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In vitro secretome analysis of primary CLL cells and genetically modified B cells

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The microenvironment plays a major role in the pathogenesis of B cell lymphomas and leukemias. Stroma-lymphoma interactions support survival and proliferation of lymphoma cells and also play an important role in the development of drug resistance. Chronic lymphocytic leukemia (CLL), the most frequent hematologic disease, is a hallmark model to analyze microenvironment-lymphoma interactions. When cultured in vitro, patient-derived CLL cells rapidly undergo apoptosis unless they are in co-culture with supporting feeder cells. We established different co-culture settings: EL08 stroma cells that mimic the microenvironment found in the bone marrow and follicuar dendritic cells overexpressing IL21 and CD40L as a model for the lymph node microenvironment, where strong proliferation is induced. These co-culture systems enable us long-term culture as well as CRISPR-mediated genetic manipulation and viral transduction of primary CLL and B cells.

In order to get a better understanding of the interplay between lymphoma cells and their microenvironment, we analyzed the secretome of primary CLL cells as well as genetically transduced human B cells as model for aggressive B cell lymphomas. We investigate the impact of specific genetic alterations, such as IGHV mutational status, on the composition of secretory proteins and their role for survival and proliferation. Moreover, we compare the secretome in different co-culture systems and also analyze the impact of drug treatment.

Therefore, patient-derived CLL cells (n= 20) and genetically transduced human B cells (n = 4) were co-cultured with supporting feeder cells in FCS-free medium for 6 and 12 hours. The secretome was analyzed by mass spectrometry. Up to 5000 proteins could be identified. Among the most abundant proteins, we found different chemokines (CXCL12, CXCL13), metalloproteinases (MMP1, TIMP1) as well as the plasminogen activator inhibitor 1 (SERPINE1).

Comparison of the secretome of genetically modified human GCB cells in co-culture with follicular dendritic cells revealed a significant upregulation of CXCL13 in the secretome of human GCB cells transduced with Bcl2 and Bcl6 compared to GCB cells that were transduced with cMyc and Bcl2 which is in line with higher germinal center activity in Bcl6 overexpressing B cells.

The analysis of the secretome of primary CLL cells is still ongoing at the time of abstract submission, the results will be presented at the symposium.