Investigating the Role of Novel MicroRNAs in Granule Cells during Mouse Cerebellar Development

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Introduction

The cerebellum is involved in key motor and non-motor functions. Gene mutations and/or environmental perturbations during cerebellar development can alter the pattern of gene expression via deregulation of epigenetic factors and result in cerebellar dysfunction and a wide range of neurodevelopmental and psychiatric disorders. miRNAs are key regulators of gene expression. Perturbations of key miRNAs can perturb development, particularly in the most numerous cell in the brain and cerebellum, the granule cells (GCs). miRNAs (and their cognate DNA sequences) important for granule cell development from early postnatal days (P) are identified to study how miRNAs regulate development.

Methods & Results

To distinguish candidate miRNAs important to the developing cerebellar granule cell; granule cells were isolated at P0, P3, P6, and P9 and m- and mi-RNAs were extracted. After RNA-sequencing, a bioinformatics exploration of the time-course transcriptional data is completed to create a catalog of miRNAs in granule cells at different developmental stages. The focus was on miRNAs that are granule cell-specific and expressed in a dynamic pattern over time.

1A

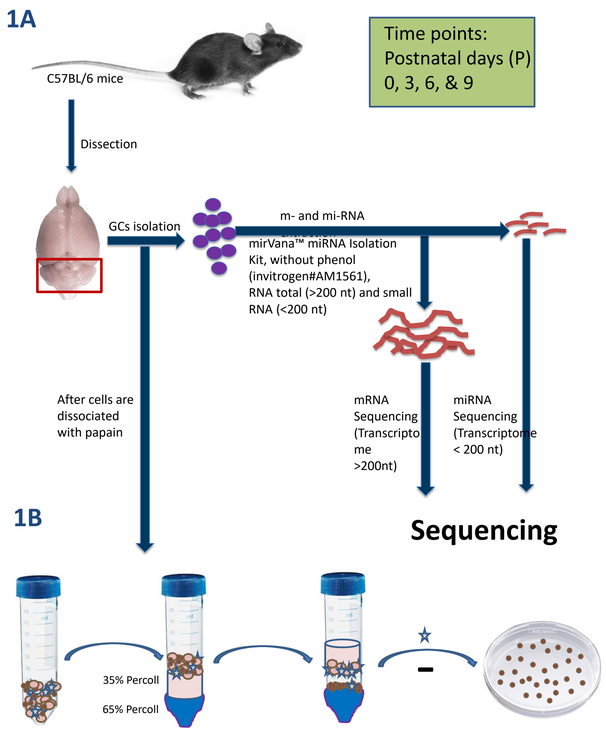
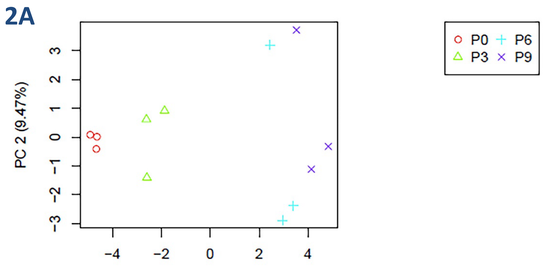


Fig.1 A) A schematic illustration of granule cell isolation, mRNA and miRNA extraction is shown. B) Granule cell isolation is done by Percoll gradient and pre-plating (Poly-D-Lysine coated plates) to reduce the number of glial cells.

Quality Control Evaluation

2A



2B

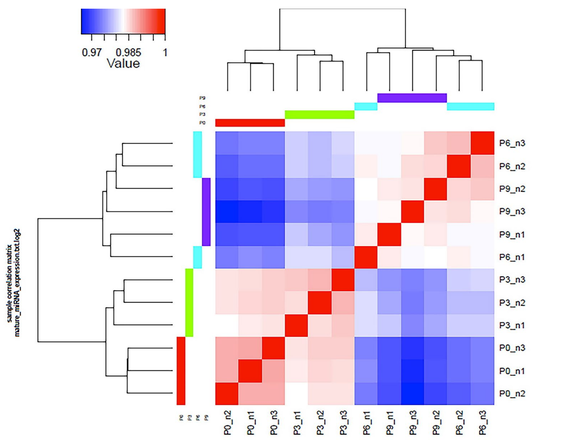
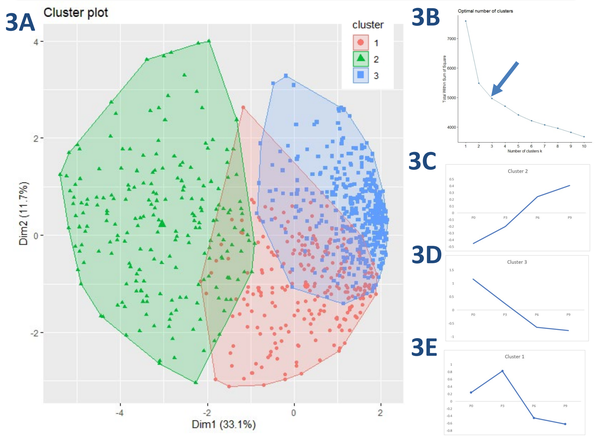


Fig.2 A) Principal Component Analysis shows similarity between P0 and P3, and between P6 and P9 (triplicate samples). B) The heatmap/correlation Matrix shows clustering in each sample and also between P0 & P3, and between P6 & P9.

