Induced Pluripotent Stem cells (iPS) can be obtained by transfecting synthetic microRNAs (miRs) into somatic cell. miRs are small regulatory RNAs that bind to transcript targets leading to translation block or mRNA degradation. In order to explore the molecular mechanisms by which miRs-106a and 302b contribute to iPS reprogramming, synthetic pre-miRs, inhibitory anti-miRs and corresponding unspecific control molecules were independently transfected into human fibroblasts and into pluripotent NTera2 lineage. After 72h, whole-genome microarrays transcriptomes were obtained. Confident miR-targets were identified by selecting experimentally modulated transcripts (downregulated by pre-miR and upregulated by corresponding anti-miR, in both cell lines) showing evolutionary conserved predicted binding sites. Reprograming-associated molecular changes were identified by comparing miR-induced changes to transcripts modulated upon iPS reprograming (as determined by comparing two distinct iPSs to the corresponding fibroblasts of origin). Pathways modulated by the miRs were identified using the DAVID Tool and selected targets were confirmed by qRT-PCR. Among pathways with a significantly enriched number of confident miR-302b targets, we found: Apoptosis, p53 and WNT signaling. Similarly, for miR-106a, we identified: Regulation of Actin Cytoskeleton, Adherens-Junction, Focal-Adhesion and MAPK Signaling. Analysis based on the shared predicted targets down-regulated by miRs-302b and 106a, in both cell lines, and down-regulated upon iPS reprograming, revealed enrichment of central pathway components of TGFbeta and MAPK signaling. We demonstrate that several components of pathways opposing pluripotency, self-renewal and reprograming, are targeted to degradation by miR-106a and miR-302b. Resulting knowledge will set the basis for further developments in the field of miR-mediated iPS reprograming.

Word Keys: pluripotency, microRNA-106a and 302b, stem cells.

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