Describing the role of microRNAs in Alzheimer's disease using a bioinformatic approach

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Overview

To understand the underlying molecular mechanisms that cause disease, it is important to know the functions of the genes involved[1]. Functional data indicates microRNAs (miRs) silence gene expression[2]. Unfortunately the functional miR data being generated by research is not being stored in a consistent manner on bioinformatic databases, where biological knowledge can be easily accessed and analysed[1]. In order to make this information available on bioinformatic databases, annotations are required. The Gene Ontology (GO) provides descriptive terms, known as GO terms, that are used to describe gene products. GO terms are utilised in the formation of annotations, where a gene product is connected to a GO term, thus allowing for a statement to be made about the function of a gene product[3]. This project looked to identify a role for miRs in Alzheimer's disease (AD). Therefore, we focused on the annotation of miRs that regulate the expression of amyloid beta clearance proteins[4] (Figure 1), as a loss of amyloid beta clearance is thought to be critical in the development of neurodegeneration in AD.

1. Huntley RP, *et al*. Expanding the horizons of microRNA bioinformatics. RNA 2018 24:1005-1017
2. Huntley RP, *et al*. Guidelines for the functional annotation of microRNAs using the Gene Ontology. RNA 2016 22:667-676
3. The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still Going strong. Nucleic Acid Res 2019 47:D330-D338
4. Jarosz-Griffiths HH, *et al*. Amyloid-beta Receptors: The Good, the bad, and the Prion Protein. J Biol Chem 2015 291:3174-3183

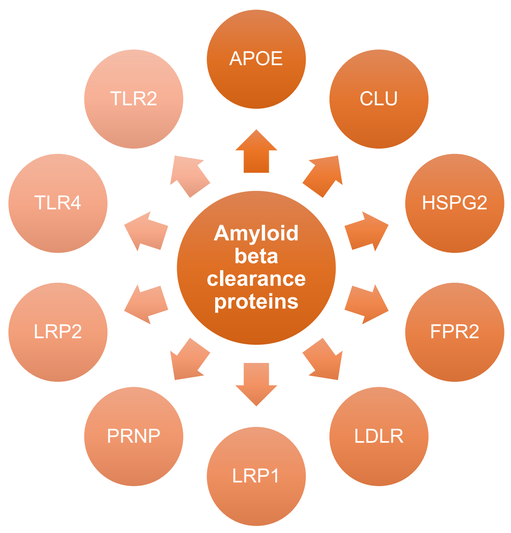

Figure 1: Amyloid beta clearance proteins. Proteins involved in amyloid beta clearance which were previously reviewed by Jarosz-Griffiths et al. (2015).

GO for miRs and annotation formation

MiRs can silence gene expression post-transcriptionally, by directly binding to messenger RNA (mRNA) transcripts and targeting them for cleavage, translational repression or deadenylation. Three GO terms describe this ability of miRs (Figure 2b). They were all used within this project to form annotations (Figure 2) when experimental data such as luciferase assays, western blots & quantitative polymerase chain reactions (q-PCRs) were available to validate a miR's function.

