Determining CD34 expression in the presence and absence of stroma

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Rationale:

Having established the ability to culture OCI-AML-20 cells that maintain CD34 expression, (and likely leukemic stem cells) we wanted to determine if CD34 expression is dependent on the stroma cells.

Methods:

OCI-AML-20 cells were cultured on OP9 stroma in IMDM media (Wisent) containing 20ng/mL GM-CSF (produced in house), 10% FBS (Wisent), primocin (InvivoGen) and 55uM beta-mercaptoethanol (Gibco). Cells were plated at a density of 125K per well in a 12-well plate that had been previously seeded with either MS5 or OP9 stroma at a density of 20-30K per well. Cells were either topped up with media or split twice a week based on cell density during the course of the experiment.

For determining CD34, CD38 expression a total of 0.5-1mL of cell culture was collected and spun down at 1500rpm for 5mins. Cells were stained with antibodies from Miltenyi at the following ratios in 50uL of PBS with 2% FBS:

CD45-VioGreen, human, clone: REA747 1:101

CD34-PE, human, clone: AC136 1:41

CD38-VioBrightFITC, human, clone: REA671 1:21

Cells were stained for 10 mins at room temperature and washed with 1mL of PBS with 2% FBS and spun down at 1500rpm for 5mins. Cells were then stained with 0.2 µM Sytox Blue viability dye. Flow analysis was performed on a MACSQuant VYB (Miltenyi) and analyzed using FlowLogic software.

Results and conclusions:

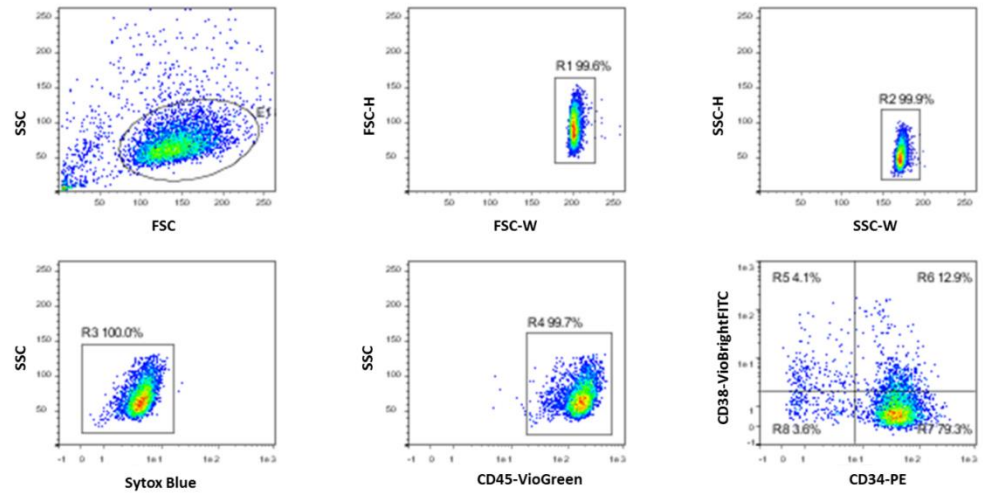

Figure 1: Gating strategy and analysis of the initial culture of OCI-AML-20. Cells were selected (SSC vs FSC) and subsequently selected for single cells (H vs W of both SSC and FSC). Viable cells (Sytox Blue negative) that expressed human CD45 (hematopoietic cells) are displayed for those that express CD38 and CD34. A large fraction of the initial culture expressed CD34 (92.2%).

