Progress Report

Author: Mr Walter Muskovic
Qualification: PhD Candidate
Institution: Children's Cancer Institute
Date: 22/01/2018

Title of Project: Unravelling the role of microRNAs in glioblastoma

Summary*:*

Despite decades of intensive research, a patient diagnosed with glioblastoma, a devastating form of brain cancer, still faces a dismal prognosis of only 15-18 months' survival. Trials of targeted therapies, while successful in other cancers, have proven ineffective for glioblastoma. This is thought to be largely due to differences between cancer cells within tumours. While some cells are sensitive to treatment and die, other, resistant cells remain and continue to grow. This scenario is like the evolution of bacterial resistance to modern antibiotics. To overcome this challenge and cure glioblastoma, alternative therapeutic candidates are needed.

One such candidate is a type of genetic material called microRNA, which regulates gene expression. Growing evidence suggests that dysregulation of microRNA regulatory networks is a key driver of glioblastoma pathogenesis, suppression of differentiation (normal cell development) and drug resistance. Processes affected by microRNAs include invasion into normal brain tissue, disrupted programmed-cell death and cell cycle regulation. However, the precise role played by microRNAs in these processes has remained enigmatic. The aim of this project was to provide insights into the nature of microRNA–target gene interactions in glioblastoma, and into the potential of microRNA as a therapeutic target in glioblastoma.

MicroRNAs, unlike messenger RNAs, do not code for proteins. Instead they interact directly with tens, or even hundreds, of genes, regulating their expression. Because many genes are abnormally expressed in cancer cells, targeting a global regulator like microRNA rather than a particular mutation (which may only be present in a portion of glioblastoma cells), may be an effective way to overcome resistance to therapy. To do this it is critical to identify which microRNA, out of the several thousand already known, would be the best therapeutic target. This requires knowing the functional role of individual microRNAs, i.e. which genes they regulate; not an easy task.

Computational predictions of microRNA target genes can be notoriously hard to interpret and often do not take actual biological conditions into account. We developed an innovative bioinformatics approach which successfully identified microRNAs that affect glioblastoma biology. Analysis revealed that, as expected,

these microRNAs are interacting with multiple genes simultaneously. Unexpectedly, our results suggested that microRNAs, contrary to current thinking, are activated at precisely the same moment as the genes they regulate. This result initially appeared contradictory. Activation of a gene accompanied by the simultaneous activation of a microRNA to turn off that gene is counterintuitive. Figure 1 summarises this finding.

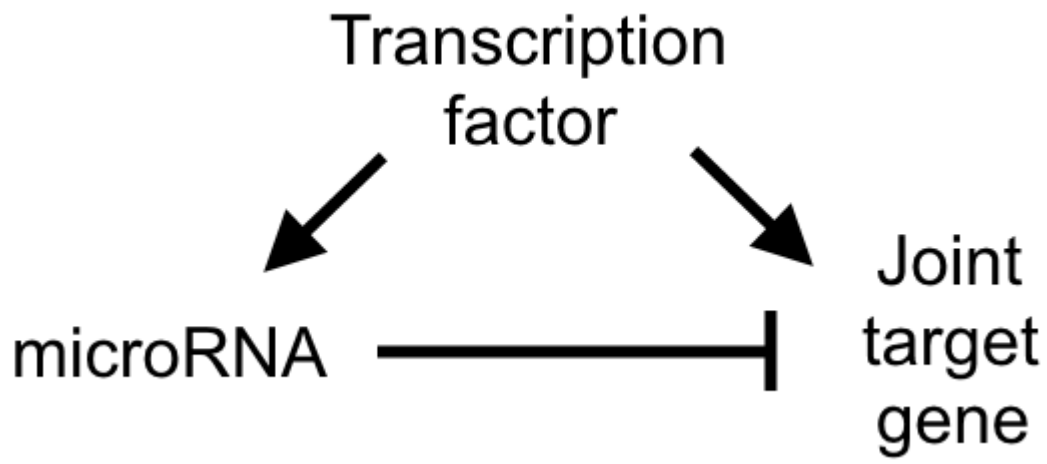

Figure 1. microRNAs are activated at the same time as the genes they regulate

The Brain Foundation grant enabled us to further investigate this finding and its biological significance. We designed a time-series experiment using cultures of synchronised cells. By tightly controlling growth conditions, we caused glioblastoma cells to proceed through the cell cycle in tight synchrony, so that every cell expressed the same genes and same microRNAs at the same time. We harvested cells at ten-minute intervals over a seven-hour period, then extracted and counted the number of each RNA species over time, using RNA-sequencing technology (RNA-seq).

