C2 Centre for Chronic
D2 Diseases & Disorders

The Role of miR-132 in microRNA processing

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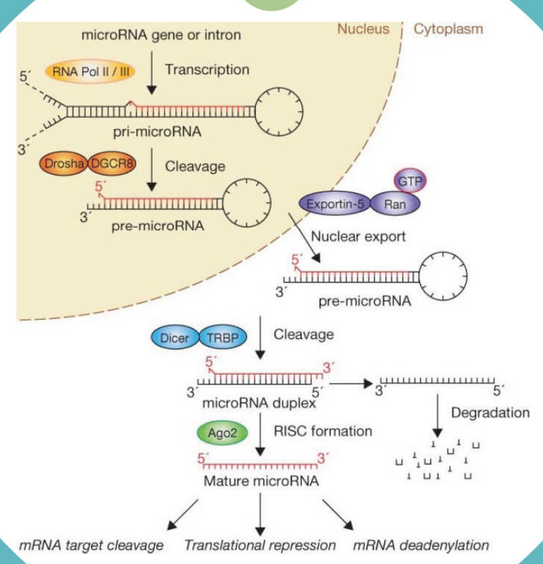
1. Introduction

MicroRNAs (miRNAs) are ~22nt short non-coding RNAs that regulate gene expression post-transcriptionally by complementary binding to target mRNA, resulting in translational repression or target degradation (1). Primary transcripts of miRNAs are processed into mature miRNAs, where they carry out their function through the formation of the RNA-induced silencing complex (RISC). A key component of RISC, Argonaute-2 (AGO2), is necessary for loading miRNAs into RISC and for the cleavage of target mRNAs (2). Additionally, AGO2 has been shown to be involved in microRNA processing (3).

Using online databases such as miRwalk, TargetScan and miRbase, AGO2 was found to be a potential candidate target for miR-132. MiR-132 has been implicated in developmental regulation, circadian rhythm control, cancer progression (4) and immunological control (5). Using the knowledge that miR-132 can potentially target AGO2, and AGO2 is involved in miRNA biogenesis and function, this project aims to investigate this interaction *in vitro*, *ex vivo* and *in silico.*

Fig. 1



Outline of miRNA biogensis and function (adapted from 1).

2. Aims of the Project

- Demonstrate that miR-132 regulates AGO2 repression
- Characterise the interaction *in vitro* using Human Dermal Lymphatic Endothelial Cells (HDLECs) and *ex vivo* in the context of miRNA biogenesis
- Develop a computational model capturing the miR-132/AGO2 interaction network and their role in miRNA biogensis

3. Methods

- *In vitro* tissue culture of HDLECs
- Transfect miR-132 mimics and inhibitors
- Endogenously induce miR-132 using PMA
- Measure target expression using qRT-PCR and Western Blot analysis

4. Results

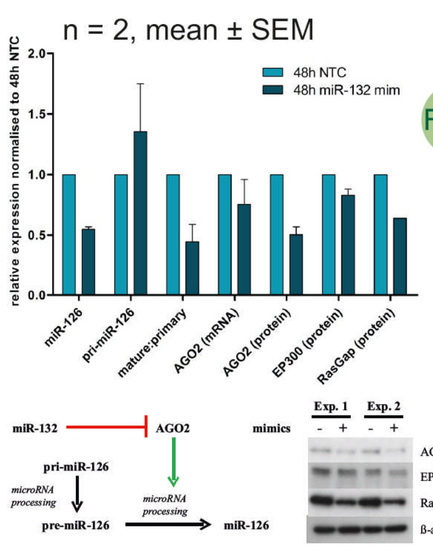


Fig. 2

miR-132 overexpression can knock down AGO2 and affect miRNA processing

- miR-132 overexpression using miR-132 mimics (25 nM) represses AGO-2, a key component of miRNA biogenesis and function
- Knockdown of AGO2 is comparable to previously published targets of miR-132 (EP300 (5), p120RasGap (6)) and results in a drop of mature miR-126 abundance but not in the pri-miR-126, indicating an effect on miRNA processing

