Functional annotation of cardiovascular microRNAs with GO

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http://www.ucl.ac.uk/functional-gene-annotation

Overview

MicroRNA (miRNA) 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 analyses tools. The association of gene products with terms from the Gene Ontology (GO) has proven highly effective for large-scale analysis of functional data, but this is currently lacking for miRNAs. In order to address this issue we have prepared a set of comprehensive guidelines for curation of miRNAs and miRNA processing proteins, in consultation with experts in the field of miRNA research, to enable biocurators to provide consistent annotation. Our plan is to build a resource comprising high-quality, reliable functional annotations for cardiovascular-related miRNAs; annotations that will be a valuable addition to the advancement of miRNA research in this field.

Curation of miRNA-processing proteins

To enable consistent curation of proteins involved in miRNA processing, the GO terms applicable to each protein in the pathway have been identified (Figure 1).

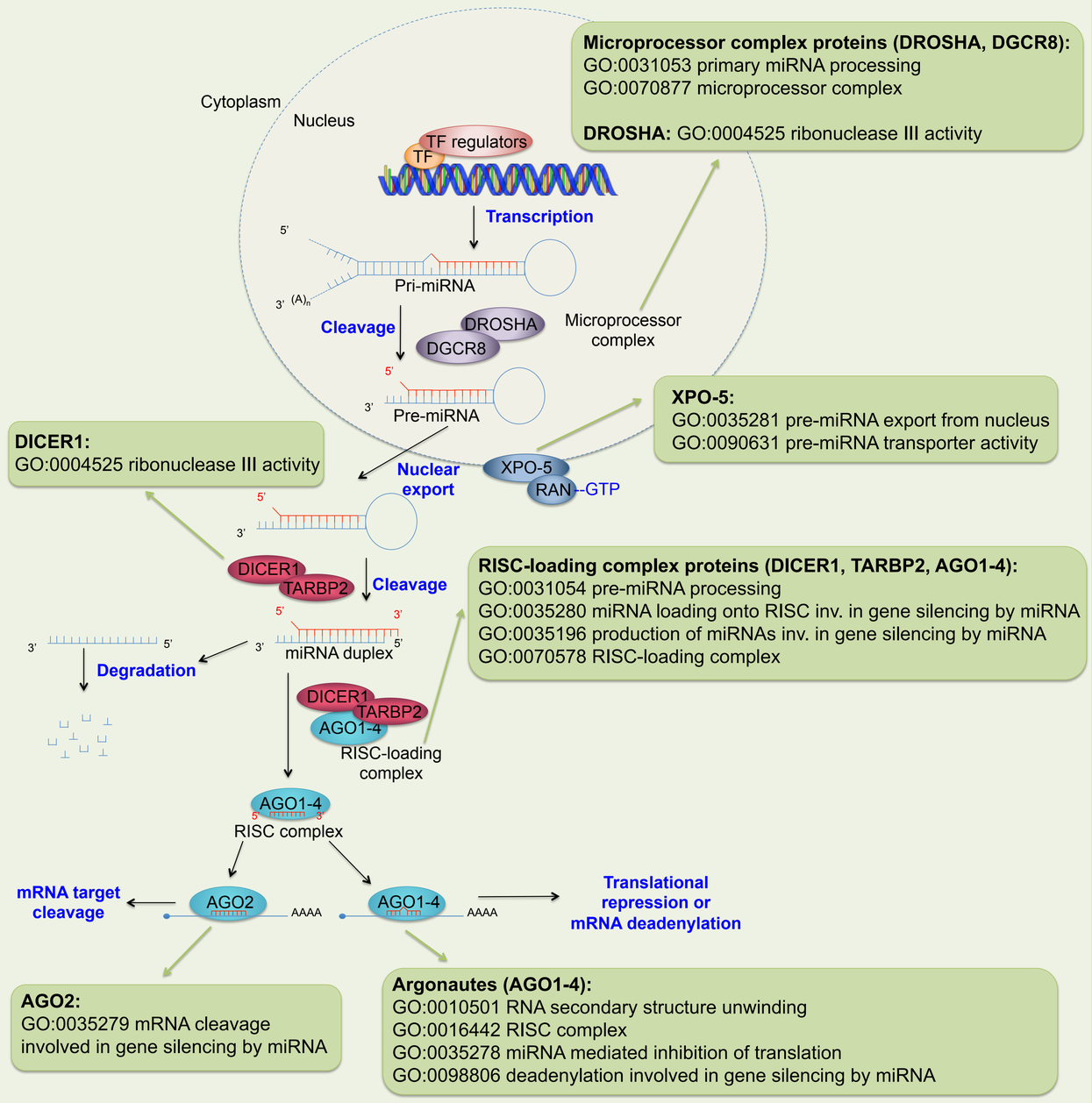

Figure 1. The canonical mammalian miRNA processing pathway. The proteins involved in miRNA processing are shown together with the GO terms that they are expected to be associated with (green boxes). *N.B. Due to space constraints, only a subset of applicable GO terms are shown.*

