Enhanced IL-9 secretion by p66Shc-deficient CLL cells modulates the chemokine landscape of the stromal microenvironment

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I this Blood manuscript Laura Patrussi and colleagues from the University of Siena address a new feature of the cross-talk between tumour and stromal cells in chronic lymphocytic leukemia (CLL).

The stromal microenvironment is central to CLL pathogenesis. However, despite extensive literature, it is not entirely clear how leukemic cells influence the stroma to enhance its chemoattraction properties. In this manuscript the authors use transgenic murine models of CLL, Eμ-TCL1 as well as Eμ-TCL1 /p66Shc-/-, to address the role of p66Shc in this interaction.

To begin with, the authors co-cultured murine splenic CD5+CD19+ cells with the murine OP9 stromal cells and discovered that chemokine expression in stromal cells is largely mediated by leukemic cell-derived soluble factors, the production of which is enhanced by p66Shc deficiency.

The results were subsequently validated in the context of organoid cultures of murine splenic
slices, where stromal cells remain in their 3D context for over 48 h without significant viability loss. Their results indicated that p66Shc deficiency in leukemic Eμ-TCL1 cells enhances the release of soluble factors that shape the stromal microenvironment to promote leukemic cell homing.

To identify relevant soluble factor candidates the authors performed a DNA microarray analysis on mRNA extracted from leukemic cells and identified differentially increased expression of IL-9 in Eμ-TCL1 /p66Shc-/- compared to Eμ-TCL1 and healthy B cells. Consistent with this observation leukemic cells from Eμ-TCL1 /p66Shc-/- mice secreted increased amounts of IL-9. The levels of homing chemokines were modulated by conditioned media containing IL-9 and this increase was neutralized by an anti-IL-9 monoclonal antibody.

The authors also noted that p66Shc modulates gene transcription through its ROS-elevating activity. Namely, the pro-oxidant adaptor p66Shc negatively regulates B-cell survival by modulating Bcl-2 family protein expression and by controlling the surface levels of homing receptors, thus affecting B-cell trafficking to and retention in pro-survival niches.

To validate these results in the context of primary human CLL, the authors used a co-culture system with human bone marrow-derived HS-5 stromal cells. They observed that, similar to OP9 cells, HS-5 cells upregulated homing chemokine expression in response to IL-9 and that this activity could be partially neutralized by an anti-IL-9 monoclonal antibody.

Furthermore, significantly higher IL-9 levels were detected in CLL cells compared to healthy B cells and were particularly high in patients with unmutated IGHV who develop the aggressive disease. The authors found that IL-9 mRNA levels inversely correlated with

p66Shc suggesting that p66Shc negatively modulates IL-9 expression in CLL cells. Importantly, conditioned supernatants from p66Shc-reconstituted CLL cells impaired the ability of HS-5 cells to express homing chemokines and promote CLL cell migration. Finally, infiltration of lymph nodes and the presence of spleen and/or liver enlargement was significantly higher in CLL patients whose leukemic cells had IL-9 mRNA levels above an arbitrarily set threshold.

In summary, these interesting data highlight a new aspect of the cross-talk between leukemic and stromal cells in CLL that is controlled by p66Shc and promotes the generation of a pro-migratory microenvironment to support leukemic cell homing as well as survival. As IL-9 expression increases with disease progression in CLL patients, and IL-9 blockade mitigates leukemic cell invasiveness in Eμ-TCL1 /p66Shc-/- mice, the authors suggest that IL-9 blockade should be explored as a potential new therapeutic approach in CLL.