



Characterization of Human Adipose Tissue Derived Hematopoietic Stem Cell, Mesenchymal Stem Cell and Side Population Cells

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Abstract

Adipose tissue represents an abundant and accessible source of multipotent adult stem cells, which appear to possess a yet-undetermined degree of plasticity. Subcutaneous adipose tissue is much studied in recent years than omentum fat tissue. Hence the objective of this work is to characterize the freshly isolated human adipose tissue derived stromal vascular fraction cell relative to passage 0 from subcutaneous fat and the omentum fat. The stromal vascular fraction of both subcutaneous and omentum fat contains hematopoietic and mesenchymal stem cell population where as in P0 the mesenchymal stem cell alone was retained. The side population ABCG2 is present in subcutaneous fat, whereas no ABCG2 expression is found in omentum fat. This study showed that the omentum fat exhibit higher percentage of hematopoietic cells compared to subcutaneous fat in both SVF and P0. It is concluded from this study that not only subcutaneous fat, but also omentum fat derived stem cells serve as a therapeutic potential in Regenerative medicine.

Keywords: Stromal vascular fraction, Adipose derived stem cells, Subcutaneous fat, Omentum fat, Hematopoietic stem cells, Mesenchymal stem cells, Side population stem cells

1. Introduction

Adult stem cells hold great promise for use in tissue repair and regeneration (Fuchs et al 2000, Kondo M et al 2003). In recent years, research on subcutaneous adipose tissue is of great interest (Tholpady Sunil S et al 2009, Kotaro Yoshimura et al 2009). Subcutaneous adipose tissues are the fat depots which are present beneath the cutaneous layer and are easily accessible and abundant. The Stromal vascular fraction (SVF) is a heterogenous cell population derived from the adipose tissue. Several recent findings demonstrates that mesenchymal stem cells with in the stromal vascular fraction (SVF) of subcutaneous adipose tissue display multilineage developmental potential in vitro and in vivo (Giuseppe Astori et al 2007, Adam J Katz 2005, Aust L et al 2004). However there is another type of fat called the omentum fat obtained from the omentum which is present beneath the abdomen and around the intestine. Singh Ashok and his team in the year 2008 isolated, cultured and characterized the omentum fat obtained from rat and concluded that these cells exhibit stem cell properties enabling to be used for repair and possibly for the regeneration of damaged tissues (Singh Ashok et al 2008). However there has been a scarcity of information on human omentum fat tissues and its implications in therapeutics. The paucity is due to lack of easy accessibility and its influence on the manifestation of pathogenesis in the bowel as omentum fat surrounds and protects the bowel beneath the intestine. Hence, as a maiden attempt, human omentum fat tissue samples have been investigated for their cytotherapeutic potentiality in contrast with subcutaneous adipose tissue by isolation and characterization of hematopoietic stem cells, mesenchymal stem cells and side population cells in stromal vascular fraction relative to passage 0 (P0). By doing so this study identifies efficient and alternative stem cell population for future therapeutics.

2. Materials and methods

2.1 Reagents

The following antibodies conjugated with corresponding fluochromes (CD34-PE; Cat No: 348057, CD117-PE-Cy7; Cat No: 339195, CD-90-PER-CP-Cy5; Cat No: 555597, Cell viability dye 7-AAD; Cat No: 555816) were purchased from BD Biosciences, (<http://www.bd.com/>). CD105-APC; Cat No: 17-1057, ABCG2-PE; Cat No: 12-8888 and CD49d-PE; Cat No: 12-0499 were purchased from eBioscience, (www.ebioscience.com). Aldefluor; Cat No: 01700 and Ficoll Paque Plus; Cat No: 07917 were purchased from stem cell technologies, (www.stemcell.com). DMEM; Cat No: AL007, Fetal Bovine Serum; Cat No: RM1112, Antibiotic antimycotic solution (Anti-anti); Cat No: A002A, Collagenase Type I; Cat No: RM2075, Trypsin-EDTA; Cat No: TCL007, Phosphate Buffer Saline (PBS); Cat No: TL1032 were purchased from Himedia.

2.2 Adipose tissue collection

Subcutaneous adipose tissue and omentum fat tissue were obtained from the obese patients undergoing bariatric surgeries to reduce their weight and who underwent abdominal surgeries respectively. The tissues were collected after the consent of the patients. The tissues were collected in a sterilized container.

