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641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Transcriptomic Analyses Indicate That Pre-Leukemic B Cells from People with Monoclonal B Lymphocytosis Are Surprisingly Much Less Active Than Normal B Cells from People with Monoclonal B Lymphocytosis and from Healthy Donors

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Introduction. Chronic lymphocytic leukemia (CLL) follows a stepwise developmental process, beginning in a specific group of normal B lymphocytes that undergo clonal expansion. These clonal expansions, known as monoclonal B lymphocytosis (MBL), are considered to be essential precursors of CLL based on immunologic, genetic, and epidemiologic studies. Since MBL represents the overabundance of a single CD5 $^{+}$ B cell, we tested how different the transcriptomes of MBL cells were from normal B cells (NBC) in people with MBL (NBC-MBL) and NBC in healthy donors (NBC-HD), expecting to identify activations pathways in the MBL cells that would explain clonal expansion.

Methods. We collected and sorted specific cell populations from 13 patients with monoclonal B lymphocytosis (MBL): CD5 $^{+}$CD19 $^{+}$CD20 ^{dim}IgL-restricted MBL cells and CD5 $^{-}$CD19 $^{+}$CD20 $^{+}$ NBC. Additionally, we obtained CD5 $^{-}$CD19 $^{+}$CD20 $^{+}$ NBC from 13 healthy donors (NBC-HD). RNA was extracted and sequenced using SMART-Seq v4 Ultra Low Input and HiSeq platform. DESeq2 was used to analyze RNA-seq data. To identify genes with differential expression between these populations, the transcriptomes of MBL cells were compared to NBC from HD, employing a cutoff of $P<0.05$ and Log2fc ratio $>\pm$ 1.5. We also used paired sample t-tests to compare 13 MBL cells to NBC from the same MBL patients. Significantly differentially expressed genes (DEG) resulting from these analyses were then subjected to Ingenuity Pathway Analysis (IPA).

Results. When comparing the gene profiles of 13 MBL B cells to 13 NBC-HD cells, we identified 2695 DEG. Next, we compared gene profiles between MBL B cells and NBC-MBL cells obtained from 13 MBL patients, identifying 4821 genes. Using IPA, down-regulated B Cell Receptor Signaling, Cytokine Signaling, and Tumor Microenvironment Pathway were identified in both analyses.

Upon overlapping the genes from these two analyses, we found that 803 genes passed the cutoff in both analyses. IPA analysis showed that 14 signaling pathways, including IL-33, IL-17, and HIF1a, were significantly downregulated in MBL cells. Moreover, the negative signaling pathway (Role of p14/p19ARF in Tumor Suppression) was upregulated in MBL cells.

PDCD1 expression was significant higher in MBL (Log2Fc 6.8, $P<0.0001$) and 11 PDCD1 target genes were significantly regulated in MBL cells. IL-6 (Log2Fc -5.4, $P<0.0001$) expression was significantly lower in MBL and 47 IL-6 target genes were significantly regulated in MBL cells. Surprisingly, genes related to cell activation, proliferation and movement were largely downregulated in MBL cells.

There were 2052 genes unique to the analysis MBL vs NBC-HD, IPA analysis showed several T-cell related signaling pathways e.g., T cell Exhaustion Signaling Pathway and T Lymphocyte Apoptosis were upregulated. In the 4018 genes unique in the analysis MBL vs NB-MBL. IPA results showed all the signaling pathways were downregulated in MBL.

Discussion. Surprisingly, our transcriptomic analyses indicate that MBL cells are much less active than NBC from people with MBL and from HD. Specifically, we found that many cytokine signaling pathways and genes related with cell activation, proliferation and movement are downregulated in MBL cells. Also, PDCD1 is highly expressed in MBL cells, and PDCD1 target genes are also significantly regulated in MBL cells. This is consistent with the immunosuppressed features of MBL cells since interaction between PD-1 on B cells and its ligands in the microenvironment negatively influence the survival, proliferation, and immune evasion of CLL and MBL cells.

Collectively, although functional studies are needed, these transcriptomic data strongly suggest that MBL cells are not in an activated state and are less active than even the NBC from the same person with MBL.

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