DROSHA: Ribonuclease 3; DGCR8: Microprocessor complex subunit DGCR8; XPO-5: Exportin-5; RAN-GTP: GTP-binding nuclear protein Ran; DICER1: Endoribonuclease Dicer; TARBP2: RISC-loading complex subunit TARBP2; AGO: Argonaute.

Curation of miRNAs

When curating miRNAs we capture not only the role of the miRNA in gene silencing together with its target gene, but also the effect of the silencing on the cell or organism (see "Example miRNA annotations" panel). MiRNAs can direct silencing of mRNA targets by three main mechanisms; 1) mRNA cleavage, 2) mRNA deadenylation and 3) translational repression. GO terms are available for each of these mechanisms (Fig. 2).

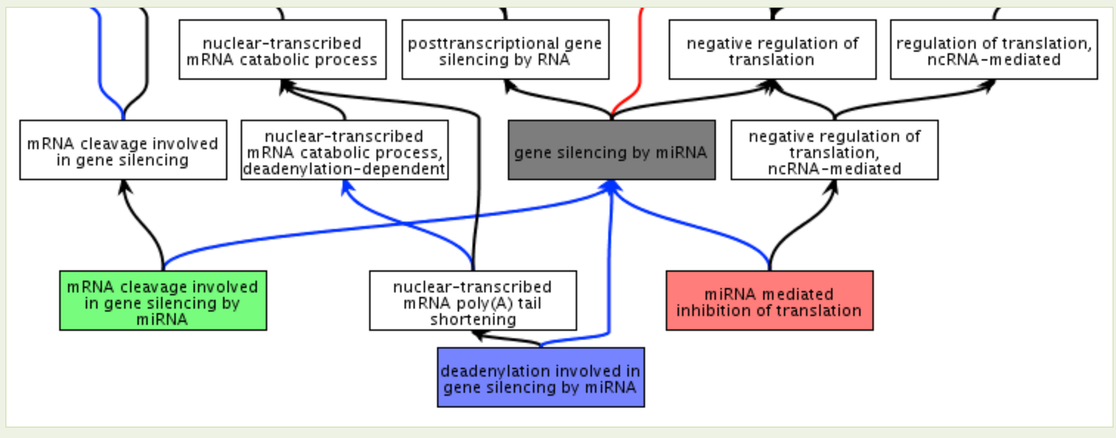

Figure 2. QuickGO view of the GO terms used for curation of the miRNA's role in gene silencing. If the exact mechanism of silencing is demonstrated, i.e. translational repression, deadenylation or mRNA cleavage, it is recommended to use the appropriate child terms of "gene silencing by miRNA". (www.ebi.ac.uk/QuickGO)

Example miRNA annotations

Capturing the role of a miRNA in gene silencing

Adhikari *et al*. demonstrated by dual luciferase assay that transfected miR-16 can directly bind to and silence the mRNA encoding vacuolar sorting protein VPS4a[1] (Fig. 3). This is a specific effect since miR-16 did not alter the luciferase activity when a mutated version of the 3'UTR of VPS4a was used.

