

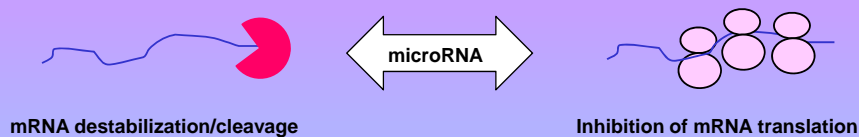
Cellular reprogramming by microRNAs

Inbar Friedrich Ben-Nun¹, Candace Lynch¹, Pauline Lieu², Andrew Fontes², Jon Chesnut²,
Louise C. Laurent¹ and Jeanne F. Loring¹

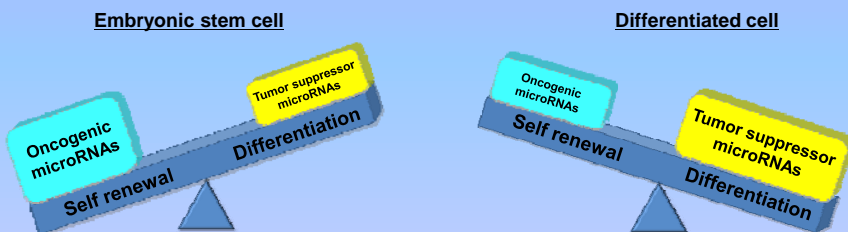
¹Center for Regenerative Medicine, The Scripps Research Institute, La Jolla, CA. ²Invitrogen, Inc, Carlsbad, CA

SUMMARY: MicroRNAs are small, non-coding RNAs that regulate gene expression through post-transcriptional gene silencing, either by inhibiting translation, by mRNA cleavage, or by mRNA destabilization. These small RNA's are involved in regulation of many critical biological processes, including cell proliferation, differentiation, apoptosis, morphogenesis and metabolism (Box 1). We mapped 800 microRNAs in pluripotent and differentiated cells, and identified several that are consistently and specifically up-regulated or down-regulated in hESCs compared to fibroblasts (Laurent *et al.*, Stem Cells, 26:1506, 2008). Notably, we found that many of the significantly upregulated microRNAs in hESCs had the same consensus seed sequence (Box 2). Our strategy is to overexpress "hESC-specific" microRNAs and repress "fibroblast-specific" microRNAs in human fibroblast cells. We expect that the changes in microRNAs will alter the transcriptional profile, which we predict will lead to cell reprogramming toward pluripotency. We have developed a human foreskin fibroblast cell line that expresses the TET-repressor and a POU5F1/OCT4 promoter-driven GFP cassette reporter. Specific microRNAs and microRNA sponges have been cloned into a TET-inducible lentiviral vector, which then can be inducibly expressed in the cells (Box 3). Reprogrammed colonies will be identified by the expression of the POU5F1/OCT4 promoter-driven GFP and the stem cell surface marker, TRA-1-81 (Box 4). One practical advantage of a microRNA approach is that the small size of the molecules may allow us to avoid using vectors to delivery the molecules. After validating the approach using the lentiviral vectors, we will introduce the microRNAs directly into cells, with the goal of inducing cellular reprogramming without creating permanent genetic changes that may raise concerns for the safety of the cells when they are put to clinical use.

1A MicroRNAs are epigenetic regulators which affect gene expression by translational inhibition and mRNA destabilization

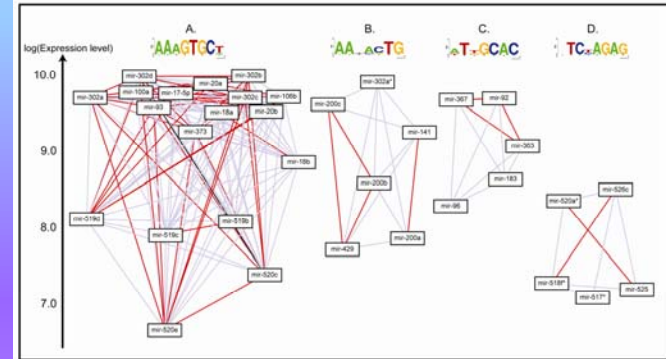


1B Possible role of microRNAs as regulators of self-renewal and pluripotency



HESCs express a unique set of microRNAs. Oncogenic microRNAs are over-represented in hESCs. Tumor suppressor microRNAs are down-regulated in hESCs compare to differentiated cells

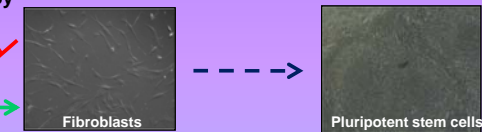
2 MicroRNAs upregulated in human ES cells share a common seed sequence



MicroRNAs upregulated in hESCs are grouped by seed sequence. Red lines connect microRNAs that have identical seed sequences. Blue lines connect microRNA with six of seven matches in the seed sequence.

3 Generating induced pluripotent cells (iPSCs) by microRNAs

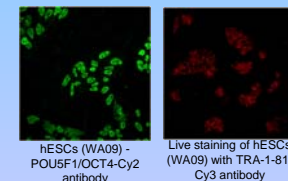
- Silence "fibroblast-specific" microRNA by "Sponges"
- Express "hESC-specific" microRNAs



To change the microRNA content of fibroblasts, we are up-regulating "hESC-specific" microRNAs using expression vectors, and down-regulating "fibroblast-specific" microRNAs using "sponges" that hybridize to specific microRNA species. We are also testing the ability of microRNAs to trigger reprogramming in cells expressing single transcription factor reprogramming elements.

4 Identification of reprogrammed cells

POU5F1/OCT4 is a hESC-associated transcription factor. Reprogrammed cells will be selected for expression of POU5F1/OCT4-GFP expression.



TRA-1-81 is a cell-surface antigen expressed on hESCs.

Reprogrammed fibroblasts are identified by ESC-like morphology, expression of a POU5F1/OCT4 promoter-driven GFP reporter, and presence of the TRA-1-81 cell surface antigen.