Do MicroRNAs Drive Glioblastoma Cell States?

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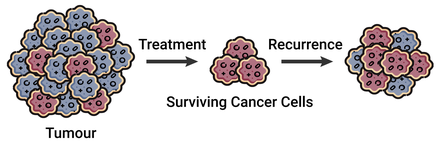
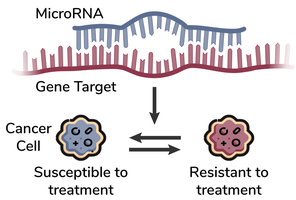
Introduction

Glioblastoma is an extremely aggressive and incurable brain cancer

2000 Australians are diagnosed a year with an average survival time of 12-15 months

One of the key challenges with treating glioblastoma is that it is extremely heterogenous – within the same tumour there are populations of cancer cells with different characteristics such as growth rates, invasiveness, or susceptibility to treatments. This type of heterogeneity can be driven by genetic variations or non-genetic mechanisms such as **cancer cell plasticity** and is considered one of the major factors preventing successful treatment of glioblastoma, with nearly all patients experiencing tumour recurrence.





Cancer Cell Plasticity refers to the ability for cancer cells to reversibly change their identity or state in response to environmental queues such as neighbouring cell signals or exposure to drugs. The genes which govern this behaviour are poorly understood.

MicroRNAs are small non-coding RNAs which regulate the expression of other genes. Their dysregulation is almost universally observed in cancer and many miRNAs have already been identified as contributors to glioblastoma oncogenesis. As a single miRNA gene can regulate hundreds or thousands of other genes, they are well positioned in gene circuits to coordinate the changes in gene expression required for cell state transitions.

How can studying microRNAs contribute to glioblastoma research?

Although single cell sequencing methods have recently been developed for detecting miRNAs, research has not utilised them to understand their contribution to different cell states in glioblastoma. This type of research may unearth novel mechanisms that glioblastomas may use to adapt and survive when targeted by drugs, providing us with new gene pathways to target. Also, since microRNAs are highly specific to cell types and often released into blood, they have huge potential as biomarkers which may be able to identify tumour states quickly and non-invasively.

Methods

1 Data Collection

Single cell datasets from 1 small RNA sequencing (smRNA-seq) and 4 single cell RNA sequencing (scRNA-seq) studies on glioblastoma were downloaded and used in this study.

2 Sequence Alignment and Gene Quantification

For small RNA-seq data, reads were aligned to the miRNA transcriptome and quantified using miraligner. For RNA-seq data, reads were aligned to the human genome and quantified using bowtie and featureCounts.

3 Identification of Novel Cell Subpopulations

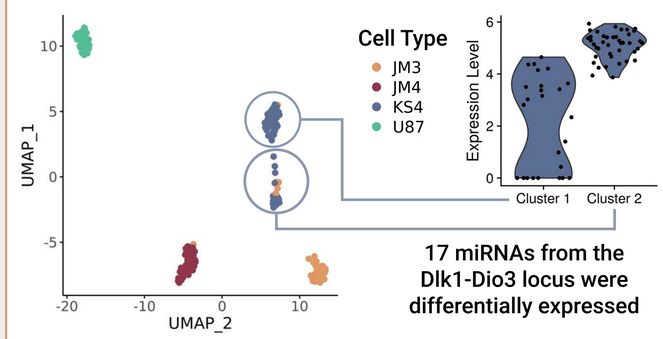
Cell subpopulations were identified by first reducing dimensionality of cell data (PCA/UMAP) and then clustering (using Seurat). Clusters were compared to identify differentially expressed genes.

4 Correlation of Genes with Glioblastoma States

To expand our study with single cell RNA-seq data - cells were assigned scores based on their expression of known cell states (cell states/scoring method from Neftel et al[1]). Scores were correlated against miRNA-related genes.

Results

Unsupervised clustering using miRNA levels identified two potential cell states in KS4 Glioblastoma cells



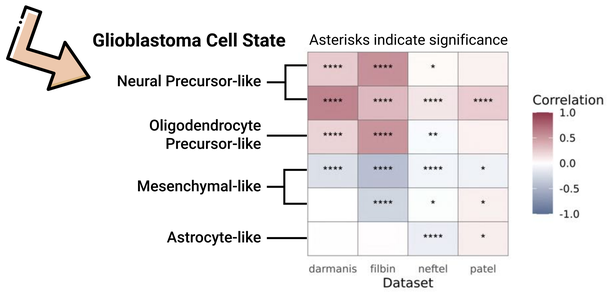
17 miRNAs from the Dlk1-Dio3 locus were differentially expressed

We identified the presence of two cell populations within a single glioblastoma cell line and found these populations were distinguished by miRNAs that were nearly all located in a single genome cluster called the Dlk1-Dio3 locus.

Because of limitations in available data, single cell RNA-seq data was used to connect glioblastoma cell states with Dlk1-Dio3 gene expression.

The long non-coding RNA MEG3 was used as a marker for Dlk1-Dio3 miRNA expression as it is coexpressed as a primary transcript with nearly all miRNAs from this locus.

Genes from the Dlk1-Dio3 locus (MEG3) correlate with a neural precursor-like state



- We found a positive correlation with MEG3 and a neural precursor-like state, consistent across the 4 datasets we looked at.
- This cell state has previously been linked to a reduction in cell proliferation and better patient survival.
- To our knowledge this is the first example of miRNAs being associated with this state.

Conclusions

1. **miRNAs can be used to identify cancer cell subpopulations**
2. **miRNAs from the Dlk1-Dio3 locus are associated with a neural precursor-like cell state**
3. **This provides further support that miRNAs are involved in driving glioblastoma cell states**
4. **Validation of our findings in the lab using glioblastoma tumour samples is ongoing**

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References: 1. Neftel, C. et al (2019), An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma, Cell, vol. 178, no. 4, pp. 835-849