Functional annotation of dementia-related miRNAs using Gene Ontology

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Overview

To understand the basis of disease it is crucial to know the functions of the genes involved and the pathways they act in. The role of microRNAs (miRNAs) in the regulation of developmental and cellular processes is a relatively new field of study, however, the data generated from such research has, so far, not been organised optimally to allow inclusion of this data in pathway and network analysis tools. The association of proteins with terms from the Gene Ontology (GO)[1] has proven to be highly effective for large-scale analysis of functional data, but the equivalent data is currently lacking for miRNAs[2]. The GO resource provides dynamic, controlled vocabularies that allow consistent descriptions of the functional attributes and subcellular locations of all gene products. We are now focused on the annotation of microglial proteins[3] implicated in neuroinflammatory processes relevant to dementia and the miRNAs[4] that regulate expression of these proteins.

[1] Ashburner M, *et al*. Gene Ontology: tool for the unification of biology. *Nature Genetics* 2000 25:25-9.
[2] Huntley RP, *et al.* Expanding the horizons of microRNA bioinformatics. *RNA* 2018 24:1005-1017.
[3] Kramarz B, *et al.* Gene Ontology annotation of microglial proteins associated with dementia. *ARUK Conference 2019* **Poster P12.3.**
[4] Huntley RP, *et al.* Functional annotation of dementia-related miRNAs using Gene Ontology. *ARUK Conference 2019* **Poster P12.9.**

Gene Ontology for miRNAs

miRNAs can directly silence mRNA targets by three main mechanisms: mRNA cleavage; mRNA deadenylation; translational repression. GO terms are available which describe each of these mechanisms (Figure 1).

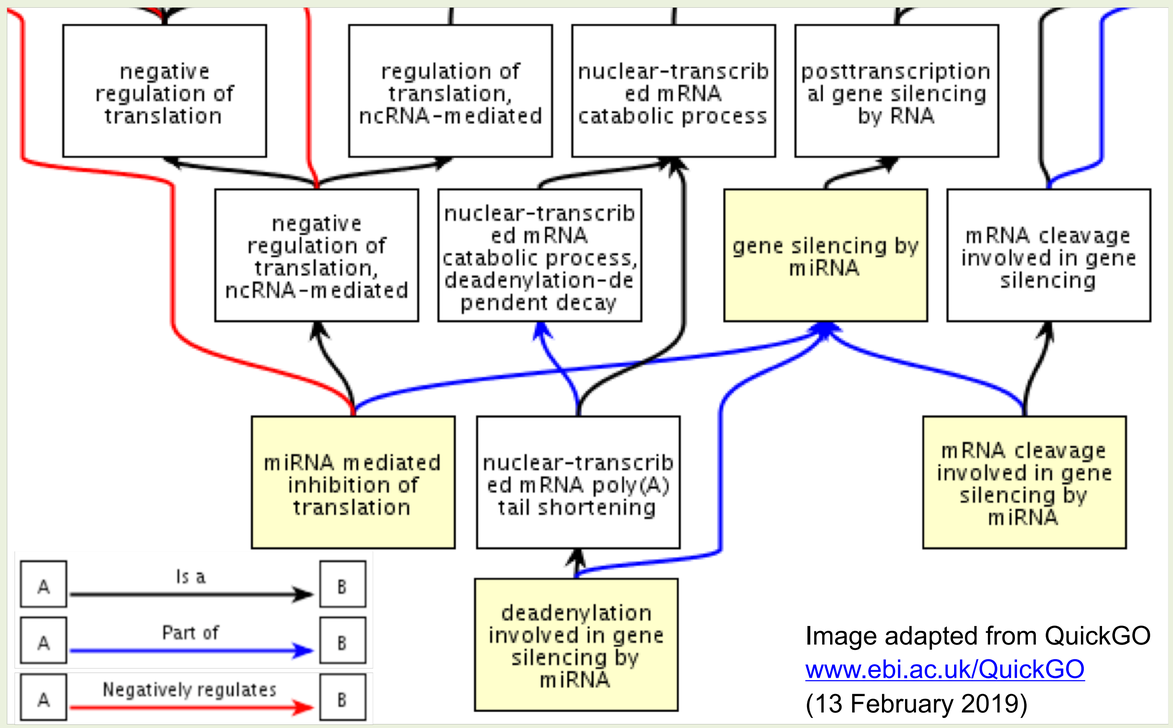

Image adapted from QuickGO
www.ebi.ac.uk/QuickGO
(13 February 2019)

Figure 1. QuickGO view of the GO terms used for curation of the miRNA's role in gene silencing. If the exact mechanism of silencing is experimentally demonstrated, i.e. translational repression, deadenylation or mRNA cleavage, the GO curators include the appropriate child terms of "gene silencing by miRNA" in the annotation.

miRNA Gene Ontology annotation

GO annotations and their contextual information can only go so far in describing a process or pathway. The GO Consortium have developed a tool, Noctua, which can be used to link together GO annotations and their associated evidence in order to give a more complete picture of the biological roles and activities of gene products (Figure 2).

