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POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

CLL Cell-Derived Exosomes Impair Immune Function and Normal Hematopoiesis in CLL

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CLL is characterized by immune and hematopoietic abnormalities such as T-cell dysfunction, hypogammaglobulinemia, increased levels of inflammatory cytokines, aberrant monocyte polarity, anemia, neutropenia, and low-grade bone marrow fibrosis. As a result, CLL patients become susceptible to increased rates of infection and/or second neoplasms.

Exosomes are nanovesicles that participate in bidirectional communication between neoplastic cells and their microenvironment. Using electron microscopy, we found that CLL cells produce and release CD19 $^{+}$/CD5 $^{+}$exosomes, and in agreement with other investigators, we detected high levels of CLL cell-derived exosomes in the plasma of patients with CLL. By using bead-assisted flow cytometry, we found that at least 50% of circulating extracellular vesicles are CLL cell-derived exosomes, identified as CD63 $^{+}$/CD41a $^{-}$ particles co-expressing CD19 $^{+}$/CD5 $^{+}$, whereas only a small proportion of CLL patients' plasma-derived exosomes expressed T-cell or endothelial cell surface markers. Furthermore, we found a positive correlation between levels of CLL cell-derived exosomes in the plasma of patients with CLL and the patients' peripheral white blood cell counts, indicating that CLL cells are the primary source of circulating exosomes.

To investigate the effects of CLL cell-derived exosomes, we conducted an analysis of CLL cells and CLL cell-derived exosomes from a cohort of 78 previously untreated patients. To identify the molecular profiles of CLL cell-derived exosomes, we analyzed their RNA transcripts using RNA-seq and qRT-PCR. Our data revealed that CLL cell-derived exosomes expressed significant levels of oncogenic micro-RNAs. Additionally, we identified 1052 differentially expressed genes, and enrichment analysis further unveiled the significant upregulation of gene clusters associated with p53, NF-κB, MAPK, mTOR, and interleukin signal transduction pathways, consistent with their neoplastic cell origin.

The distinctive transcriptional profile of CLL cell-derived exosomes led us to explore their effects on normal cell populations. We labeled ultra-centrifuged CLL cell-derived exosomes with fluorescent markers and incubated them with various cell types in culture conditions conducive to their physiological activity. Using confocal microscopy and flow cytometry, we observed significant uptake of CLL cell-derived exosomes by normal monocytes, fibrocytes, B cells, T cells, and CLL cells. Furthermore, incubation with CLL exosomes resulted in notable alterations in normal CD14 $^{+}$ monocyte polarity and subset-specific shifts in the expression of inflammatory cytokines. Remarkably, CLL cell-derived exosomes induced overexpression of the T-cell makers CD5 and CD8 in normal CD19 $^{+}$ cells, suggesting that CLL exosomes may promote immune-evasive reprogramming of normal B cells. Moreover, when normal T cells were incubated with CLL cell-derived exosomes, CyTOF analysis using 46 markers revealed elevated levels of immune checkpoints, including PD1, CTLA4, and LAG3, in both CD4 $^{+}$ and CD8 $^{+}$ subsets, indicating that exosomes derived from CLL cells induce T-cell dysfunction.

Given their profound effect on immune cells, we wondered whether CLL exosomes affect hematopoietic cells. We previously found that normal monocyte derived fibrocytes stimulate hematopoietic colony proliferation. In sharp contrast to control fibrocytes, fibrocytes preincubated with CLL exosomes and CLL cell-derived exosomes inhibited normal BFU-E and CFU-

GM colony formation, suggesting that CLL exosomes might contribute to CLL patients' anemia or neutropenia. Wondering whether CLL cell-derived exosomes affect the viability of other cell types, we found that they induced a dose-dependent apoptosis of $CD19^+$ B cells, CLL cells but not normal T cells.
In summary, CLL cell-derived exosomes exert a profound effect on gene and cell surface protein expression in immune and tissue-remodeling cells, leading to functional modifications, alteration of inflammatory cytokine levels, and suppression of normal hematopoiesis. The impact of CLL exosomes may vary across different cell types; however, as the leukemic cell mass increases, exosome levels escalate, invariably affecting CLL cell environment. The identification of exosomes with distinct molecular signatures holds promising potential for novel therapeutic interventions in future treatment of CLL.

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