Low expression of C-type lectin-like molecule-1 and reduced oxidative stress define a subset of acute myeloid leukaemia cells with 'stem-like' properties

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Background:

Leukaemic stem cells (LSCs) are an important therapeutic target in acute myeloid leukaemia (AML) as failure to fully eradicate these cells is thought to drive disease relapse. Therapeutic targeting of LSCs has been hindered by the controversy surrounding their phenotypic characterisation. So, in this study, we built a multi-colour immunophenotyping panel, which included C-type lectin-like molecule-1 (CLL-1), to address the possible shortcomings of using CD34 and CD38 alone as LSC markers. CLL-1 is preferentially expressed on the majority of AML cells, but not on haematopoietic stem cells. Here we identified heterogeneity of CLL-1 expression within the CD34$^+$CD38$^-$ compartment of the KG1a AML cell line, which led us to explore whether this marker could more accurately identify LSCs.

Aims:

We set out to phenotypically and functionally characterise the 'stem-like' sub-population in the KG1a cell line. Ultimately, we aim to determine whether we can preferentially target these 'stem-like' cells for therapeutic benefit.

Methods:

We used the AML KG1a cell line as it contains a sub-population of CD34$^+$CD38$^-$ 'stem-like' cells. A 9-colour immunophenotyping panel (comprised of CD34, CD38, CD47, CD71, CD90, CD117, CD123, HLA-DR & CLL-1), was used to look for heterogenous expression of 'stemness' markers in KG1a cells. Subsequently, we used fluorescence activated cell sorting (FACS) to purify phenotypically distinct sub-populations and compared their growth kinetics, relative viabilities and cell cycle distributions in hypoxia and normoxia. We also compared their metabolic activity using the redox-sensitive probe CellROX to assess relative levels of reactive oxygen species (ROS) as a readout of oxidative stress.

Results:

Most of the immunophenotypic markers in our flow cytometry panel showed homogeneous expression patterns in KG1a cells. In contrast, we observed heterogeneity in CLL-1 expression, particularly in the CD34$^+$CD38$^-$ sub-population. CD34$^+$CD38$^-$CLL-1$^-$ cells were smaller in size than their CD34$^+$CD38$^-$CLL-1$^+$ counter-parts and represented approximately 2% of the entire CD34$^+$CD38$^-$ subset. Cell sorting allowed us to isolate highly purified CD34$^+$CD38$^-$CLL-1$^-$ & CD34$^+$CD38$^+$CLL-1$^+$ sub-populations (Figure 1). After four days in culture, we found a 5.5-fold preferential expansion of the CD34$^+$CD38$^+$CLL-1$^+$ cells relative to the CD34$^+$CD38$^-$CLL-1$^-$ cells (P<0.0001). However, both subsets expanded and remained viable in culture. The lower growth rate observed in the CD34$^+$CD38$^-$CLL-1$^-$ sub-population was associated with a significantly higher percentage of G_0/G_1 cells than the CD34$^+$CD38$^+$CLL-1$^+$ sub-population (91% vs 49%; P=0.0001), respectively. We then went on to show that this subset of CD34$^+$CD38$^-$CLL-1$^-$ cells were metabolically distinct from the bulk tumour. Purified CD34$^+$CD38$^-$CLL-1$^-$ cells contained significantly lower levels of ROS than CD34$^+$CD38$^+$CLL-1$^+$ cells (P=0.048; Figure 1).

Summary/Conclusion:

We identified a CLL-1$^-$ROS^{low} sub-population within the CD34$^+$CD38$^-$ compartment of the KG1a cell line that is both phenotypically and functionally distinct from the remaining tumour cells. These cells were more quiescent and showed reduced oxidative stress suggesting that they possess LSC properties.
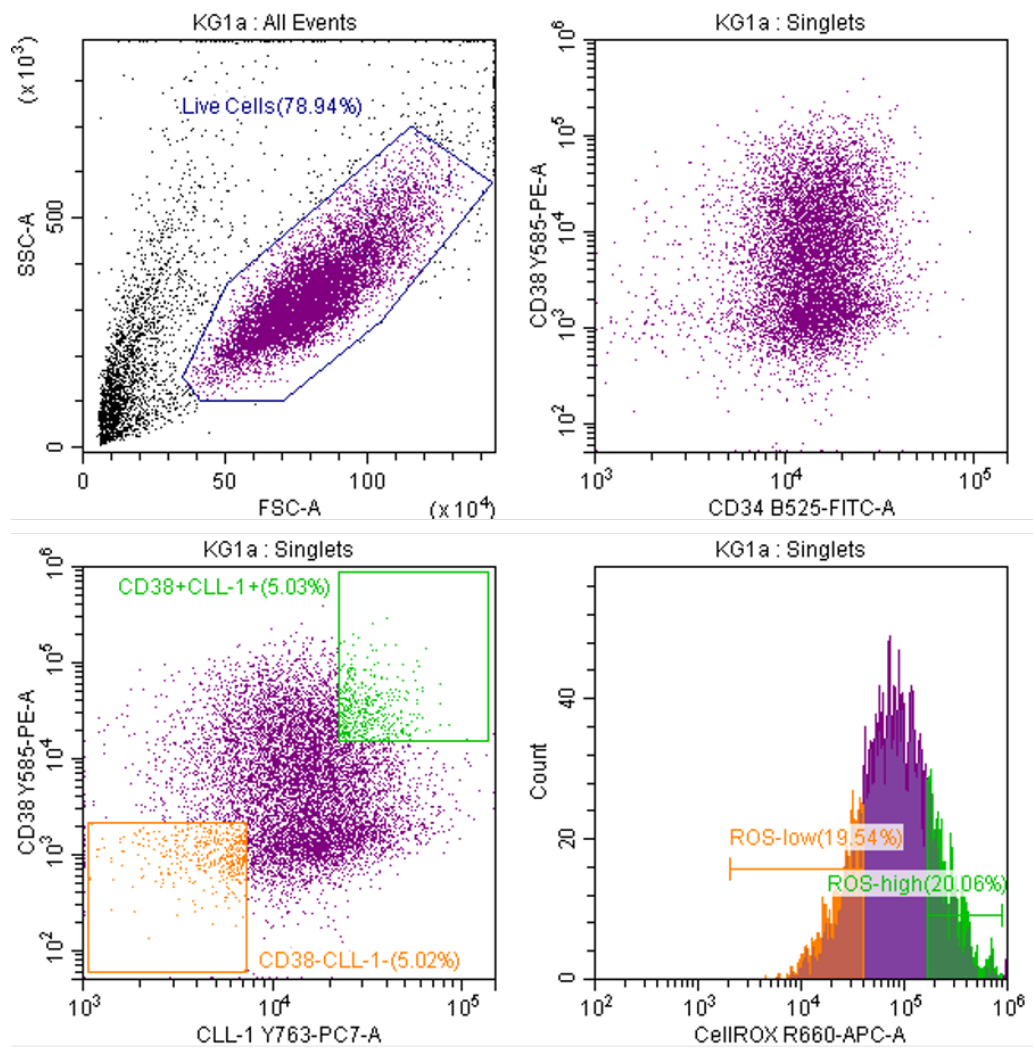
Figure 1. The gating strategy used to isolate highly purified CD34$^+$CD38$^-$CLL-1$^-$ & CD34$^+$CD38$^+$CLL-1$^+$ sub-populations and their respective levels of reactive oxygen species.