

Ring Finger Protein 2 (RNF2) Targeting by miRNAs in Breast Cancer Cell Lines

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Abstract : Ring Finger Protein 2 (RNF2) is a member of polycomb repressive complex 1 (PRC1), which is one of the epigenetic regulators in the genome. When RNF2 combines with other PRC1 members, it mediates the mono-ubiquitination of Histone 2A (H2A). In breast cancer, RNF2 is commonly overexpressed, and also it promotes metastasis and invasion in other aggressive tumors like melanoma, prostate, and hepatocarcinoma. The role of RNF2 in the metastasis and invasion of breast cancer has not yet been elucidated. Our aim is to observe the role of RNF2 in metastasis and invasion in this study by miRNA mediated RNF2 gene silencing in breast cancer cell lines. We selected miRNAs, targeting to RNF2 by searching online databases. miR-17-5p, miR20a-5p, and miR-106b-5p were transfected to breast cancer cell lines (MCF-7, MDA-MB-231, SK-BR-3, and ZR-75-1), and also we used normal breast epithelial cell line (hTERT-HME1) to compare RNF2 gene expression level. After 48-72 hours post-transfection, mRNAs were isolated from the cells, and gene expressions were measured by RT-qPCR after from cDNA syntheses. We observed that RNF2 was highly expressed in SK-BR-3 and MDA-MB-231 cell lines opposite to MCF-7 and ZR-75-1 cell lines. RNF2 was downregulated 5, 5 and 7 fold by miR17-5p, miR20a-5p and miR106b-5p respectively in MCF-7. However, in SK-BR-3 and ZR-75-1 cell lines, miRNAs did not affect significantly RNF2 gene expression level. miR20a-5p decreased RNF2 3 fold and miR17-5p and miR106b-5p did not affect MDA-MB-231. After gene expression analysis, we performed metastasis and invasion assay in MCF-7 cells. For metastasis, we used both wound healing assay and Transwell Cell Migration Assay, and we used Transwell Cell Invasion Assay for invasion. The data of this assay showed that miR17-5p and miR20a-5p decreased both invasion and metastasis level, but miR106b-5p has no effect. We would like to conclude that RNF2 can be targeted by miR17-5p, miR20a-5p and miR106b-5p in MCF-7 cells and also RNF2, which is one of the upregulated genes in aggressive tumor, can be decreased by using these miRNAs. In future, we would like to confirm these results at the protein level and also whether these miRNAs are direct target of RNF2 or not.

Keywords : breast cancer, epigenetic, microRNAs, RNF2

Conference Title : ICMMD 2018 : International Conference on Molecular Medicine and Diagnostics

Conference Location : Dublin, Ireland

Conference Dates : February 15-16, 2018