

Cellular and Molecular Biology 2018- Alteration of proteins which are targeted by miRNAs involved in megakaryopoiesis during megakaryocyte commitment of hematopoietic stem cells

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MicroRNAs(miRNAs) are small noncoding RNAs that regulate gene expression by mRNA silencing, consequently have an impact on many biological processes. Previous studies approved the role of miRNAs in megakaryopoiesis. These molecules target specific protein mRNAs. In this study, we analyzed the expression of MAPK, DYRK1A, CDC7, RUNX1 and CDK during megakaryopoiesis. These proteins are targeted for at least two miRNAs which are involved in this pathway. BM derived CD133+ Hematopoietic stem cells (HSCs) were sorted and cultured in cytokines stimulating Mk differentiation media. Differentiation was evaluated by CD42/CD61/CD41 expression and colonogenic capacity in Megacult media. Total RNA was extracted from CD133+HSCs and Megakaryocytic cells. Quantitative Polymerase Chain Reaction (qPCR) was done on synthesized cDNAs. The expression alteration of target proteins was evaluated by flow cytometry as Mean Fluorescent Intensity (MFI) that shows the antibody reacted proteins. The qPCR results illustrated meaningful down-regulation of MAPK-DYRK1A and CDC7 mRNAs, that are targeted by up-regulated following miRs: miR-22, miR-188, miR-1246, miR-148a, miR-224, miR-486-5p, miR-886

Materials and Methods

In this experimental study, human CD133+ hemato-

poietic stem cells were collected from three human umbilical cord blood (UCB) samples, and then differentiated to the megakaryocytic lineage and characterized by flow cytometry, CFU-assay and ploidy analysis. Subsequently, microarray analysis was undertaken followed by quantitative polymerase chain reaction (qPCR) to validate differentially expressed miRNA identified in the microarray analysis.

Results

A total of 10 and 14 miRNAs were upregulated (e.g. miR-1246 and miR-148-a) and down-regulated (e.g. miR- 551b and miR-10a) respectively during megakaryocyte differentiation, all of which were confirmed by qPCR. Analysis of targets of these miRNA showed that the majority of targets are transcription factors involved in megakaryopoiesis.

Conclusion

We conclude that miRNA play an important role in megakaryocyte differentiation and may be used as targets to change the rate of differentiation and further our understanding of the biology of megakaryocyte commitment.

Keywords: Cord Blood, Hematopoietic Stem Cells, Megakaryocytes, Microarray Analysis, MicroRNAs