Identification of MSCs in Trinity Evolution® Explant Cultures using FACS Analysis

INTRODUCTION

Adult mesenchymal stem cells (MSCs) are often characterized by evaluating the expression or lack of expression of specific cell surface markers. For example, previous studies have shown that adhesion molecules and cytokine receptors such as CD44, CD90, CD105, and CD166 are typically present in MSCs[1,2]. In contrast, MSCs are known to not express the CD45 antigen, which can elicit an immune response and is required for T cell and B cell activation[1]. In this study, cells derived from explants of Trinity Evolution tissue were analyzed for expression of CD105 and CD166 and the absence of CD45 in order to provide evidence that these cells are MSCs. Explanted cells were stained with antibodies for these specific markers and flow cytometry with fluorescence activated cell sorting (FACS) was used to identify cell populations. Cells that were positive for CD105 and CD166 but negative for CD45 were considered to be MSCs and were quantified as a percentage of the entire cell popluation.

METHODS

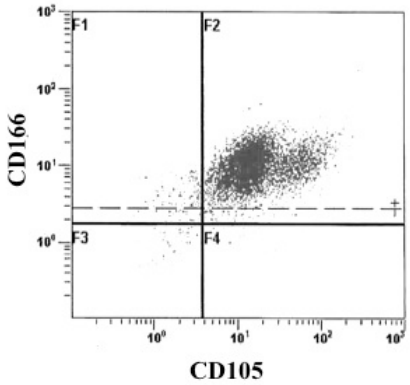
Explanted cells3 from Trinity Evolution tissue were expanded and then re-suspended in a staining buffer containing bovine serum albumin (BSA). CD105-FITC (Serotec), CD166-PE (BD Pharmingen), and CD45-APCCy7 (BD Pharmingen) fluorescent antibodies were added to the cell suspension. To exclude dying or dead cells, 7-Aminoactinomycin D (7-AAD) (BD Pharmingen) was also added to the staining tube. The molecule penetrates the membranes of dead cells and emits a fluorescent spectrum distinguishable from that of the antibodies. The cells were incubated on ice protected from light for 30 minutes to allow for antibody attachment, and then washed and resuspended in a fresh volume of buffer. The stained cells were kept on ice for a maximum of 1 hour until analysis in the flow cytometer.

Flow cytometry was used to assess the surface marker expression of cells from Trinity Evolution tissue for CD45 negative ($CD45^-$), CD166 positive ($CD166^+$) and CD105 positive ($CD105^+$) using simultaneous labeling with antibodies to perform a three-color analysis. The analysis was conducted on FACS Cytomics FC-500 (Beckman Coulter) running CXP analysis software. The cells were gated for $CD45^-$ and $CD166^+$ and $CD105^+$ markers on a dot plot with isotype as controls.

RESULTS

The FACS analysis revealed strong expression of CD166 and CD105 and very low expression of CD45 as shown in *Figures 1 and 2*. The purity was 96.29% and 98.13% respectively. *Figure 3* provides histograms of $CD166^+$ and $CD105^+$ expressing cells.

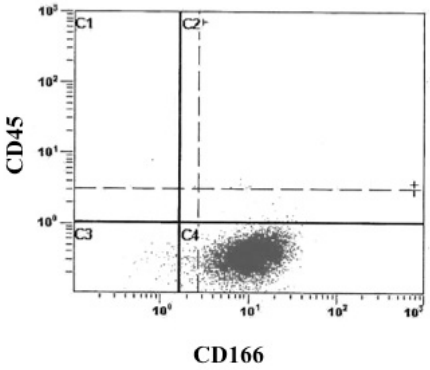
Dot Plot of CD 166+ and CD105+ Cells



Quadrant	% Gated
All	100.00%
F1	2.46%
F2	96.29%
F3	0.93%
F4	0.32%

Figure 1: A dot plot demonstrating double positive expression of CD166 and CD105 in cells from Trinity Evolution tissue. The purity of this population of cells is 96.29% (as shown in quadrant F2).

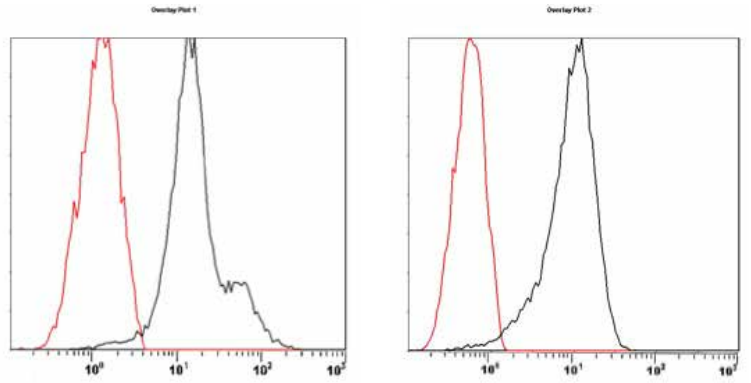
Dot Plot of CD 166+ and CD45- Cells



Quadrant	% Gated
All	100.00%
C1	0.05%
C2	0.71%
C3	1.11%
C4	98.13%

Figure 2: A dot plot showing negative expression of CD45 in cells that are positive for CD166 in Trinity Evolution tissue. The purity of this population of cells is 98.13% (as shown in quadrant C4).

Histogram of CD 105+ and CD166+ Cells



Red = unstained
Black = stained

Figure 3: Histograms highlighting CD105⁺ and CD166⁺ expressing cells (stained cells in black) compared to isotype controls (unstained cells in red). shown in quadrant C4).

DISCUSSION

FACS analysis of explanted cells from Trinity Evolution tissue demonstrated that the majority of the cells expressed two surface markers that are indicative of MSCs, CD105⁺, and CD166⁺ at a >96% purity. In addition, cells that were CD166⁺ lacked expression of CD45 (>98% purity) suggesting that these cells are nonimmunogenic. Taken together, these results suggest that Trinity Evolution tissue contains a relatively homogenous population of cells that exhibits some of the key characteristics of adult MSCs.

REFERENCES:

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