2.3 Cell isolation procedure

Cells were isolated from adipose tissue using a procedure modified from Zuk et al 2001. The collected tissues were washed two to three times with phosphate buffer saline (PBS). The tissues were taken in a sterile petridish and minced in to pieces using forceps and surgical blade with 1-3 mm in diameter. The minced tissues were then treated with 0.075% collagenase type I prewarmed to 37°C with continuous agitation for 1-2 hours and centrifuged at 600g for 10 minutes. The supernatant containing the mature adipocytes was discarded. The pellet was identified as the SVF cells. Ficoll density gradient centrifugation technique was performed on the isolated stromal vascular fraction cells. This is a modified step without filtration. The usage of Ficoll technique without filtering the sample in a 100 μ cell strainer and 40 μ cell strainer is to avoid all the debris and using this protocol, high yield of stem cells were obtained than filtration method.

2.3.1 Ficoll method

The washed pellet termed the Stromal Vascular Fraction (SVF) isolated from Adipose tissue was layered on to Ficoll density gradient medium slowly at the sides of the tube at 45° angle and centrifuged. After centrifugation at 400 g for 30 min at room temperature in a swinging bucket rotor, the SVF was collected from the adipocyte -Ficoll interface. Further, cells were washed twice with phosphate buffer saline (PBS) at 450 g for 10 minutes at room temperature to remove residual Ficoll and other contaminants. The cells were resuspended with RBC lysis buffer solution to remove any left over erythrocytes (RBC's) for 10 minutes and immediately treated with 0.9% cold NaCl to stop the lysis reaction and centrifuged at 300 g for 5 minutes at 4° C. Cell viability was determined using the Trypan blue dye exclusion method using hemocytometry. The Stromal vascular fraction cells were characterized for various hematopoietic, mesenchymal and side population cell surface markers using flowcytometry.

2.4 Cell culture of adherent cells of adipose tissue

The cells were suspended with DMEM, 10% Fetal Bovine Serum and 10 % antibiotic- antimycotic solution (anti-anti) and plated immediately in 110 mm culture dish (Cat No: PWO46; Himedia). The cells were incubated at 37° C, 5% CO₂, in a humid air. This initial passage of the primary cell culture was referred to as passage 0 (P0). The medium was replaced every 3 days. The adherent cells at P0 were removed by 0.25% Trypsin-EDTA and characterized for various surface marker analysis using flowcytometry.

2.5 Characterization of SVF cells using flowcytometry

1X10⁶ cells from stromal vascular fraction and P0 cells were taken and characterized for various hematopoietic, mesenchymal and side population markers using BD FACS Aria. The CD34+ hematopoietic population, the CD34-CD117-CD90+CD105+CD49d+ mesenchymal markers and the CD117+ABCG2+ALDH+ side population cells were used for characterization in flowcytometry.

2.5.1 Flowcytometric protocol for characterisation

Flow cytometry was performed on a Becton, Dickinson FACS Aria (<http://www.bd.com/>) using a 488-nm argon-ion LASER and 632nm red LASER for excitation; fluorescence emission was collected using its corresponding detectors. 1X10⁶ cells were stained with appropriate amount of conjugated antibodies in each of 12X75 mm falcon polystyrene FACS tube, BD Bioscience; Cat No: 352054. The quantity of each antibody conjugated with fluorochromes added to the cells in each tube were 20µl of CD34-PE, 5µl of CD90- PER CP CY5, 20µl of CD 105-APC, 5µl of CD117-PE CY7, 20µl of ABCG2-PE, 20µl of 7-AAD (BD Via probe), 20µl of CD49d-PE respectively. All tubes were incubated for 20 minutes in dark. After incubation, cells were washed in phosphate buffer saline to remove the unbound antibodies. The pellet was further resuspended to 500µl. Data analysis and acquisition was then performed using DIVA Software, Becton Dickinson. Flow cytometer instruments were set using unstained cells. Cells were gated by forward versus side scatter to eliminate debris. The number of cells staining positive for a given marker was determined by the percentage of cells present within a gate established. A minimum of 10 000 events was characterized and recorded.

3. Results

3.1 Flowcytometric analysis of subcutaneous fat

Flowcytometric analysis of both SVF and P0 of subcutaneous fat showed that hematopoietic stem cell populations were present in both SVF and P0. Cells expressing CD90+CD105+CD49d+ mesenchymal stem cells population increases in P0 compared to SVF in subcutaneous fat (Figure 1, Line diagram1).

