PLURIPOTENCY AND DIFFERENTIATION CONTROLING MICRORNAS SCREENING AND TARGET CHARACTERIZATION

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BACKGROUND

MicroRNAs (miRNAs) are small molecules responsible for gene silencing by either inducing cleavage of the target mRNA or by inhibiting its translation into protein (Fig. 1). In mammal embryos, miRNA processing enzyme Dicer lack is lethal and promotes the loss of the stem cell compartment. Embryonic stem cells (ESC) Dicer$^{-/-}$ exhibit impairment in proliferation and differentiation.

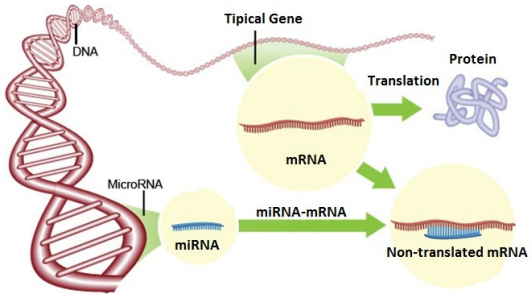


Figure 1. Schematic representation of miRNA-based gene silencing mechanism.

Groups of miRNAs enriched in ESC decrease as cells differentiate as well other miRNA groups down-regulated in these cells increase upon differentiation, implicating these molecules in the control of the differentiation state of cells. Indeed, miR-302b and miR-372 cluster increased the mouse and human somatic reprogramming efficiency, i.e. the generation of induced pluripotent stem cells (iPS), after the insertion of viral vectors of Oct4, Sox-2, Klf-4 and c-Myc transcription factors.

The expression of the miR-302/367 cluster was able to reprogram somatic cells without the use of viral vectors. A library screening study identified the miR-130/301/721 family as important regulators of induced pluripotency, which specific silencing of their mRNA target (Meox-2/Gax) resulted in the same effect on the reprogramming. These results highlight the importance of identifying the downstream targets of miRNAs involved in pluripotency and differentiation. This will consistently contribute to our knowledge of the RNA-based control system of undifferentiated cellular states and will possibly permit the manipulation of cell machinery for disease modeling and therapeutic uses, such as generation of iPS cells.

AIM

The aim of this work is to evaluate the effect of miRNA precursors (pre-miR) and miRNA antagonists (anti-miR) library on the expression of a reporter gene under the control of different promoters (Oct-4, Nanog, and RAR) in terminally differentiated cells (BJ Human Fibroblasts) and in an human embryonic carcinoma cell line (N-Tera2) in order to determine their mRNA targets.

METHODS

❖*Cell lines establishment:* BJ and N-Tera2 lines will be transduced with lentiviral particles (Ambion®). Ideal multiplicity of infection (MOI) is being experimentally determined in various cell types. As experimental control, cells will first receive a renilla-luciferase reporter gene under control of constitutive CMV promoter. Firefly-luciferase reporter gene will be under control of different promoters (Fig. 2).

❖*MicroRNA screening:* Pre-miR and anti-miR molecules will be transfected into cells in the final concentration of 50 nM (experimentally determined) using Polybrene. After 72h, dual-glo luciferase assay will be conducted to evaluate changes on the expression of reporter genes in response to miRNAs.

❖*Microarray:* The Whole Human Genome Oligo Microarray kit and Agilent platform will be used to detect changes on approximately 44.000 transcripts levels upon transfection of screened miRNAs.

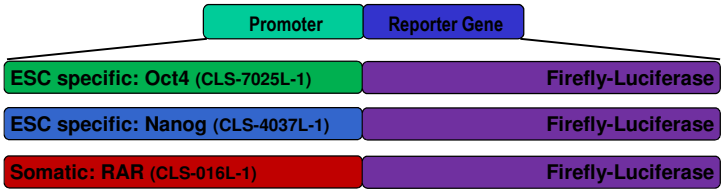


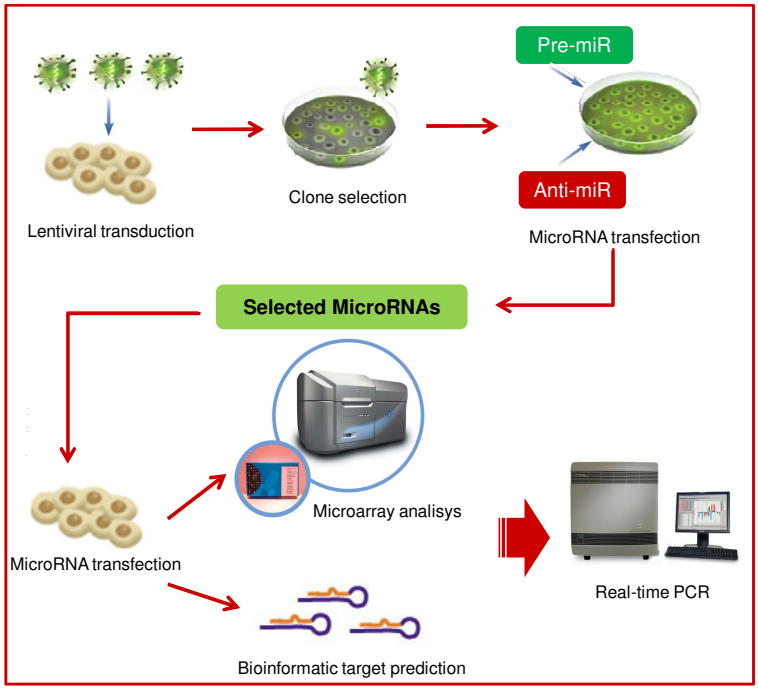
Figure 2: Representation of lentiviral vectors used to establish reporter cell lines

❖*Bioinformatic target prediction:* The online toll at www.microrna.org will be used to predict targets of the miRNAs capable to change the expression levels of reporter gene.

❖*mRNA target validation:* Significantly modulated mRNAs will have their levels measured by real-time PCR after the transfection of miRNAs in BJ and N-Tera2 cells.

❖Statistical analysis: Performed using the GraphPad Prism 5.0, Mann-Whitney test and significance level of 5%.

EXPERIMENTAL DESIGN



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