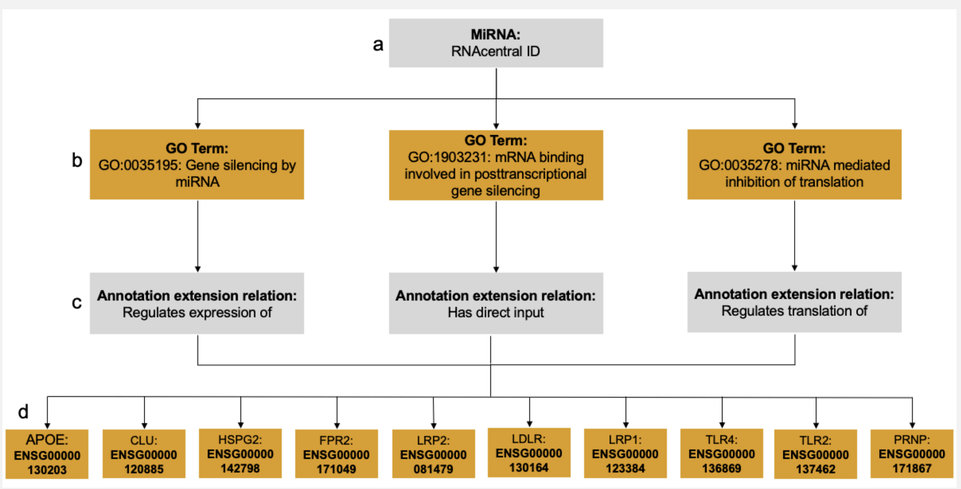

Figure 2: GO for miRs and annotation formation. A flow chart illustrating how annotations were formed (based on experimental data) to capture the role of miRs in silencing the expression of amyloid beta clearance proteins. (a) Firstly a RNAcentral ID unique to a specific miRNA is connected to a (b) GO term that describes the ability of a miR to silence gene expression. (c) A relation is then added between each GO term and the (d) Ensembl gene ID for the gene of a protein involved in amyloid beta clearance. (d) Indicates the gene being silenced by the miR applied to (a).

Annotation Summary

A total of 39 miRs were found to silence the expression of proteins involved in amyloid beta clearance (Figure 1) by directly interacting with mRNA transcripts. Through the curation of experimental data this information was captured by 88 GO annotations.

Example of a GO annotation

Gene Product	Qualifier	GO Term	Evidence	Reference	Taxon	Assigned By	Annotation Extension
RNAcentral:URS00005DBAF3_9606 hsa-miR-193b-3p	involved_in	GO:0035195 (P) gene silencing by miRNA	ECO:0000314 IDA	PMID:30451334	9606 Homo sapiens	ARUK-UCL	regulates_expression_of (ENSEMBL:ENSG00000171867) (PRNP)

Figure 3: Example of GO annotation. An example of a GO annotation formed within this project by ARUK-UCL. Here hsa-miR-193b-3p was annotated to the GO term 'gene silencing by miRNA' alongside the annotation extension 'regulates expression of PRNP (Ensembl gene ID)'. The experimental data to support this annotation came from Pease et al. (2019)[5] where a luciferase assay showed transfection of hsa-miR-193b-3p to significantly reduce the expression of PRNP's 3'UTR.

5. Pease D *et al*. Genome-wide identification of microRNAs regulating the human prion protein. Brain Pathol 2019 29:232-244

Network analysis of miRs and GO enrichment

Cytoscape[6] was utilised to visualise the miR:mRNA interactions captured by annotations made within this project (Figure 4). The nodes represent either miRs or mRNAs whilst the edges between them indicate an interaction. Within Cytoscape, a GO enrichment analysis was also conducted in order to overlay Alzheimer's relevant GO terms (Figure 4) with the gene products of the Cytoscape network. This was important as it showed other ways in which miRs may be involved in AD development and progression, outside of reducing amyloid beta clearance.

6. Demchak B, et al. Cytoscape: the network visualization tool for GenomeSpace workflows. Version 2. F1000Res. 2014 3:151

