Abstract

miR-17-92 cluster (Oncomir1) encodes seven microRNAs (miRNA, miR) regulating many biological processes including proliferation, differentiation or apoptosis. Overexpression of microRNAs encoded by miR-17-92 cluster is found in a number of tumors including acute and chronic myeloid leukemias (Dixon-McIver et al., 2008; Li et al., 2008; Venturini et al., 2007). Myeloid progenitors express miR-17-92 cluster at a high level, while macrophage differentiation associates with its downregulation. Our laboratory found, that miR-17-92 cluster is repressed by transcription factor Early growth response 2 (Egr2) upon differentiation of primary myeloid PUER progenitors, induced with transcription factor PU.1. Aim of this thesis is to further test the abovementioned data by preparing a reporter vectors set, carrying various fragments of miR-17-92 putative promoter, which enables us to study regulation of transcription of miR-17-92 cluster. This task complicated by presence of increased GC content of the miR-17-92 promoter was successfully accomplished resulting in amplification of eight fragments containing the various parts of miR-17-92 promoter including region -3.3 to 0 kb relative to the start of miR-17-5p sequence, that were inserted into pGL3 reporter vector.

Transfection of pGL3 reporter vector carrying -3.3;-0kb fragment of miR-17-92 putative promoter to NIH3T3 and HeLa cells followed by functional assay resulted in significant inhibition of miR-17-92 cluster regulatory regions by Egr2 factor. These results further documented that the repression of miR-17-92 cluster by Egr2 is independent on transcription factor PU.1, that transcriptionally activates expression of Egr2 in myeloid progenitors. The repression mechanism of miR-17-92 cluster that newly includes Egr2 is probably not limited to hematopoetic cells.

MicroRNAs and their target genes can create mutually reciprocal regulatory circuits. Three microRNAs encoded by miR-17-92 cluster (miR-17-5p, miR-20a and miR-92-1) are predicted (TargetScan) to inhibit expression of the transcription factor Egr2, presumably by binding to 3´ untranslated region (3'UTR) of Egr2 mRNA . We therefore prepared reporter vectors carrying (3'UTR) of Egr2 , containing either predicted binding sites of miR-17-92 cluster or their mutants. Cotransfection of the reporter vectors carrying 3'UTR of transcription factor Egr2 together with the expression vector carrying and overexpressing miR-17-92 cluster into HeLa cells resulted in inhibition of the reporter expression thus confirmed earlier observation from myeloid progenitors, that microRNA of miR-17-92 cluster are able to inhibit Egr2 transcription factor by binding into its 3'UTR region.

In conclusion, my data support the overall conclusion found in the laboratory that miR-17-92 cluster is transcriptionally repressed by Egr2 and in turn that miR-17-92 cluster has ability to inhibit Egr2, indicating the existence of possible mutual regulation between Egr2 and miR-17-92 cluster.