Figure 4 shows the annotations we can make for the role of miR-16 in VPS4a gene silencing from this data.

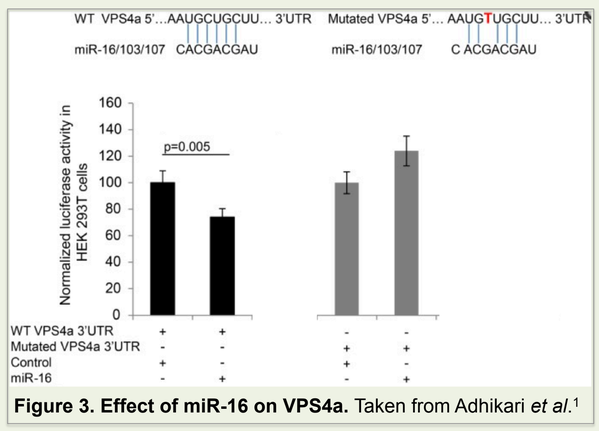

Figure 3. Effect of miR-16 on VPS4a. Taken from Adhikari *et al*.[1]

Gene/product	Gene/product name	Qualifier	Direct annotation	Annotation extension	Assigned by	Taxon	Evidence	Reference	Date
URS00004BCD9C_9606	Homo sapiens ncRNA/misc_RNA		gene silencing by miRNA	regulates expression of ENSEMBL:ENSG00000132612	BHF-UCL	Homo sapiens	IDA	PMID:25033200	20151203
URS00004BCD9C_9606	Homo sapiens ncRNA/misc_RNA		mRNA binding involved in posttranscriptional gene silencing	has_direct_input ENSEMBL:ENSG00000132612	BHF-UCL	Homo sapiens	IDA	PMID:25033200	20151203

Figure 4. AmiGO2 view of BHF-UCL annotation for miR-16. URS00004BCD9C_9606 is the RNAcentral identifier for human miR-16 and ENSEMBL:ENSG00000132612 is the identifier for the VPS4a gene. (http://amigo.geneontology.org/amigo)

Capturing the effect that a miRNA silencing event has on the cell or organism

Sabatel *et al*. demonstrated that miR-21 can inhibit the GTPase activity of RhoB, a putative regulator of angiogenesis, in endothelial cells[2] (Fig. 5). The context in which a miRNA acts is key information since its effects may be different in different environments, e.g. one cell type may have different miRNA targets to another, therefore we also capture contextual information.

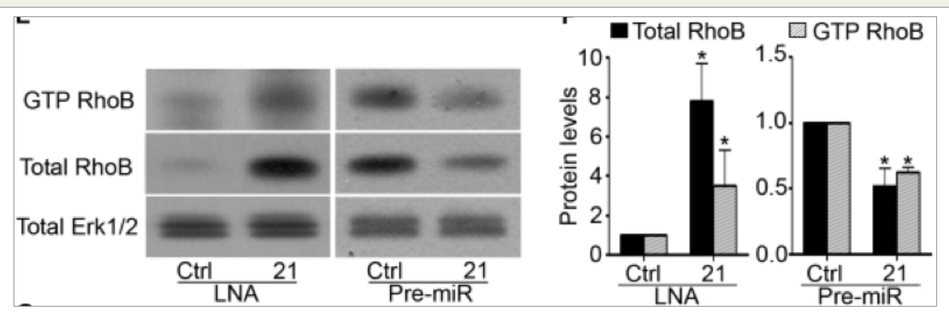

Figure 5. Effect of miR-21 on GTPase activity of RhoB. Decreasing levels of miR-21 by using Locked Nucleic Acid (LNA) causes an increase in levels of active RhoB (GTP RhoB), whereas increasing levels of miR-21 using precursor miR-21 (Pre-miR) causes a decrease in levels of active RhoB. Taken from Sabatel *et al*.[2]

Fig. 6 shows how we capture miR-21's inhibition of active RhoB in endothelial cells

