Supplemental Methods

Immunophenotyping

Between 1 and 2 x 10^4 MSC, detached using the "Non-enzymatic Cell Dissociation Buffer" (Mediatech), were incubated for 20-30 min at 4^o C in 100 μl of PBS/5% FBS/0.1% sodium azide (staining buffer) with the following anti-human-antigen monoclonal antibodies (mAbs) from Beckton Dickinson: anti-CD14-Phycoerytrhin (PE), anti-CD29-PE, anti-CD34-PE, anti-CD31-fluorescein, ant-CD44-PE, anti-CD45-PE, anti-CD105-PE or anti-D7-FIB-PE from AbD Serotech, or with their appropriate isotype controls. Cells were washed twice in staining buffer, fixed in 3.7% formaldehyde in PBS, and subsequently analyzed by flow cytometry using a FACSCalibur (Becton Dickinson) and the FlowJo software (Tree Star). A similar staining protocol was followed for the determination of CXCR4 expression levels on $CD19^{Pos}CD5^{Pos}$ CLL cells, except that 2x10^5 CLL cells were used per staining. The antibodies, anti-CD19-Peridinin chlorophyll protein-Cyanin 5.5 (PerCP-Cy5.5), anti-CD5-PE, and anti-CXCR4-Allophycocyanin (APC) were purchased from BD. The expression levels of ZAP-70 in CLL cells were determined by flow cytometry, as previously described.[1] The cutoff was set at 20%: >20% of CLL cells expressing ZAP-70 were defined as $ZAP\text{-}70^{Pos}$ and ≤20% were defined as $ZAP\text{-}70^{Neg}$.

Immunoblot Analyses

MSC generated in 5% O_2 were cultured in parallel in either 5% O_2 or 21% O_2 for 4 or 9 days, at which point the cells were collected and lysed for protein extraction. MSC were lysed for 20 min. on ice with RIPA lysis buffer (10 mM Tris pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 5 mM EDTA (ethylenediaminetetraacetic acid) supplemented with 1 mM PMSF (phenylmethylsulfonyl fluoride), Halt phosphatase inhibitor (Thermo Fisher Scientific), 1 mM sodium vanadate,

1 mM sodium fluoride, and protease inhibitor cocktail (Roche). Protein concentration was determined using the DC (Detergent compatible) protein assay (Bio-Rad). The lysates were snap frozen and stored at -80ºC until analyzed. Equal amounts of protein lysates (40µg) were size-separated by gel electrophoresis using a NuPAGE Novex 4-12% Bis-Tris Midi Gel (Invitrogen) and transferred to PVDF (polyvinylidene fluoride) membranes (Bio-Rad). Membranes were washed with 1xTBST (Tris-Buffered Saline Tween-20), blocked for 1h at room temperature in 5% milk/TBST, and probed overnight for $p21^{WAF/Cip}$, $p16^{INK4}$ or β-actin as loading control using antibodies from Cell Signaling Technology. The next day, membranes were washed with 1xTBST and incubated with goat-anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) in 5% milk/TBST for 1h at room temperature. Antibodies were detected either using an enhanced chemi-luminescence (ECL) detection kit (GE Healthcare) or SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific). For densitometry analysis, the intensity of each band was determined using the free NIH ImageJ software (http://rsbweb.nih.gov/ij/), normalized to the intensity of control protein β-actin.

CXCL12 secretion measurement

CXCL12 detection in MSC CM was performed using a Quantikine Immunoassay from R&D System, according to manufacturer's instructions.

Reference

1. Rassenti LZ, Huynh L, Toy TL, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. *N Engl J Med.* 2004;351(9):893-901.