

MicroRNA EXPRESSION PROFILE OF CD34+ CELLS IN A COCULTURE OP9-DL1 SYSTEM

Lucila Habib Bourguignon Oliveira^{1,3}; Josiane Lillian dos Santos Schiavinato^{2,3}; Amélia Góes Araújo³; Patrícia Vianna Bonini de Palma³; Camila Cristina de Oliveira Menezes Bonaldo; Dimas Tadeu Covas³; Marco Antonio Zago^{1,3}; Rodrigo Alexandre Panepucci^{2,3}

¹ Departamento de Clínica Médica da Faculdade de Medicina de Ribeirão Preto-Universidade de São Paulo, FMRP-USP
² Departamento de Genética - FMRP-USP
³ Instituto Nacional de Ciências e Tecnologia em Células-Tronco, INCTC e Centro Regional de Hemoterapia, HCFMRP-USP

Introduction

The development of T lymphocytes requires a specific microenvironment of the thymus, in which hematopoietic progenitor cells (HPC) receive signals that activate Notch signaling pathway. Despite the growing attention of microRNA (miRNA) in process of hematopoietic differentiation, the set of miRNA with specific roles in early involvement of HPC with the T-lymphoid lineage, remains unknown.

Objective

In attempt to explore miRNA potential regulatory mechanisms of Notch in commitment and differentiation of T lymphocytes in vitro, we use a recent cocultive system (Immunity 17: 749-756, 2002), based on OP9 murine stromal cells, expressing the Notch ligand Delta-like-1 (OP9-DL1).

Methods and Materials

The study was approved by the Institutional Ethics Committee. CD34+ HPC were immunomagnetic selected from umbilical cord blood (n=4) after informed consent. After 12 hours of cocultivation with OP9-DL1 (test) or OP9 (control), CD34+ cells were sorted and miRNA expression profiles were performed using Agilent miRNA microarrays. Scanned images were analyzed using the Agilent Feature Extraction software followed by R Programming for Bioinformatics analysis. Targets genes of selected miRNA were predicted by at least two prediction tools (TargetScan and PicTar).

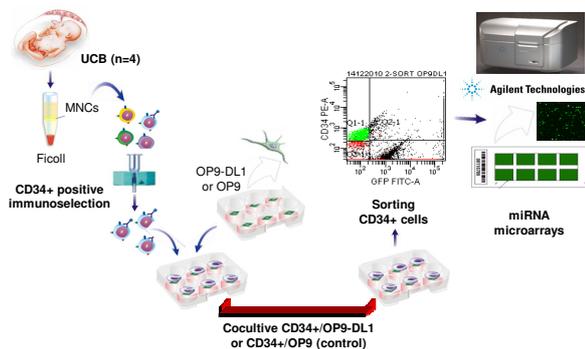


Figure 1: Experimental desing.

Results

Comparison between both groups resulted in a set of 14 miRNA up regulated in CD34+ cells cocultivated with OP9-DL1 (paired T-test and fold change 1.45).

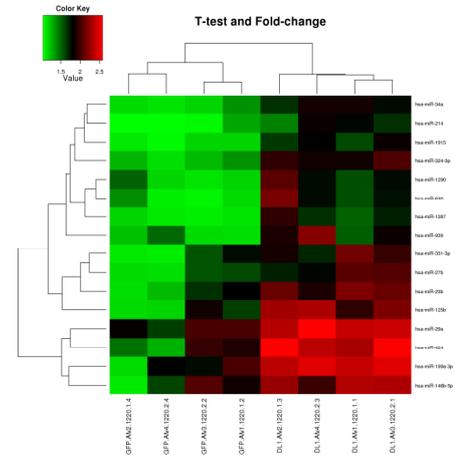


Figure 2: Hierarchical cluster. The heatmap depicts expression values from all selected miRNA, wich were used to group transcription profiles according to their similarities. .

Up-regulated mir-29a were selected and evaluation of targets genes revealed biological processes related to DNA methylation, beta-amyloid metabolic process and proteolysis.

Process	Target Gene	Sources			
		Validated	TargetScan	miRanda	PicTar
beta-amyloid metabolic process	BACE1	✓	✓		✓
DNA methylation	DNMT3A	✓	✓		
DNA methylation	DNMT3B	✓	✓		
membrane protein ectodomain proteolysis	BACE1	✓	✓		✓
proteolysis	BACE1	✓	✓		✓

Figure 3: mir-29 a associated processes.

Conclusion:

These results contribute to the characterization of miRNA-Notch stimulated roles, in the regulation of the first steps of lineage commitment and differentiation in T-lymphoid cells. Additional experiments such as validation by real-time PCR and functional assays using pre-and anti-mir mir are underway to validate and further explore these findings.