Gene/product	Gene/product name	Direct annotation	Annotation extension	Assigned by	Taxon	Evidence	Reference	Date
URS000039ED8D_9606	Homo sapiens ncRNA/misc_RNA	negative regulation of GTPase activity	has_regulation_target UniProtKB:P62745 occurs in endothelial cell	BHF-UCL	Homo sapiens	IDA	PMID:21347332	20151109

Figure 6. AmiGO2 view of BHF-UCL annotation for miR-21. URS000039ED8D_9606 is the RNAcentral identifier for human miR-21 and P62745 is the UniProtKB accession for human RhoB.

Future work

We aim to provide functional annotation that is useful for miRNA research, therefore we will take a process-centric approach to our curation, prioritising any miRNAs that are currently in clinical trial. The first process we endeavour to comprehensively curate is ***cardiovascular regeneration***. Discovering how cardiac regeneration is controlled is a major priority for scientists since this may be used to treat common cardiac diseases. Increasing evidence shows that miRNAs control many aspects of cardiac function and by manipulating their levels complex pathways, including cardiac regeneration (Fig. 7), can be switched on and off[3].

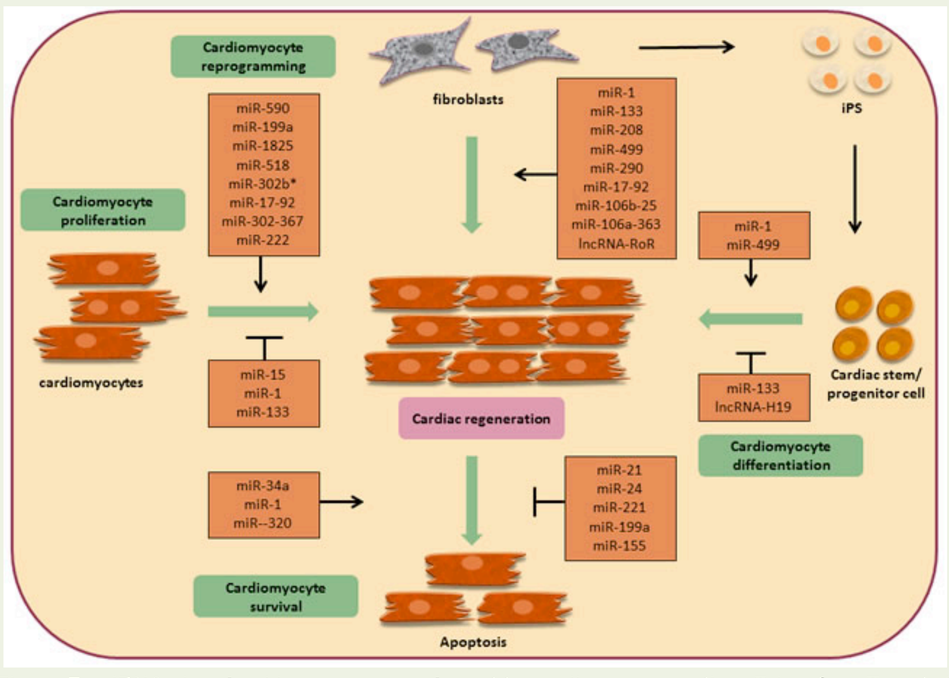

Figure 7. miRNAs involved in processes relevant to cardiac regeneration. Taken from Tao *et al*.[4]

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References:

1. Adhikari *et al*. Identification of a new target of miR-16, Vacuolar Protein Sorting 4a. PLoS One. 2014 Jul 17;9(7).
2. Sabatel *et al*. MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. PLoS One. 2011 Feb 10;6(2).
3. Giacca and Zacchigna. Harnessing the microRNA pathway for cardiac regeneration. Journal of Molecular and Cellular Cardiology. 2015. 2015 Dec;89(Pt A):68-74.
4. Tao *et al*. Non-coding RNAs in cardiac regeneration. Oncotarget 2015 Dec 15;6(40):42613-22.