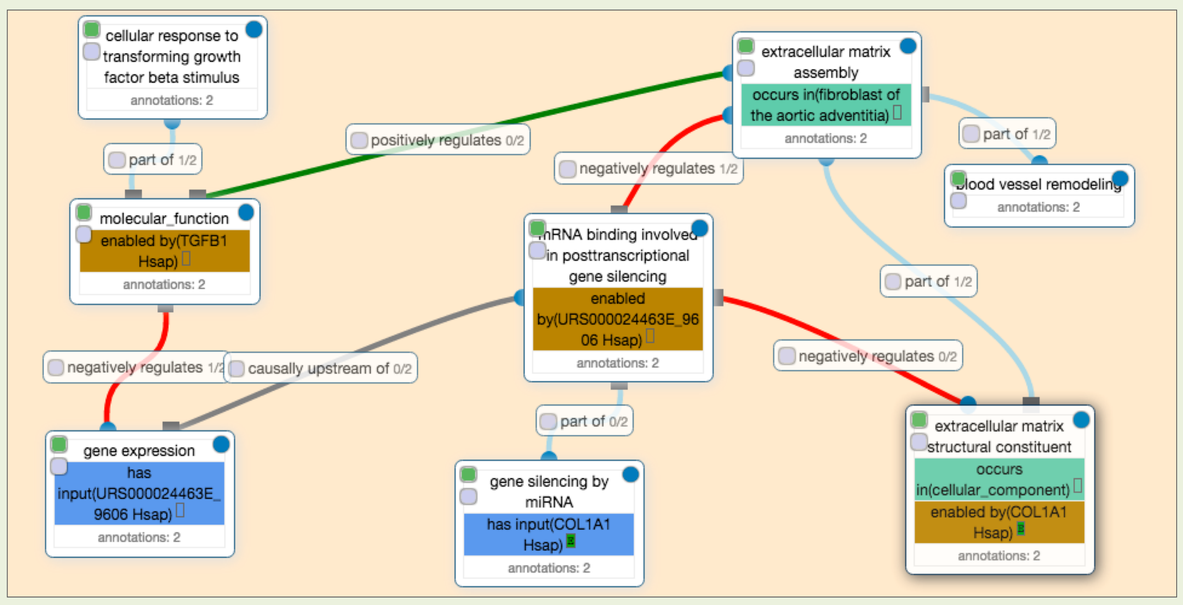

Figure 2. TGFβ1:miRNA-29b regulation of extracellular matrix assembly pathway model. miRNA-29b negatively regulates the expression of collagen 1A1 (COL1A1) and thus reduces extracellular matrix assembly. TGFβ1 (TGFB1) increases extracellular matrix assembly by negatively regulates the expression of miRNA-29b (URS000024463_9606) and thus increasing collagen 1A1 production. The model was created using Noctua (noctua.berkeleybop.org).

GO curation of dementia-related miRNAs

We capture both the role of the miRNA in gene silencing and the effect that the silencing event has on the cell or organism (Table 1), enabling researchers to easily find the roles of a miRNA and interpret large datasets.

	the miRNA's role in gene silencing	dementia-relevant mRNA targets	
miR-338-3p	Gene silencing by miRNA	Regulates expression of *SMO*	ARUK-UCL
	dementia-relevant processes regulated by the miRNA	**where (or when) the regulation by miRNA occurs**	**Curated by**
miR-140-5p	Cellular response to amyloid-beta	Occurs in *neuron*	ARUK-UCL

Table 1. GO annotation of the experimentally verified roles of miRNAs.

Network analysis of miRNAs

An analysis of Alzheimer's disease-relevant miRNAs (Figure 3) identifies that the network is highly connected with many miRNAs having common mRNA targets (for example both miR101-3p and miR-155-5p target RIPK1). In addition, the network demonstrates that a single miRNA may be regulating the expression levels of multiple Alzheimer's-associated proteins (for example both APP and RIPK1 are targets of miR101-3p).

