Negatively-correlated tRF5 expressions with mRNA and protein expressions in iPSC-derived microglia cells in Alzheimer's disease

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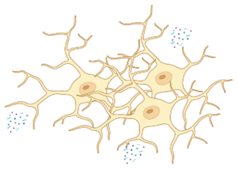
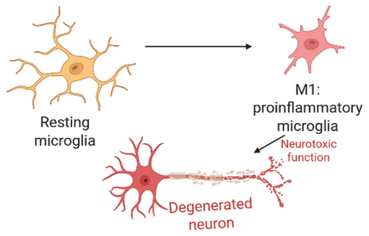
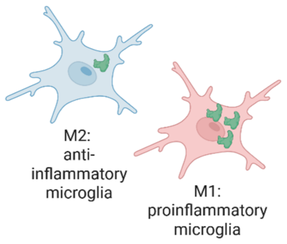
BACKGROUND



Microglia are responsible for maintaining neuron homeostasis through clearing cellular debris, misfolded proteins, and dead cells

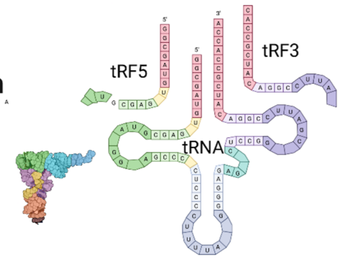
Activated M1 microglia can lead to neurotoxic function and degeneration in Alzheimer's patients





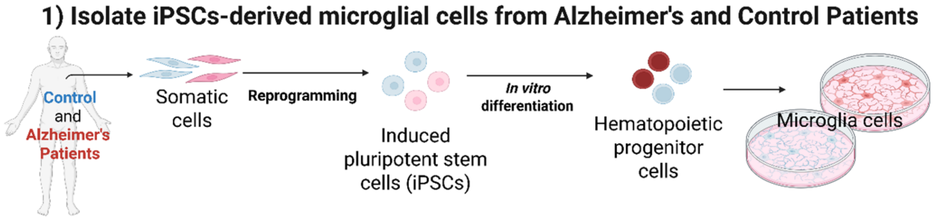
Understanding differences in gene expression between healthy and abnormal microglial cells could identify biomarkers for future therapies

tRNA fragments (tRFs) interfere with gene expression and could be a potential biomarker target



METHODS

1) Isolate iPSCs-derived microglial cells from Alzheimer's and Control Patients



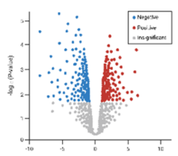
2) Extract, Measure, and Map small RNAs, total RNAs, and Proteins

small RNAseq: sRNA mapping database (miRNA, tRFs, snRNAs, snoRNAs, piRNAs)

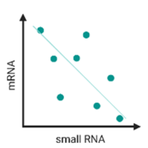
total RNAseq: mRNA mapping database was created using RefSeq transcripts

Proteomics: Protein expression measured using MALDI-TOF

3) Identify Significant Differentially Expressed tRFs, mRNA, and protein

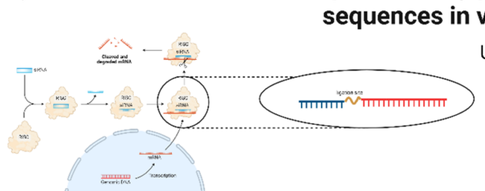


4) Identify tRFs v Protein pairs with negative correlation



tRF5s and tRF3s have been shown to silence gene expression

5) Filter for tRFs that have experimentally proven hybridization with mRNA sequences in vivo



Using data generated through cross-linking ligation and sequencing of hybrids (CLASH) methodology

Hybrids are chimeric sequences of tRF and mRNA nucleotides in the AGO protein

6) Calculate the bioenergetics hybridization energy of tRF and mRNA sequences for most favorable connections

RESULTS

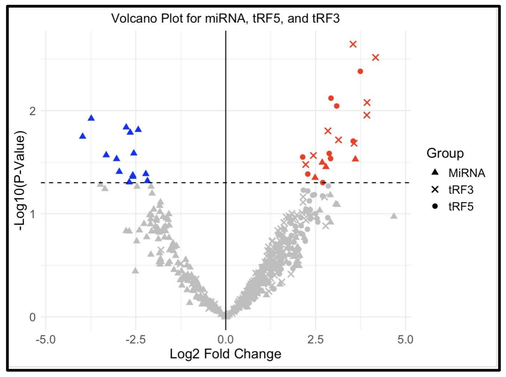


Figure 1: Volcano Plot of significant differentially expressed miRNAs, tRF3s, and tRF5s in Alzheimer's versus Control IPSC- derived microglia cells. Blue indicates significant small RNAs that are downregulated in Alzheimer's patients, Red indicates significant small RNAs that are upregulated in Alzheimer's patients. All tRFs are upregulated, while miRNAs are more downregulated

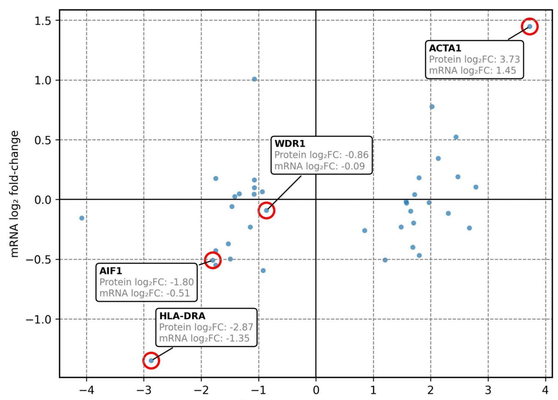


Figure 2: Correlation plot of the Log Fold Change of mRNA v the Log Fold Change of protein differences between control v Alzheimer's IPSC-derived microglia cells. Two significant mRNA/protein products, ACTA1 and HLA-DRA were identified