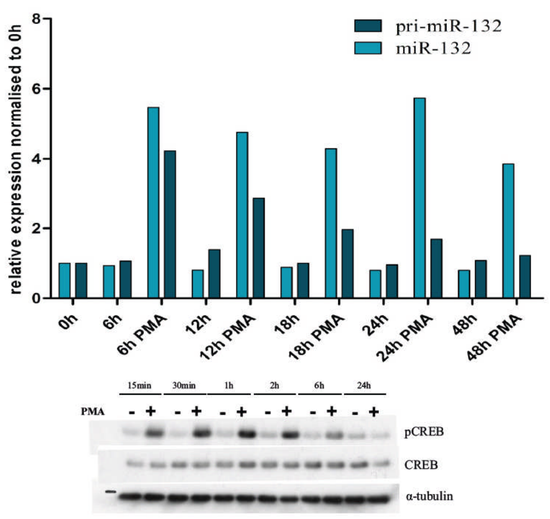


Fig. 3 PMA endogenously induces miR-132 through the activation of CREB

- miR-132 is transcriptionally induced by PMA (25 nM) through the activation of CREB
- pri-miR-132 transcriptional induction is short-lived
- mature miR-132 sustains a higher level of expression for an extended period of time

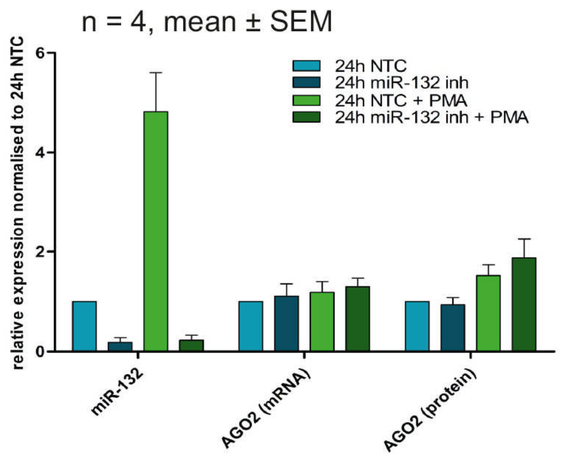
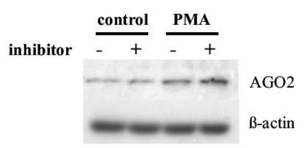


Fig. 4



miR-132 inhibition in PMA treated samples affects AGO2 expression

- LNA inhibitors effectively knock down miR-132 expression
- miR-132 inhibition in PMA-induced samples indicates a possible derepression of AGO2

5. Conclusions

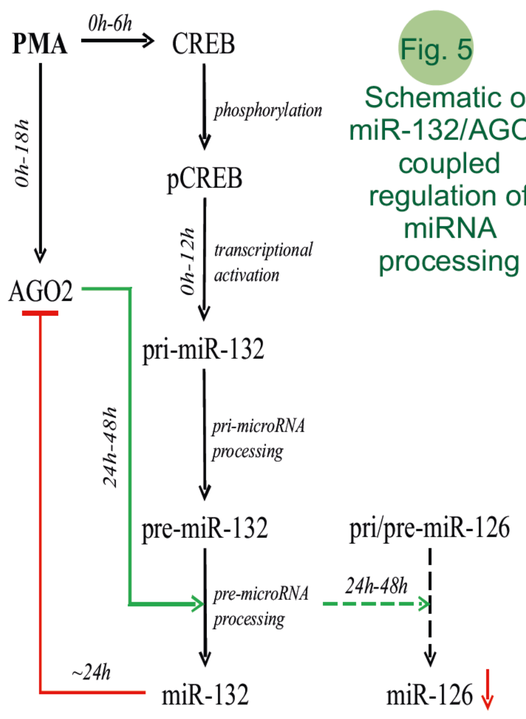


Fig. 5 Schematic of miR-132/AGO2 coupled regulation of miRNA processing

- miR-132 can target AGO2 resulting in reduced miR-126 processing (Fig 2)
- Established a system where miR-132 can be endogenously induced (Fig 3)
- Endogenous induction of miR-132 affects AGO2 expression in PMA treated samples (Fig 4)
- Previous understanding of miRNA processing has been revised based on experimental findings (Fig 5)

6. Further Work

- Additional replicates to address the variabilty between experiments when using primary cells
- Determine the levels of endogenous miR-132 induction that affect miRNA processing
- Investigate regulation of miR-132/AGO2 in wild type and miR-132 knockout mice
- Develop a stochastic computational model describing the role of miR-132 in miRNA biogenesis

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References

1. Winter J *et al.* Nat Cell Biol. 2009 11(3):228-34
2. Meister G *et al.* Mol Cell. 2004 15(2):185-97
3. Diedrichs S *et al.* Cell. 2007 131(6):1097-108
4. Wanet A *et al.* Nucleic Acids Res. 2012 40(11):4742–4753
5. Lagos D *et al.* Nat Cell Biol. 2010 12(5):513-9
6. Anand S *et al.* Nat Med. 2010 16(8):909-14

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