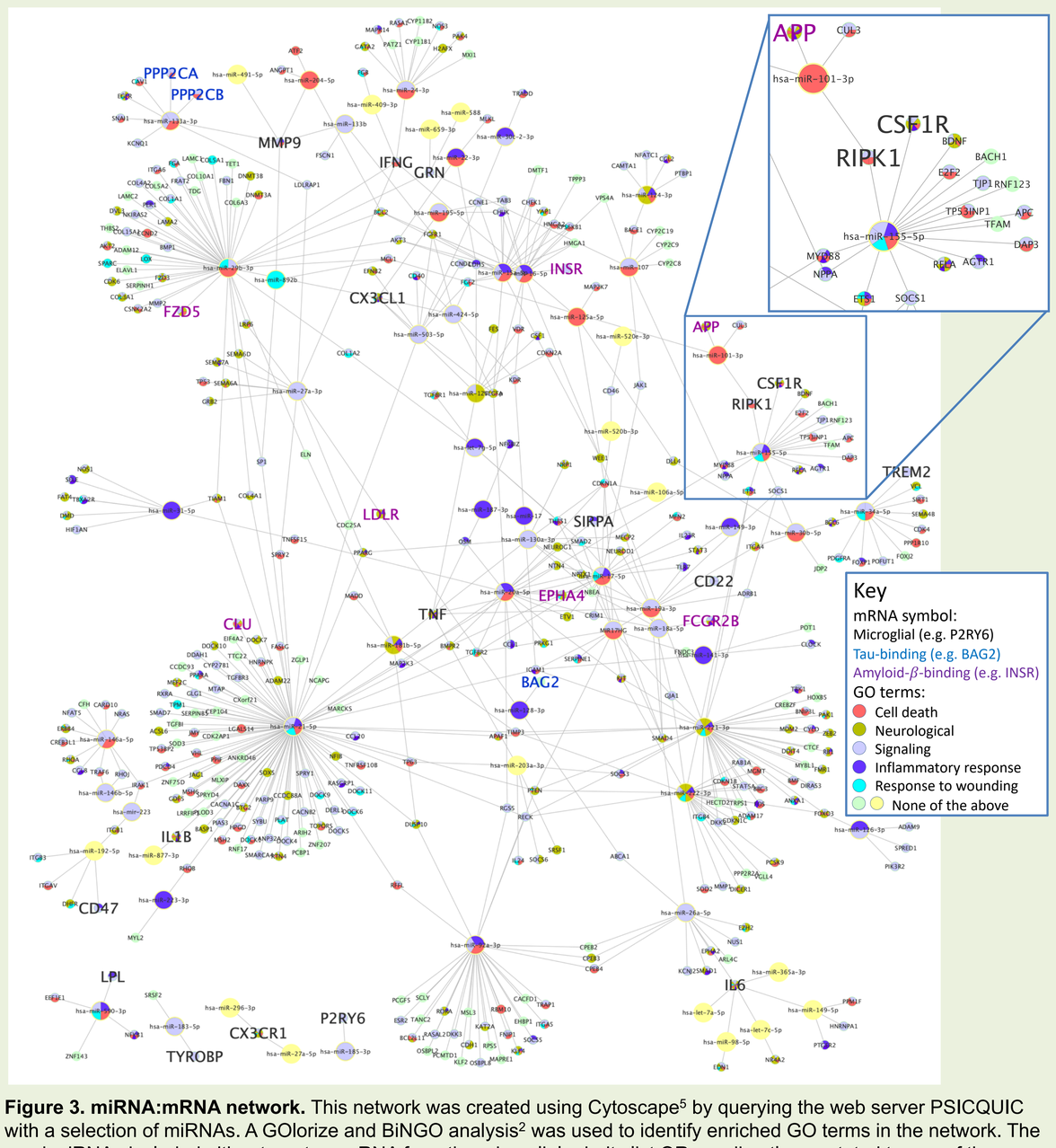

Figure 3. miRNA:mRNA network. This network was created using Cytoscape[5] by querying the web server PSICQUIC with a selection of miRNAs. A GOlorize and BiNGO analysis[2] was used to identify enriched GO terms in the network. The seed miRNAs included either target an mRNA from the microglial priority list OR are directly annotated to one of the following GO terms: microglial cell activation, immune system response and inflammatory response, or their regulation terms. The nodes represent miRNAs (large nodes) or mRNAs (small nodes), edges represent experimentally validated interactions. The highlighted symbols indicate mRNAs encoding the proteins we have included in our annotation priority lists (microglial[3], tau-binding[6], amyloid-β-binding[6]). A selection of enriched GO terms are overlaid onto the nodes (see key).

[5] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D et al. Cytoscape: a software environment for integrated models of biomolecular interaction network (2003). *Genome Research* 13: 2498-504.
[6] Kramarz B, et al. Improving the Gene Ontology Resource to Facilitate More Informative Analysis and Interpretation of Alzheimer's Disease Data. *Genes* 2018 9(12). pii: E593.

CONCLUSION

We have pioneered the GO annotation of miRNA relating to dementia. We have shown how our functional annotations can be used to visualise the roles of individual miRNAs in a dementia-relevant molecular interaction network, thereby demonstrating that this resource will be a valuable addition to the advancement of miRNA research and may be used to predict proteins with a role in dementia. This work will support the rapid evaluation of new neurological data.

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