Pattern Expression

3A



3B

3C

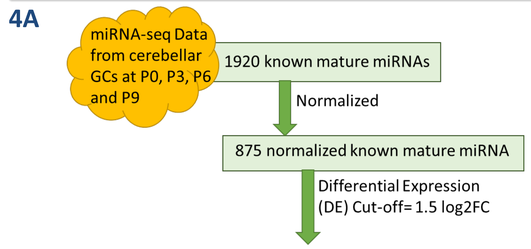
3D

3E

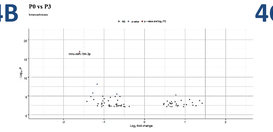
Fig.3. A) Using K-means clustering, 3 clusters of miRNAs (collection of data points aggregated together) were detected and mean expression showed three different patterns: B) Optimal number of clusters are shown as 3 clusters. C) up, D) down regulations, and E) bimodal regulation.

Differential Expression

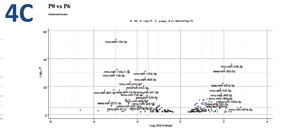
4A



4B



4C



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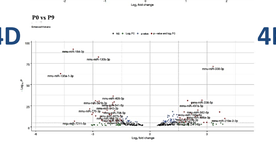

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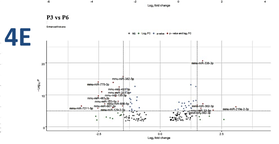

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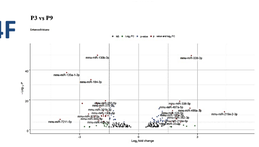

4G

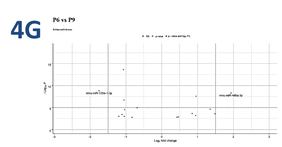

Fig.4. To define significantly, differentially expressed miRNAs. A) miRNA-seq data were prefiltered and normalized, then enhanced volcano plot (R package) was used comparing all the time points and significantly differential expressed miRNAs shown at upper-left (down-regulated miRNAs) and upper-right (up-regulated miRNAs): B) P0 vs P3 (1 down-regulated miRNA), C) P0 vs P6 (50 down-regulated and 23 up-regulated miRNAs) , D) P0 vs P9 (57 down-regulated and 35 up-regulated miRNAs) , E) P3 vs P6 (19 down-regulated and 7 up-regulated miRNAs) , F) P3 vs P9 (30 down-regulated and 17 up-regulated miRNAs) , G) P6 vs P9 (1 down-regulated and 1 up-regulated miRNAs). Through time progress from P0 to P9 there is a dynamic up- and down-regulation of miRNAs. Number of miRNAs in each comparison is 875 variables. x-axis shows log2FoldChange; y-axis shows –log10pvalue. Cutoff for log2FoldChange was 1.5 and p-value is 0.05.

Candidate miRNAs

Candidate miRNAs and their possible targets (Table 1) were elucidated from the list of miRNAs which showed significant differential expression and followed further filters shown in fig. 5.

Candidate miRNAs	mRNA targets
mmu-miR-541-5p	Adamts4, Camta1
mmu-miR-382-5p	Camta1 & Nfia
mmu-miR-770-5p	Camta1
mmu-miR-667-5p	Galr1
mmu-miR-669h-5p	Camta1
mmu-miR-139-5p	Nfib

Table 1. List of candidate miRNAs and their possible targets expressed in developing cerebellar granule cells.

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miRNA-mRNA interaction

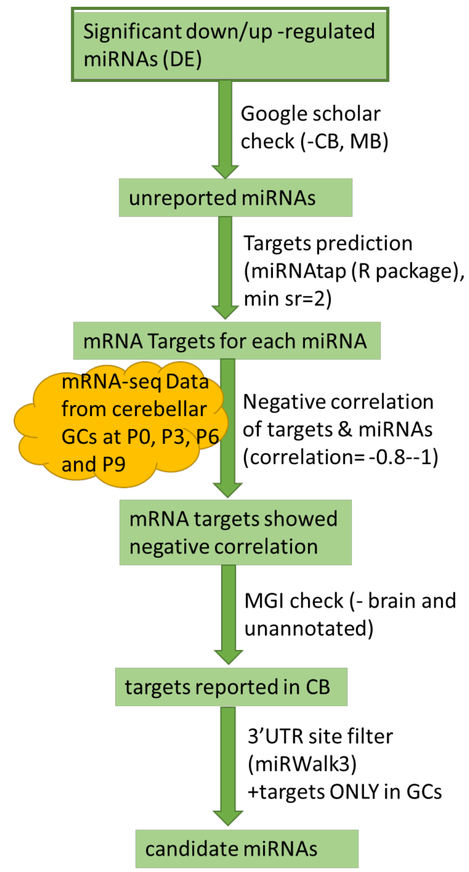

Fig.5. A flowchart represent a pathway used to find novel miRNAs involved in cerebellar development. Different Google scholar search helped to find unreported and omit already reported miRNAs in cerebellum (CB), and medulloblastoma (MB). In parallel to miRNA-seq analysis we conducted mRNA-seq data on GCs at same time points. The stringent negative correlation (-0.8 - -1) was found between miRNAs and their targets (mRNA-seq data). Mouse Genome Informatics (MGI) was categorized targets expression in brain, CB, and unannotated . miRWalk filtered targets for 3'UTR.

Future Direction

To validate the candidate miRNAs that are specific to the developing cerebellar granule cell: The miRNAs will be further filtered for those that are quantitatively replicated with qRT-PCR and spatially validated with *in situ* hybridization. Then to evaluate the miRNA targeting sites a Luciferase reporter gene assay will be utilized. To perturb the best validated candidate miRNAs and understand the importance of these miRNAs in cerebellar granule cell development and disease I plan to perturb gene expression in granule cell cultures and mice by stereotaxic surgery to knockdown or over-express these miRNAs. The deliverable will be novel roles of miRNAs and their target sequences from which they are transcribed to show their importance in granule cell development and disease.