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**Second Term Progress Report**

Investigating non-coding RNAs underlying temozolomide resistance in glioblastoma multiforme.

The overarching research objective of this project was to better characterize the involvement of long non-coding RNAs (lncRNAs) in glioblastoma multiforme (GBM) formation, development and resistance to the alkylating agent temozolomide (TMZ). GBM is a highly aggressive and prevalent brain tumour. The estimated median time of survival following diagnosis remains between 12 to 15 months. This short survival time is associated with multiple molecular mechanisms notably contributing to TMZ resistance. As outlined in the first term progress report, the expression of a number of lncRNAs was quantified in a panel consisting of 6 normal brain tissues, 19 GBM primary samples as well as 7 glioma cell lines. Interestingly, lncRNA HOTAIR displayed elevated levels in several primary GBM samples and in the LN319 GBM cell line. During this second term, attempts to confirm the RNAi-mediated HOTAIR knockdown in LN319 cells approach undertaken last summer (Figure 1) was conducted by assessing HOTAIR expression via qRT-PCR. Unfortunately, successful and reproducible HOTAIR knockdown in LN319 cells could not be achieved. On the other hand, primers were designed to amplify and quantify additional lncRNAs in the models mentioned above. Results of relative expression of quantified lncRNAs are presented below (Figures 2 to 7). In addition, experimental protocol design and amplification of another family of non-coding RNAs was undertaken during this term. Circular RNAs (circRNAs) are non-coding RNAs that have recently garnered interest in various models of cancer. Nevertheless, information is lacking regarding these non-coding RNAs in GBMs. A circRNA extraction and cDNA synthesis protocol was optimized and a few circRNAs were amplified setting the stage for their investigation in primary GBM tissues. Overall, future plans for the project are numerous and will be pursued by two 4th year undergraduate biochemistry students during the 2016-2017 academic year. The proposed plans include: (1) Reduction of lncRNA HOTAIR levels in LN229 cells, another model with strong endogenous Hotair levels, and evaluation of this modulation on various cellular phenotypes (2) Quantification of lncRNA expression in GBM cell lines treated with TMZ and (3) Quantification of circRNAs in GBMs and investigation of their involvement, if any, in TMZ resistance.

As a proud recipient of a Brain Tumour Foundation of Canada studentship, I am extremely thankful to Brain Tumour Foundation of Canada for the amazing opportunity I had these past two summers. This experience has been immensely rewarding on an intellectual, professional and personal level. Working in a biochemistry and molecular biology-focused laboratory, I have learnt many biomolecular concepts and techniques that are sure to become advantageous in the future. Such an experience gave me an opportunity to further the learnings I had been confronted with while studying the nervous system in the neurology unit of my medical program. I have fell in love with the depths and complexity of the human brain and its connection to other systems of the human body. In hopes that our paths cross once again in the near future, thank you to Brain Tumour Foundation of Canada, my supervisor Dr. Pier Jr. Morin and the entire research team at Université de Moncton.

Appendix : Figures

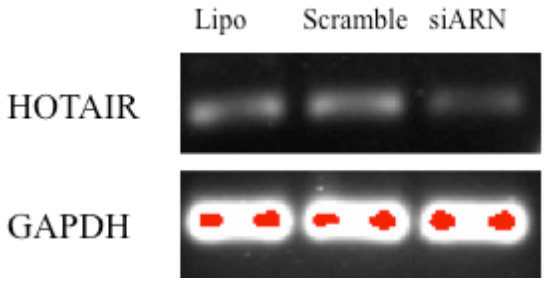


**Figure 1**: RNAi-mediated HOTAIR knockdown in LN319 cells detected by semi-quantitative PCR (2015). HOTAIR knockdown was further evaluated by qRT-PCR and adequate knockdown could not be confirmed (2016).

