Advanced 3D culture systems revealed the dynamic regulation of Podoplanin in CLL cells within the microenvironment

TT1-05

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Chronic Lymphocytic Leukemia (CLL) is the most prevalent adult leukemia in developed countries and is characterized by the clonal expansion of CD19 CD5 mature B cells disseminating in the blood and lymphoid organs. A key feature of CLL is its strict bidirectional interaction with the tumor microenvironment (TME), which can promote disease progression and be therapeutically exploited. Current in vitro and in vivo models often fail to fully recapitulate tissue complexity or human pathology. To overcome these limitations, we developed a 3D in vitro model of lymph node and bone marrow to mimic CLL-niches. By using a millifluidic system we recapitulated leukemic cell recirculation and generated functional lymphoid tissues in which CLL cells responded to biochemical and mechanical cues by modulating key markers as CXCR4, VLA-4, and IgM (Barozzi et al. 2025). During scaffold analysis, we noted the expression of podoplanin (PDPN) in leukemic cells, besides the stroma. While its expression in B lymphocytes and CLL cells is not well-established, its overexpression in inflammation and neoplastic contexts in various TME has been reported. By flow cytometry we are screening patients' cells (n=24), observing marked inter-patient heterogeneity in PDPN+ cells within the neoplastic clone (4–90%), with no significant clinical correlations so far, unlike the more homogeneous expression seen in healthy donor B cells. We confirmed PDPN expression by real-time qPCR and imaging. Preliminary results on 2D and 3D co-cultures with lymph node stroma revealed an increase in PDPN+ cells in different samples (n=3), reinforcing the hypothesis that PDPN is involved in CLL-TME crosstalk. Since regulation of this microenvironment-dependent protein may be linked to disease progression, transformation and dissemination, an in-depth analysis is ongoing integrating biochemical and mechanical analyses of the CLL microenvironment, correlating with clinical outcome and response to targeted therapies.