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A novel role for miRNA let-7b target FZD4 in the regulation of developmental megakaryocytopoiesis

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Background: Neonatal thrombocytopenia is common among sick infants and it is known that developmental differences between neonatal and adult megakaryocytes (MKs) contribute to the higher vulnerability of neonates to develop prolonged and severe thrombocytopenia. The regulatory mechanisms underlying these developmental differences are unclear. Recent reports indicate a critical role of small non-coding microRNAs (miRNAs) in the regulation of megakaryocytopoiesis. miRNAs regulate the target gene expression on a post-transcriptional level by binding to the target mRNAs which, in turn, leads to translational arrest. Our studies found that out of 88 miRNAs involved in the stem cell development, let7b was the only miRNA downregulated in human cord blood (CB-) MKs compared to peripheral blood (PB-). Let7b has not been previously described in MKs, however reduced expression of let-7b was found in several human cancers, suggesting that it functions as a tumor suppressor.

Objective: To investigate the differential expression of miRNAs in neonatal and adult megakaryocytes, and its contribution to the phenotype.

Design/Methods: We cultured human CB- and PB- derived $CD34^+$ cells in the presence of thrombopoietin for 14 days. At day 14, >90% of cells were megakaryocytes ($CD41^+$) as measured by flow cytometry. miRNA was prepared using the miRNeasy mini kit (Qiagen) and expression analysis of 88 miRNAs was performed using a quantitative PCR-based array kit (SA Biosciences). Web-based computational approaches (TargetScan, PicTar and MiRanda) were used for putative target prediction.

Results: All CB- and PB-megakaryocyte samples expressed detectable amounts of all 88 screened miRNAs. Nevertheless, the specific miRNA expression levels differed significantly between CB- and PB-derived megakaryocytes. Ten miRNAs were expressed at significantly higher levels in CB MKs (2 to 22 fold), while only one, let-7b, was down-regulated (10% of PB). All of these differences reached statistical significance ($p<0.05$). Specific targets of let-7b were predicted by web-based computational approaches (TargetScan, PicTar and MiRanda) and identified FZD4, a Wnt family protein as a potential target. The complex Wnt family molecules are generally thought to be important to sustain large-scale production of blood cells, delivering critical signals to stem cells and progenitors as they reside in specialized niches. Wnt proteins are also known to be involved in malignancies and are causatively involved in the development of several types of leukemias.

Conclusions: Our results indicate that neonatal and adult megakaryocytes show a differential expression pattern of miRNAs that are likely to regulate some or all of the molecular mechanisms underlying their phenotypic differences. Our findings could unveil a novel role for let-7b target FZD4 in the regulation of megakaryopoiesis, and implicate developmental differences in this axis as one of the molecular mechanisms responsible for the high proliferative rate of neonatal MKs. This might explain the susceptibility of neonates to develop myeloproliferative disorder in the presence of trisomy 21 and GATA-1s mutations.

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A study on proneural genes in the development of olfactory local interneurons in Drosophila

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Certain morphological, physiological and molecular characteristics are shared by all neurons, however, despite these similarities; neurons constitute the most diverse cell population. Genetic studies in Drosophila have provided that small number of proneural genes which encode the transcription factors of the basic helix – loop helix (bHLH) class, are necessary in the context of ectoderm to initiate the development of neuronal lineages. We studied the role of atonal, acheate, amos and scabrous genes in the development of olfactory local interneurons of Drosophila using UAS- Gal4enhancer trap lines, RNAi , splinkerette PCR, MARCM ,Immunohistochemisty and Confocal analysis.

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