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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 ($p<0.05$). Furthermore, RUNX-1 and CDK mRNAs were up-regulated during megakaryopoiesis ($p<0.05$). These mRNAs are subject to target by Let-7b, miR-10a, miR-125b, miR-99a, miR-155, miR-181a, Let-7c, miR-146b, miR-363, miR551b. The MFI of reacted target proteins that were detected by flowcytometry showed increase in RUNX-1 and CDK and decrease in MAPK- DYRK1A and CDC7 expression, in agreement with qPCR results. These data confirmed the regulation of some target transcription factors during megakaryocyte commitment of HSCs that achieved by miRNAs.

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