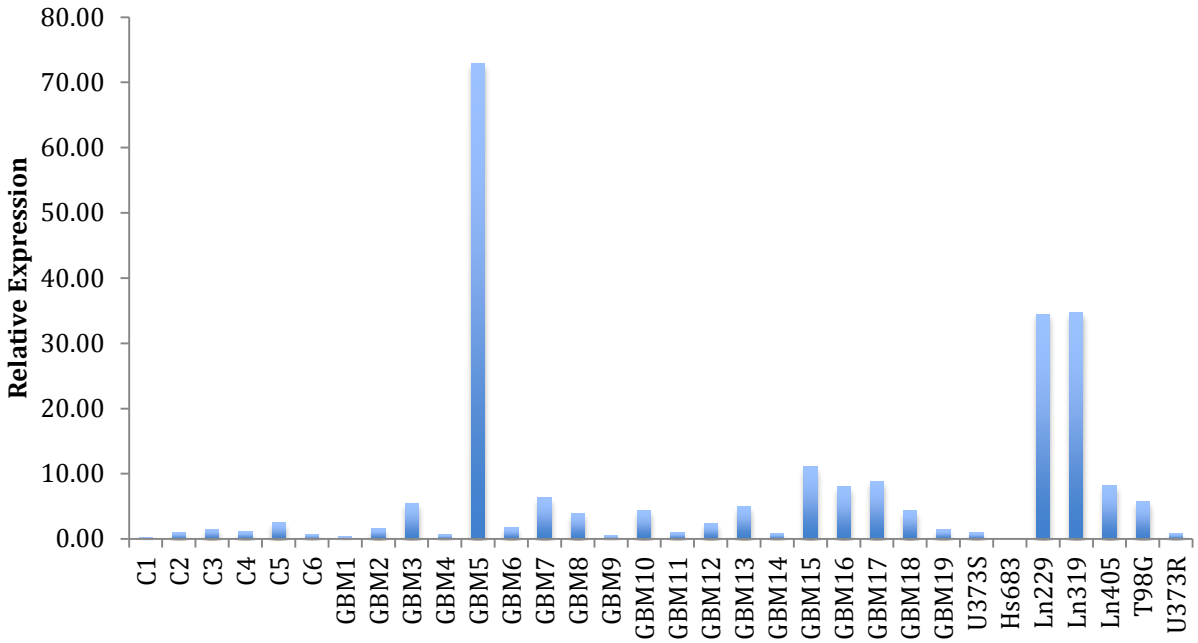


**Figure 2**: Relative expression of lncRNA HOTAIR in glioma samples as measured via qRT-PCR. C1-C6 represent the primary control samples, GBM1-GBM19 represent primary GBM samples and remaining samples represent glioma cell lines.