When side population cells are considered, it is clear that SVF cell expresses higher percentage of CD117+, ALDH+ cells in SVF compared to P0 whereas ABCG2+ cells shows no significant changes in both SVF and P0 (figure 2 and bar diagram 1).

3.2 Flowcytometric analysis of omentum fat

Flowcytometric analysis of both SVF and P0 of omentum fat showed that hematopoietic stem cell population was found to be higher in SVF. Cells expressing CD90+CD105+CD49d+ mesenchymal stem cell population is found to be higher in P0 compared to SVF (Figure3 and Line diagram 2).

When side population cells are considered, it is clear that stromal vascular fraction expresses more percentage of CD117+, ALDH+ cells compared to P0, whereas no expression of ABCG2+ cells were found in both SVF and P0 (figure 4 and Bar diagram 2).

3.3 Comparative flowcytometric results of both subcutaneous and omentum fat

Flowcytometric analysis of SVF and P0 of both subcutaneous and omentum fat showed that the omentum fat contains higher percentage of hematopoietic stem cells compared to subcutaneous fat in both SVF and P0 (Line Diagram 3).

4. Discussion

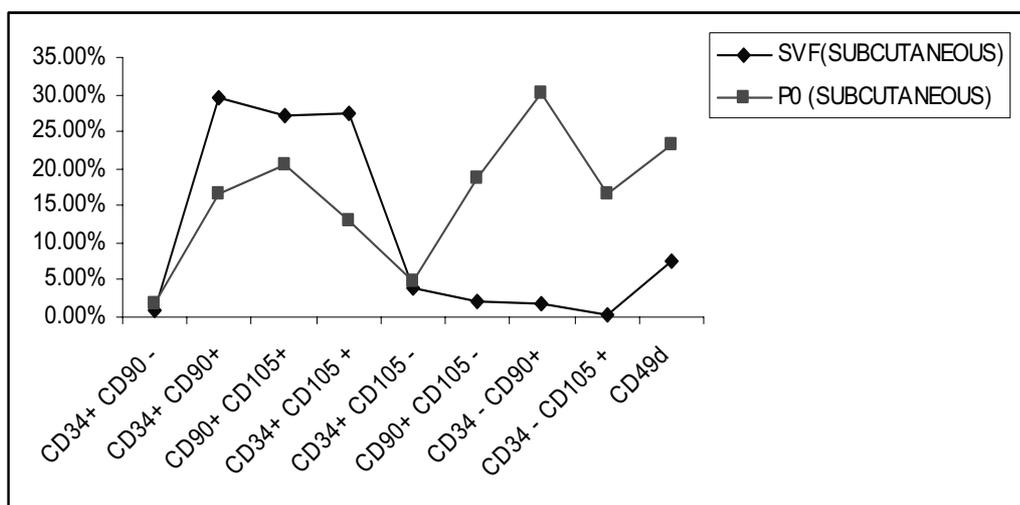
The stromal vascular fraction derived from the adipose tissue is not a homogenous cell population and usage of exact markers for characterization for therapeutic purpose remains unknown (Giuseppe Astori et al 2007). Several researchers have proved that adipose tissue derived stem cells is of great use in tissue repair, regeneration and organ transplantation (Kotaro Yoshimura et al 2009, Planat-Benard V et al 2004). Though extensive research on hematopoietic stem cells and mesenchymal stem cell exists (Gimble et al 2003, Zuk PA et al 2002), very few studies pertaining to side population characterization exists (Zhou S et al 2001, Kim M et al 2002). Moreover there is paucity in human omentum fat derived stem cell research because of the niche where the omentum fat lies and the difficulty in complications of the bowel after isolation. However we feel that the human omentum fat has multiple differentiation potential and plasticity after the findings of Singh et al and his team who found that rat omentum fat tissue serves as a potential source of tissue repair and regeneration (Singh Ashok et al 2008). Thus we chose to pursue the study on characterization of human omentum fat derived stem cell population in contrast to subcutaneous fat tissue with certain definitive markers in flowcytometry. From the results obtained, we found that side population stem cell ABCG2 is present in SVF and P0 of subcutaneous fat. Whereas research from Katz et al suggests that the SVF of subcutaneous fat lacks sidepopulation ABCG2 (Adam J Katz et al 2005). Additionally, we found that omentum fat contains high percentage of hematopoietic stem cells compared to subcutaneous fat tissue in both SVF and adherent P0 population. The advantage of omentum fat derived stem cell isolation is that the SVF of this omentum fat contain maximum of homogenous hematopoietic stem cell population and lack heterogeneity. Whereas the disadvantage of SVF of subcutaneous fat tissue is that it suffers from heterogeneity (Giuseppe Astori et al 2007). Thus from this study we found that subcutaneous and omentum fat tissues are qualitatively and quantitatively different. Hence, it is concluded from the results that not only subcutaneous fat, but also human omentum fat can be used as a high therapeutic potential in regenerative medicine.

