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Project Number
38307

Project Title

The Application of Exosomes for Diagnostics and Prognostics in Brain Tumors: Medulloblastoma and Glioblastoma

Abstract

Objectives/Goals

My project aims to develop a simple Central Nervous System (CNS) Tumor test by investigating exosome expression profiles on the transcriptional and protein level for Medulloblastoma (MB) and Glioblastoma(GBM). A clinically applicable liquid biopsy for CNS tumors is needed to overcome limitations in screening methods like MRI. The techniques employed have been designed to be easily transferable to a clinical setting.

Methods/Materials

For Medulloblastoma tumor samples were from the Group 3 MB Model Tumors and blood was collected weekly from Group 3 MB tumor bearing mice via retro orbital, as well as a final blood collection after 3 weeks. Exosomes were pelleted with SBI's Exoquick solution. RNA was isolated from the exosomes samples as well as the tumor samples followed by RT-qPCR for c-Myc and Dominant Negative p53(Dnp53) in addition to controls (B-actin). For Glioblastoma, GBM39 cell culture and GBM39 tumor bearing mice, where blood was collected after about 4 weeks of tumor progression, had ELISA performed for EGFRviii, EGFR, and CD81 and qPCR for EGFR and EGFRviii (with techniques similar to those used for MB samples). Western blot and additional qPCR is to be performed on the HDMB03 cell lines, among other human MB Group 3 Lines, and the Group 3 MB model tumor to identify possible biomarkers.

Results

The MP Model for Group 3 MB from tumor samples showed significant overexpression of c-Myc and Dnp53 compared to Sonic Hedgehog (a subgroup of MB) and the control (non-tumor bearing mice) indicating high specificity and sensitivity. The targets (Myc and Dnp53) in the exosomes collected from MB tumor bearing mice were unable to be detected. The ELISA and qPCR for GBM yielded similar results, however this indicates the need for further optimization of antibodies and of the qPCR reaction.

Conclusions/Discussion

Because the targets that were expected to be overexpressed in the exosomes were difficult to detect in vivo, in vitro studies are currently being done on cell lines to investigate more viable biomarker targets as a way to backtrack and apply to future in vivo studies. The results indicate that there is need for further optimization of the blood sample targets. The additional studies can provide biomarkers that are important for following diagnostic studies since the ability of exosomes to pass the BBB provides for a good liquid biopsy option.

Summary Statement

I was able to develop the framework for a clinically applicable liquid biopsy method for CNS tumors such as GBM and MB through the employment of exosomes by analyzing the expression profiles of key proteins and transcriptional elements.

Help Received

The Wechsler-Reya and Furnari Labs provided materials and supplies. Lianne Chau from the Wechsler-Reya Lab at the Sanford Consortium trained me in RT-qPCR and performed the mouse tumor transplants. Dr. Tomoyuki Koga performed GBM xenografts and trained me in ELISA.