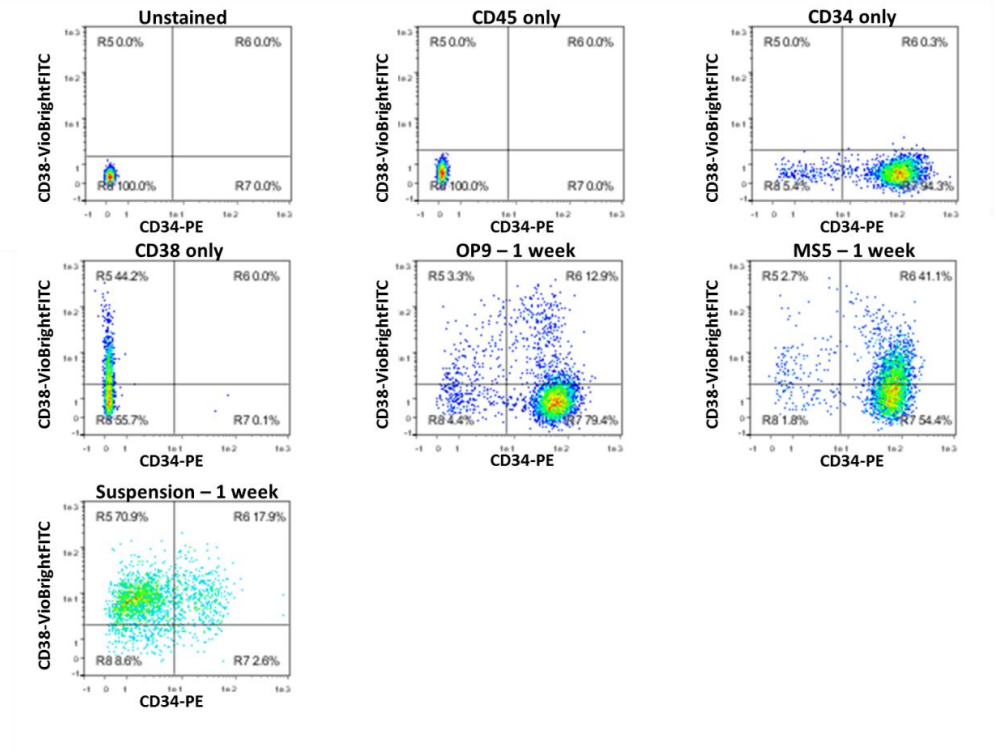

Figure 2: Using the gating strategy from Figure 1, representative flow plot images from unstained, single stained and cells grown on either OP9 or MS5 stroma or in suspension culture. Cells cultured on stroma maintain CD34 expression, but it is lost in cells grown in suspension.

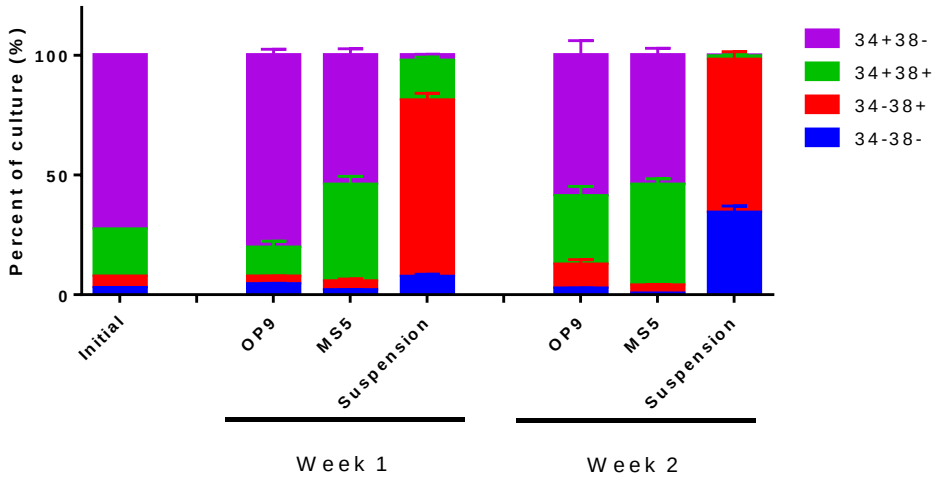

Figure 3: Summary of CD34 and CD38 expression in OCI-AML-20 cells grown with and without stroma over two weeks. OCI-AML-20 require stroma for CD34 expression maintenance. Figure is a summary of 3 technical replicates.

In conclusion, OCI-AML-20 cells require stroma to maintain CD34 expression in culture.