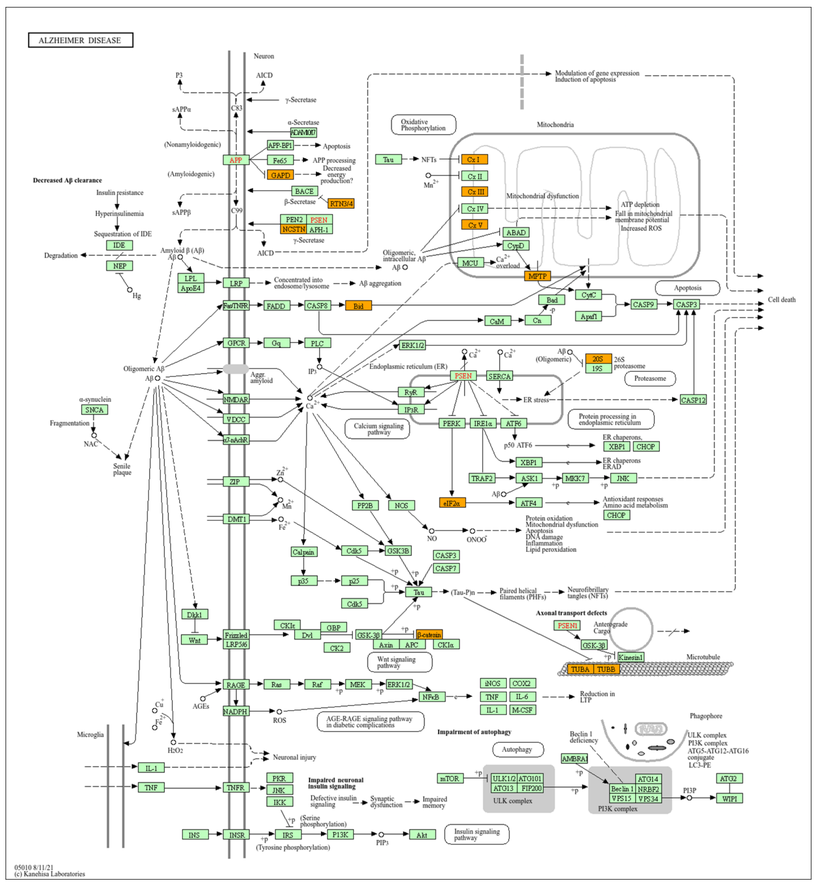


Figure 3: KEGG figure of Alzheimer's Disease pathway and implicated genes. Proteins colored in orange indicate protein targets that were negatively correlated with differentially expressed tRFs in this analysis.

KEY FINDINGS

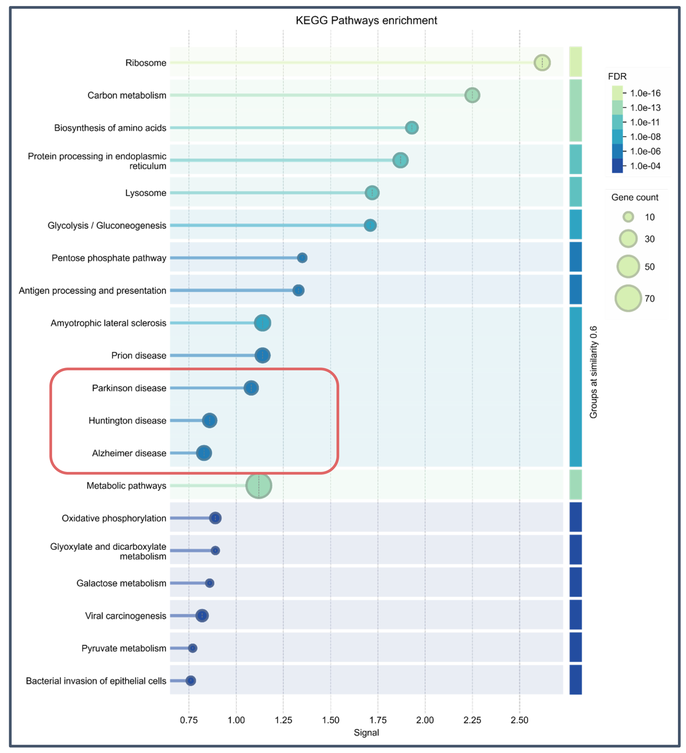


Figure 4. KEGG Enrichment calculated using String-db.org. Top enrichment pathways indicate a relationship between the tRF-mRNA targets identified in this study and neurodegenerative disease. A total of 319 mRNA targets were used for the string-db analysis.

CONCLUSION

- Analysis of iPSC-derived microglia revealed significant in vitro tRF5 expression differences and related mRNA/protein targets.
- HLA-DR and WDR1 proteins were identified as down-regulated and negatively correlated with multiple tRFs (correlation $p < 0.05$), while their respective mRNA UTRs and tRFs have strong interaction sites, suggesting tRF5 regulation
- HLA-DR and AIF1 have previously been implicated in the regulation of the brain microenvironment suggesting potential key roles in disease pathology.

ACKNOWLEDGEMENTS

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