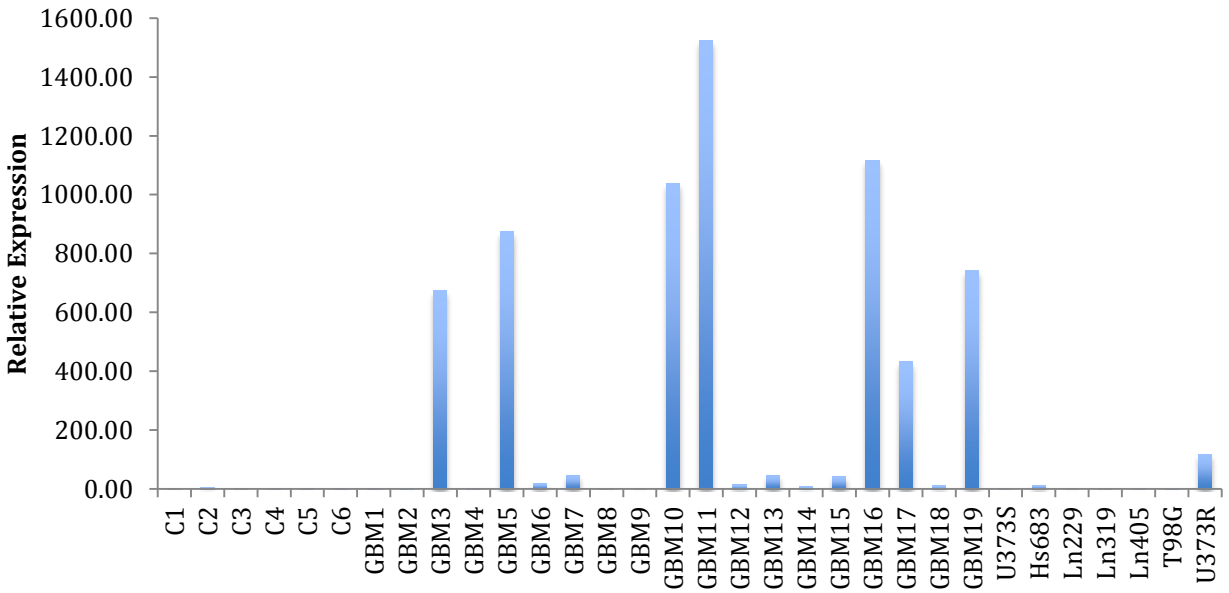


**Figure 3**: H19 transcript expression in GBMs. Relative expression of lncRNA H19 as quantified by qRT-PCR. C1-C6 represent the primary control samples, GBM1-GBM19 represent primary GBM samples and remaining samples represent glioma cell lines.

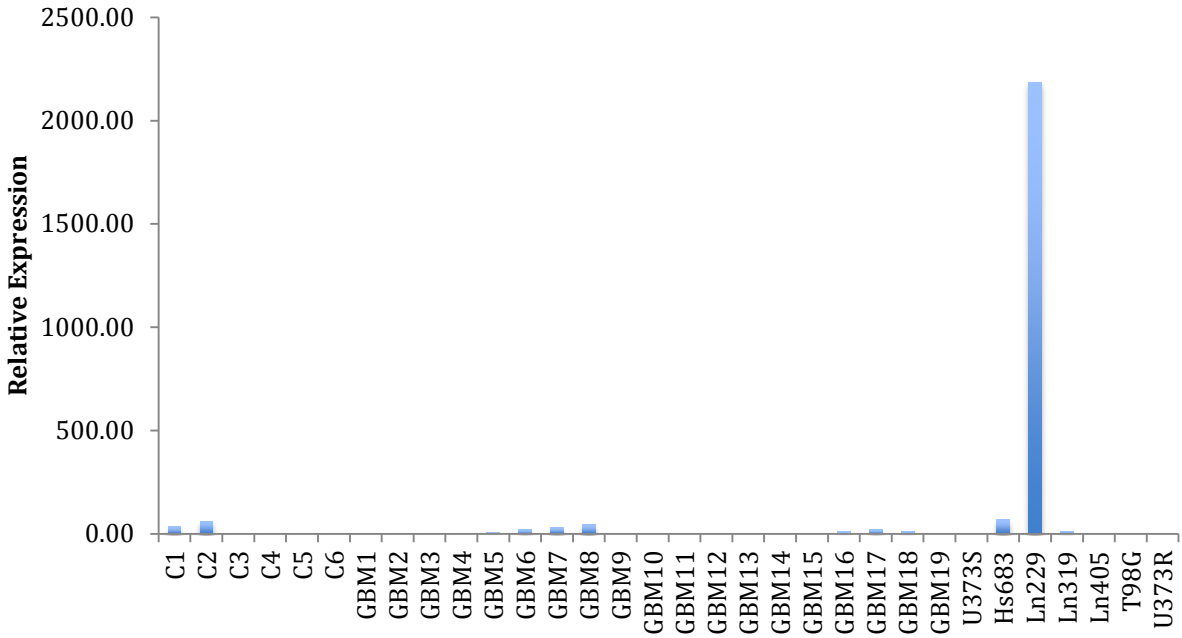


**Figure 4**: Xist lncRNA transcript expression in GBMs. Relative expression of lncRNA Xist as quantified by qRT-PCR. C1-C6 represent the primary control samples, GBM1-GBM19 represent primary GBM samples and remaining samples represent glioma cell lines.

