figure 1c is a simple bar chart of several metabolites combined into a single graph, comparing the WHO grade 1 versus WHO grade 2 tumors in blue and red, respectively. In addition, the authors indicate P-values in the graph using brackets. What is not explicitly stated—but is a necessary analytical precursor to the graph below—is screening all the metabolites included in the metabolomics experiment to determine which 10 metabolites should be included in the graph.

Figure 2a is also a simple bar chart, but instead demonstrates a stable isotope tracing experiment using 13-Carbon labeled Glutamine. The red and blue colors represent two different cell cultures, while the different values along the x-axis reflect the number of 13-Carbons in the respective metabolite. I do not currently have 13C-Glutamine tracer data but am in the process of obtaining this in radiated patient-derived xenografts, so will hopefully have these data by the end of this course.

Chart

Description automatically generated

Chart

Description automatically generated with low confidence

**Project Idea 2:** Masalha, W., Daka, K., Woerner, J. *et al.* Metabolic alterations in meningioma reflect the clinical course. *BMC Cancer* 21, 211 (2021). <https://doi.org/10.1186/s12885-021-07887-5>

I would plan to re-create the heatmap in Figure 2A. This will also require an unsupervised clustering analysis of the primary schwannomas and/or meningiomas from which we have metabolomic data. Unsupervised cluster analysis is a commonly-used technique in modern multi-omics papers in the Neuro-Oncology literature, being used to demonstrate previously undescribed/unknown molecular sub-types of tumors. Ideally, the molecular sub-types are also combined with some form of clinical or histopathological information to demonstrate that the molecular groups have clinical significance. The paper in question used NMR-based metabolomics to describe two metabolic sub-groups in meningiomas. To my knowledge, no such analysis has ever been done for Schwannomas, nor has a similar analysis been done for mass spectrometry-based metabolomics in meningiomas. Thus, this appears to be ripe for discovery. I already have the metabolomic data that would be used for this – however, I am unsure whether in my data we would find clustering as clear as that seen in the paper referenced here.

Chart

Description automatically generated

**Project Idea 3: Automated script of the steps for analysis of metabolomics data from mouse xenograft schwannomas & meningiomas, currently done manually using Excel and GraphPad then exported to PowerPoint.**

Raw values formatted from source (Excel worksheet) into new Excel file containing only the desired metabolites, plus associated clinical or patient information. Raw metabolite values then used to make group-specific Fold Change calculations within same sheet (Example formula:

[cell AC8] “=IF(F8=ISBLANK(TRUE),"",F8/AVERAGE(F$6:F$8))”

Notably, for each different mouse group (i.e. different patient tumors of origin), the term seen above as “F$6:F$8” had to be manually changed to correspond to the correct set of control (0 Gy) xenograft samples (two or three control samples per group). Fold Change values then copy-pasted as values only to new sheet. These then have to be sorted by radiation dose then copy-pasted from Excel to GraphPad Prism. Next, GraphPad is used to perform outlier analysis (Grubb’s test, alpha = 0.01), then test for normality, log transform if lognormal data, then perform t-test vs ANOVA. This is notably done manually for every individual metabolites included, which limits reproducibility and the efficiency of analysis. Then column graphs are manually generated in GraphPad Prism.

I would plan to create a scripted R file to perform this automatically, generate the graphs, and generate lists of the relevant values for each metabolite that can be easily reviewed to find which variables are significantly different.