RNA-Seq enables the identification of all the genes that are activated in a cell, and measurement of the levels to which each is activated. Unlike the older, microarray-based approaches, RNA-Seq does not require advance knowledge of which microRNAs or genes are activated. Genes and microRNAs can all be captured across a large dynamic range of detection, allowing accurate quantification with high resolution. With a small pilot time-series experiment of eight time points we confirmed the validity of this approach, and determined that the quality of the data was excellent and suitable for our purposes.

We have recently completed the higher time-resolution RNA-seq experiment. Examples of the gene profiles we obtained are shown in Figure 2. They demonstrate the high degree of precision we achieved in capturing the dynamics of gene expression. This precision gives us confidence in the output of our computational model.

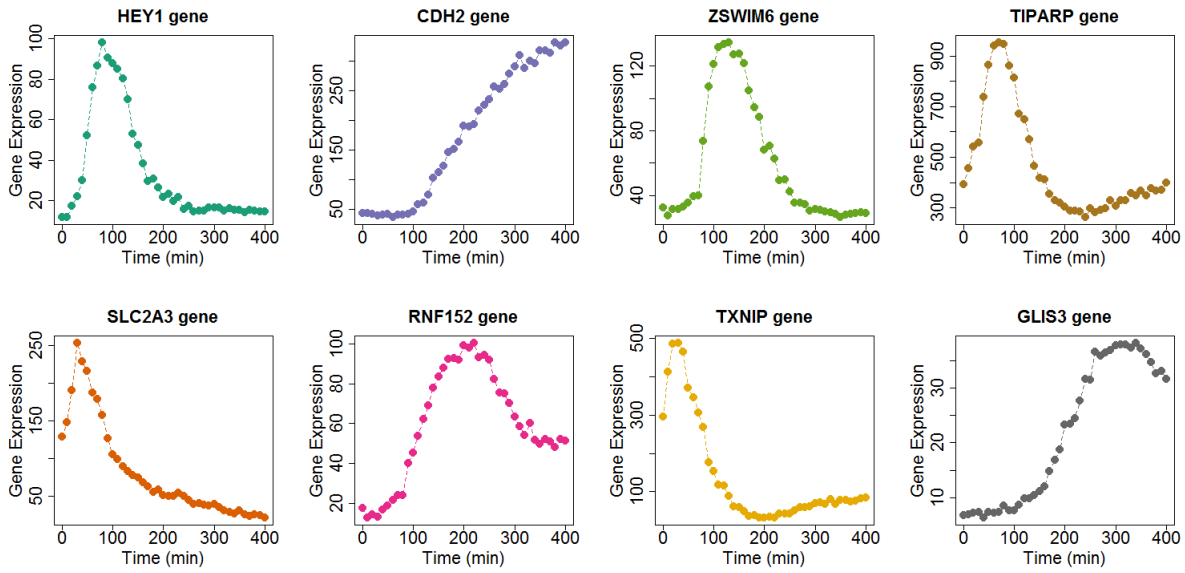

Figure 2: Gene expression profiles from the higher time-resolution RNA-seq experiment

Hypothesis vs Findings

Analysis of gene and microRNA expression profiles in glioblastoma cells using our model is confirming our hypothesis that microRNAs are co-expressed with their target genes. Furthermore, the unprecedented time-resolution of this experiment enabled detection of a much larger number of genes involved in these processes than had been previously identified.

Unanswered Questions

The RNA-seq time series experiment enabled us to link expression of microRNAs and their target genes, and thus infer functional interactions. We are currently investigating these microRNA-target gene relationships further using a more sophisticated modelling approach. This will enable us to connect individual microRNAs to their target genes. By mapping this network of interactions throughout the glioblastoma cell cycle, we hope to design rational therapies to disrupt these relationships.

What these research outcomes mean

This work has provided a key insight into the nature of microRNA–target gene interactions in glioblastoma. Comparing our own analysis to published observations, we have shown that while all microRNAs repress gene expression, in the context of a dynamic network of genes a more nuanced view is required. Rather than simply switching off target genes, individual microRNA-target interactions fine-tune the timing and level of target gene expression. These novel findings have yet to be

presented as they are being prepared for peer review publication where the support of the Brain Foundation will be acknowledged.