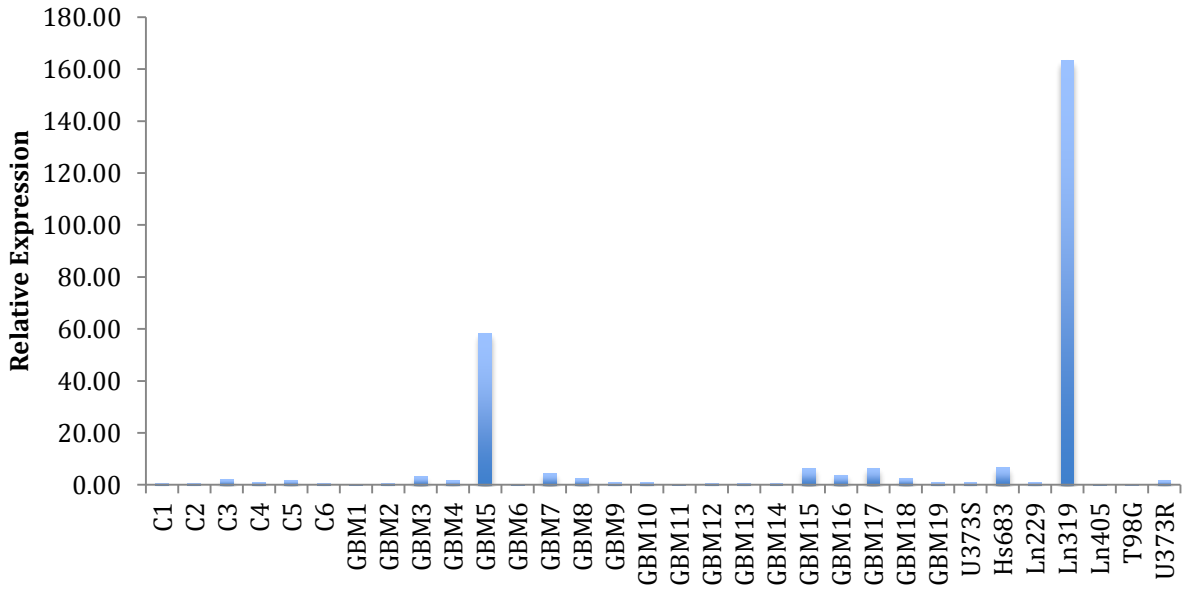


**Figure 5**: LncRNA CCAT2 levels in multiple glioma samples as assessed by qRT-PCR. C1-C6 represent the primary control samples, GBM1-GBM19 represent primary GBM samples and remaining samples represent glioma cell lines.