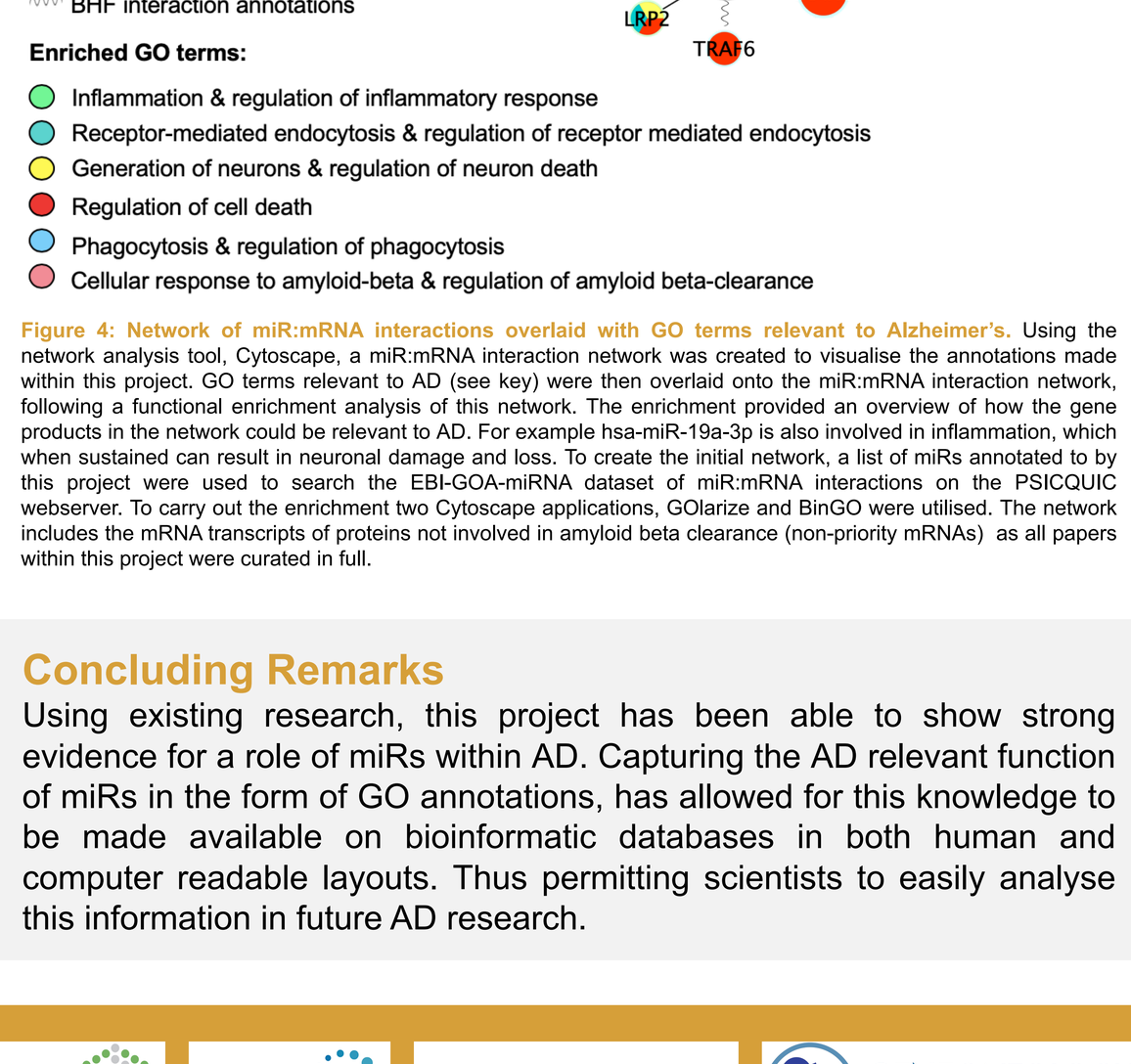

Figure 4: Network of miR:mRNA interactions overlaid with GO terms relevant to Alzheimer's. Using the network analysis tool, Cytoscape, a miR:mRNA interaction network was created to visualise the annotations made within this project. GO terms relevant to AD (see key) were then overlaid onto the miR:mRNA interaction network, following a functional enrichment analysis of this network. The enrichment provided an overview of how the gene products in the network could be relevant to AD. For example hsa-miR-19a-3p is also involved in inflammation, which when sustained can result in neuronal damage and loss. To create the initial network, a list of miRs annotated to by this project were used to search the EBI-GOA-miRNA dataset of miR:mRNA interactions on the PSICQUIC webserver. To carry out the enrichment two Cytoscape applications, GOlarize and BinGO were utilised. The network includes the mRNA transcripts of proteins not involved in amyloid beta clearance (non-priority mRNAs) as all papers within this project were curated in full.

Concluding Remarks

Using existing research, this project has been able to show strong evidence for a role of miRs within AD. Capturing the AD relevant function of miRs in the form of GO annotations, has allowed for this knowledge to be made available on bioinformatic databases in both human and computer readable layouts. Thus permitting scientists to easily analyse this information in future AD research.

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