A novel approach to identify primitive CD34+ stem cells

Stem cells are important for living organisms for many reasons, as they are capable of renewing themselves as well as to expand to repair and regenerate damaged tissues. Adult stem cells exist throughout the body after embryonic development and have been found in many tissues, such as the brain, bone marrow, blood, muscles, skin, and the liver. A stem cell transplant offer new possibility for treating diseases, and it has been extensively used to regenerate diseased bone marrow. Nowadays, numerous clinical trials and stem cell treatments have attempted to provide potential cell therapies with focus on neurological disorders, diabetes, heart disease, and genetic disorders.

The CD34 cell surface marker is widely used to both identify and isolate hematopoietic stem/progenitor cells. CD34+ marrow cells are capable of reconstituting hematopoiesis, the process of blood formation. Therefore, CD34+ cell counting is crucial to efficient engraftment. Quantitative flow cytometry is the preferred method to determine the number of CD34+ cells, and is used to define an optimal dose in stem cell transplantation.

Representative CD34+/DCV measurements in peripheral blood using flow cytometry. CD34+ cells can be subdivided into two different subpopulations of CD34+ cells, that are classified as CD34+/DCV high (right window) and CD34+/DCV low cells (left window). CD34+/DCV low cells are enriched in primitive progenitors with long-term engraftment potential.

Conventional flow cytometry uses hydrodynamic focusing to move and maintain particles or cells in the center of the center of the interrogating laser, to read scatter and fluorescence signals from the particles or cells. Acoustic focusing flow cytometry uses ultrasonic waves, rather than hydrodynamic forces, to position cells into a single, focused line along the central axis of a capillary, to achieve sample-throughput rates up to 10 times faster than traditional cytometers.

Although several methods for CD34+ quantification have been proposed, there is a lack of consensus on a method to count this rare population. Thus, the aim of our research was to study the numbers of circulating CD34+ cells in peripheral blood and marrow cell specimens, by using a rare-cell adapted system that provides results with minimum possible sample perturbation. In order to discriminate nucleated white cells from non-nucleated red cells, unlysed whole blood samples were stained with the DNA-selective and cell membrane-permeant Vibrant DyeCycle Violet stain (DCV). This method is ideally suited to study the numbers of circulating CD34+ cells in combination with CD45, the common leukocyte antigen expressed by almost all nucleated white cells.

CD34+ populations usually show a well-defined cluster of fluorescent cells on a flow cytometer. Only bone marrow CD34+ cells as well as malignant CD34+ progenitor cells show different CD34 fluorescent intensities, which can be associated with a differential antigen expression. However, the distribution of DCV-stained CD34+ cells helped to identify two main cluster groups, with significantly different fluorescent intensities. Based on their different DCV intensity, we termed these two subpopulations as CD34+/DCV^{high} and CD34+/DCV^{low} cells, and in general, DCV^{high} cells (R1) were 12-times brighter when compared with DCV^{low} cells (R2).

Under our experimental conditions we obtained a dual CD34 fluorescent profile that can be explained in terms of P-glycoprotein (P-gp) activity. P-gp is a multidrug efflux pump expressed in practically all hematopoietic progenitor CD34+ cells. Hence, DCV can also be differentially effluxed by CD34+ cells with different expression levels of P- glycoprotein; the more primitive progenitors express P- gp at a high levels, giving the lowest fluorescent intensity and not ruling out a differential feature of CD34+ cells, which can be based in P-gp expression but also in the chromatin heterogeneity of progenitors cells. If true, CD34+/DCV^{low} cells should also be enriched in long-term human hematopoietic stem cells, making this protocol useful to monitor the frequency of these two subpopulations after stem cell mobilization and their association with long-term reconstitution, engraftment, and graft failure.

Taken together, our data suggest that DCV staining may be used to discriminate subsets of CD34+ cells by means of different functional properties that can be related to the characterization, resolution, and purification of primitive hematopoietic stem cells in combination with specific useful markers for multicolor flow cytometric measurements.

Publication

Vybrant DyeCycle Violet stain discriminates two different subsets of CD34+ cells.
Nunez-Espinosa C, Garcia-Godoy MD, Ferreira I, Rios-Kristjansson JG, Rizo-Roca D, Rico LG, Rubi-Sans G, Palacio C, Torrella JR, Pages T, Ward MD, Viscor G, Petriz J
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