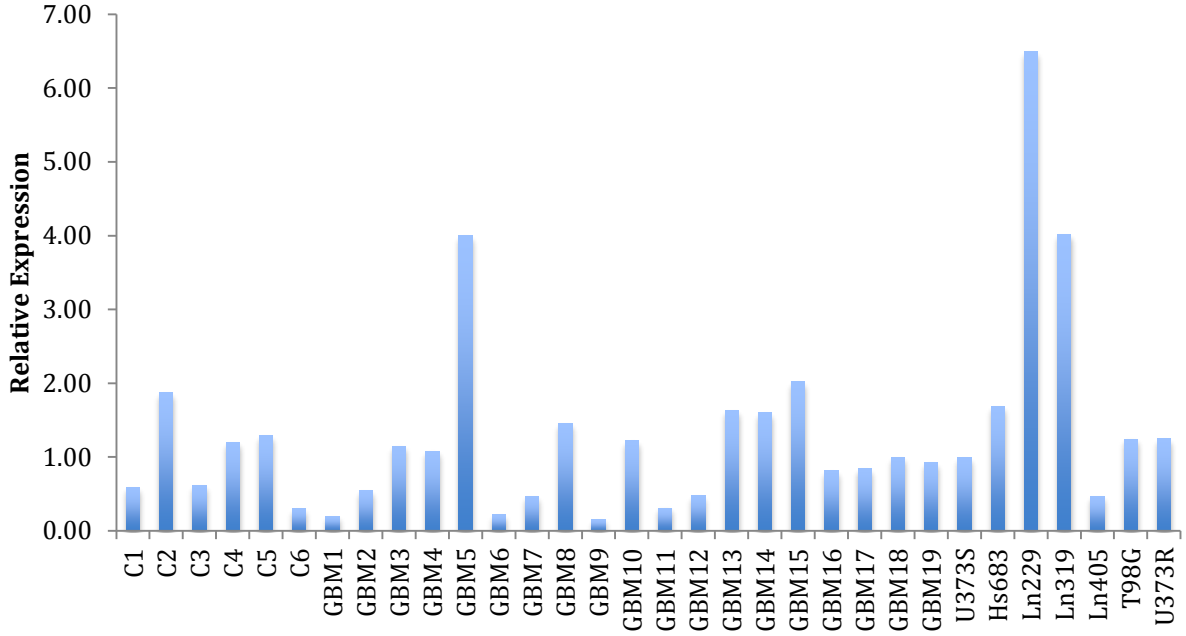


**Figure 6**: Relative expression of lncRNA Tug1 in glioma samples as measured via qRT-PCR. C1-C6 represent the primary control samples, GBM1-GBM19 represent primary GBM samples and remaining samples represent glioma cell lines.

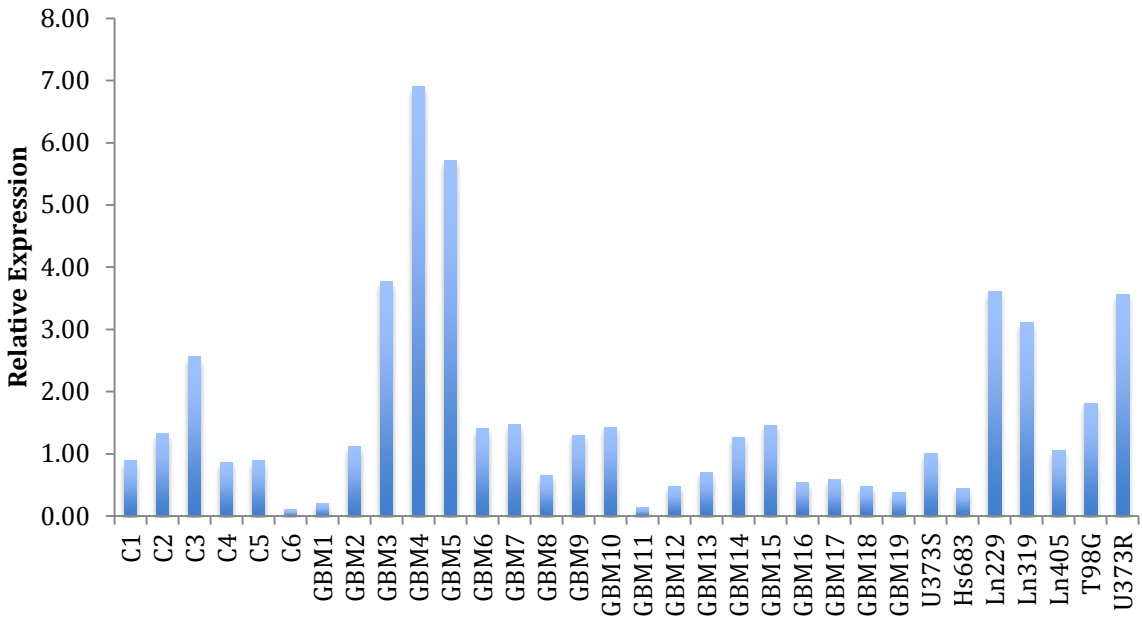


**Figure 7**: Panda transcript expression in GBMs. Relative expression of lncRNA Panda measured by qRT-PCR. C1-C6 represent the primary control samples, GBM1-GBM19 represent primary GBM samples and remaining samples represent glioma cell lines.