Molecular regulation of the oncogenic miRNA-17-92 cluster

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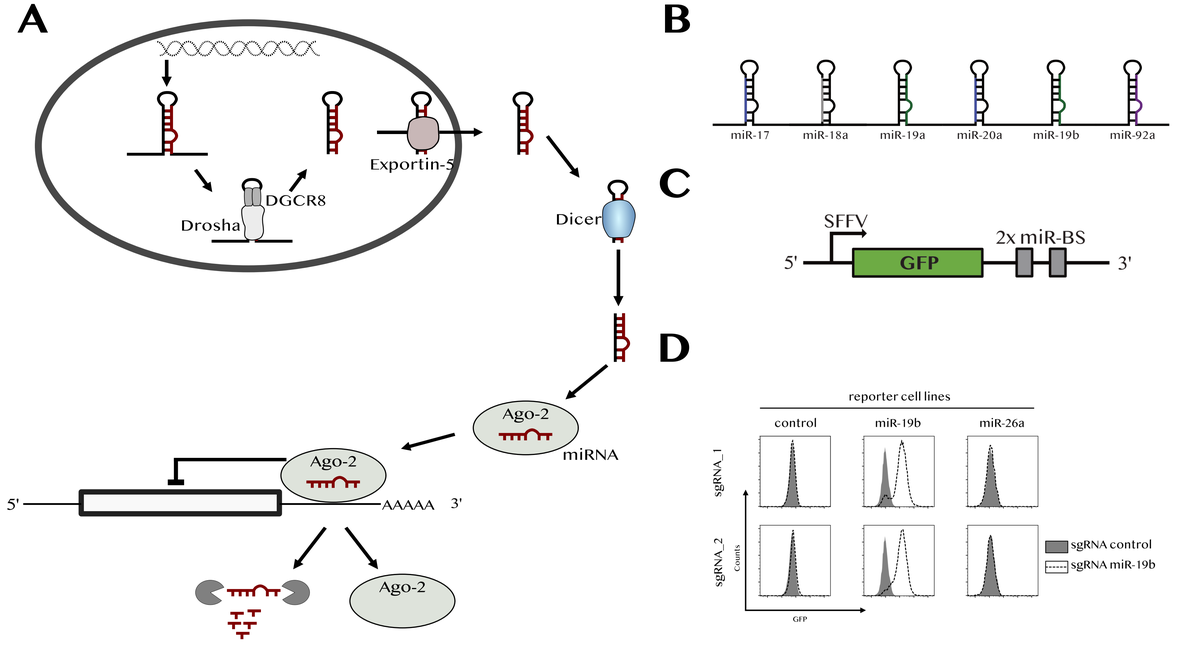
The polycistronic miR-17-92 cluster, frequently overexpressed or amplified in cancer, is transcribed as one long primary transcript that is then processed into six individual miRNAs. In the beginning, this genetic organization suggested that all members of the cluster functionally cooperate and drive tumor progression synergistically. However, recent data demonstrate that the cluster is not oncogenic per se, but rather encodes tumor-promoting as well as tumor-suppressing functions. In consequence, it appears that an imbalanced expression of the cluster members, with a shift towards the oncogenic components, is crucial to drive tumor cell formation in both mouse and men. Of note, it is mostly unclear how these imbalanced expression patterns are established, thereby warranting an in-depth analysis.
We have performed a set of genome-wide CRISPR/Cas9 loss-of-function screens to generate a comprehensive understanding of the molecular mechanisms that regulate miR-17-92. The genome-wide screens generated a list of about 200 novel genes that have not been associated with the regulation of miR-17-92 cluster before. While still ongoing, the validation of these hits already confirmed a clear regulatory role for 15 of those genes (out of 60 tested), i.e. deletion of these genes has a statistically significant impact on individual or groups of cluster miRNAs.

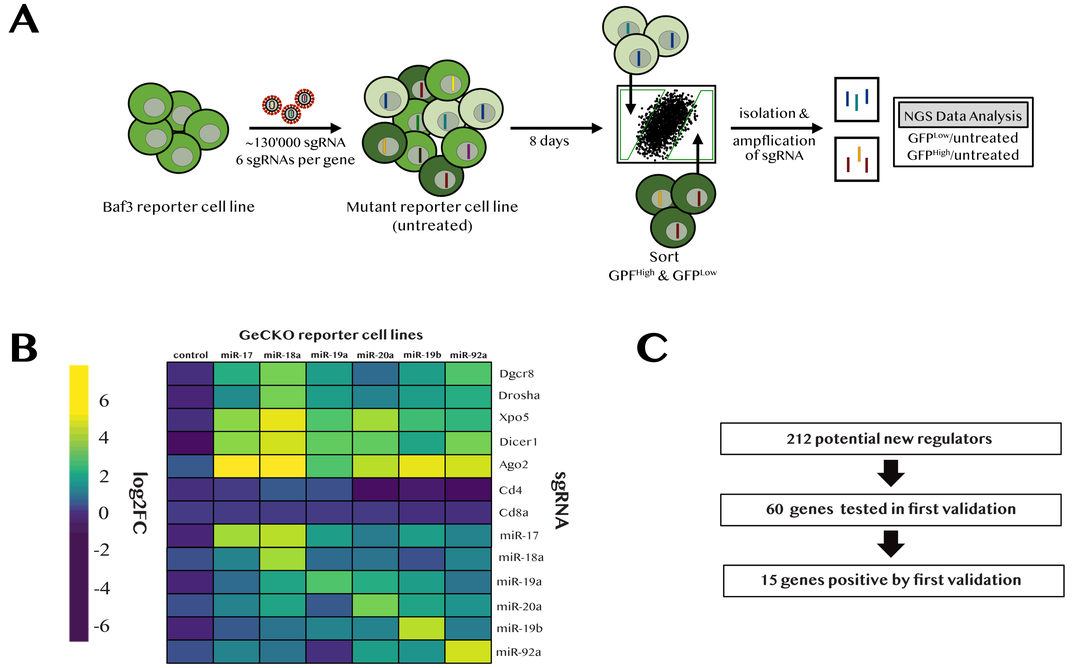
The miR-17-92 cluster members - diverse expression levels & potential levels of regulation



A. Schematic illustration of the miRNA biogenesis. **B.** Schematic illustration of the miR-17-92 cluster. The miR-17 family depicted in blue and the miR-19 family depicted in green. **C.** Schematic illustration of the GFP reporter plasmid, sensing miRNA upon miR- binding site (BS) in the 3'UTR. **D.** Analysis of mean-fluorescence-intensity (MFI) of GFP in Baf3 cells expressing reporter and sgRNA constructs as indicated.

ML is recipient of a DOC Fellowship of the Austrian Academy of Sciences at the IDI.

Genome-wide CRISPR Cas9 screen to elucidate potential new regulators of the miR-17-92 cluster



A. Schematic illustration of a pooled genome-wide CRISPR/Cas9 loss-of-function screen with the functional miRNA readout by a GFP reporter plasmid. **B.** Heatmap of sgRNA fold change (log_2FC) of GFP-High population normalized to unsorted cell lines after next generation sequencing. The genome wide CRISPR Cas9 loss-of-function screen was performed in Baf3 reporter cell lines. (6 sgRNAs per gene n=4) **C.** Schematic illustration of genes retrieved after bioinformatic data processing (number of potential new regulatory genes, tested genes and positive genes after first validation (single CRISPR/Cas9 loss-of-function experiments) are displayed.