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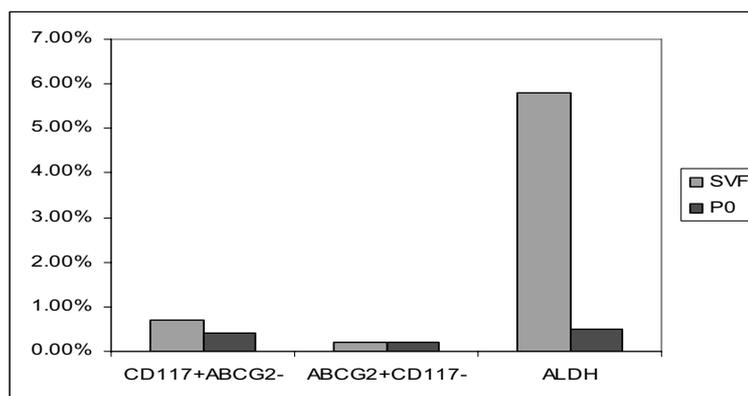
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Note: The first and second author contributed equally to this work.

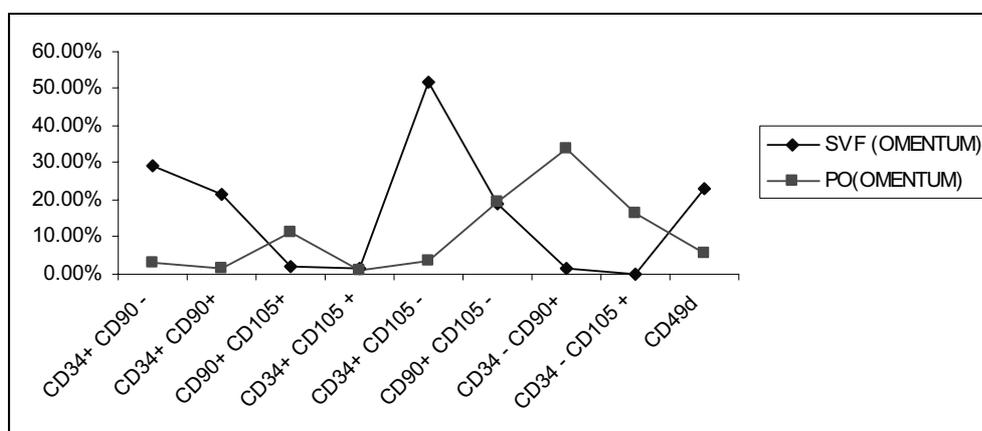
Line diagram 1. Comparison of HSC and MSC of SVF and P0 of subcutaneous fat



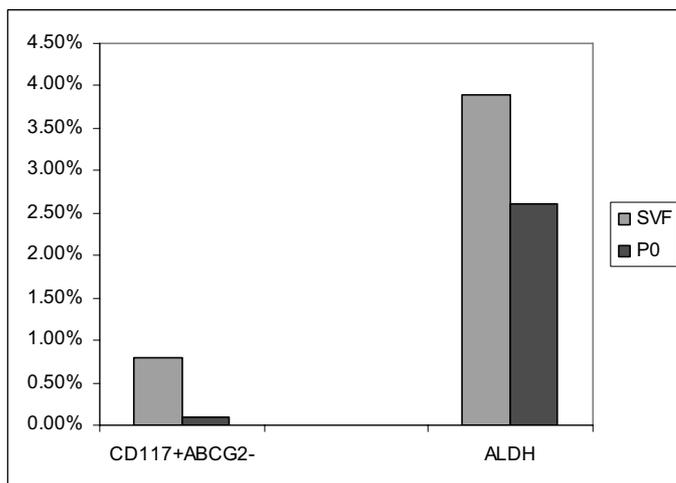
Bar diagram 1. Comparison of side population cells of SVF and P0 of subcutaneous fat



Line diagram 2. Comparison of side population cells of SVF and P0 of omentum fat



Bar diagram 2. Comparison of side population cells of SVF and P0 of subcutaneous fat



Line diagram 3. Comparison of SVF and P0 of subcutaneous and omentum fat

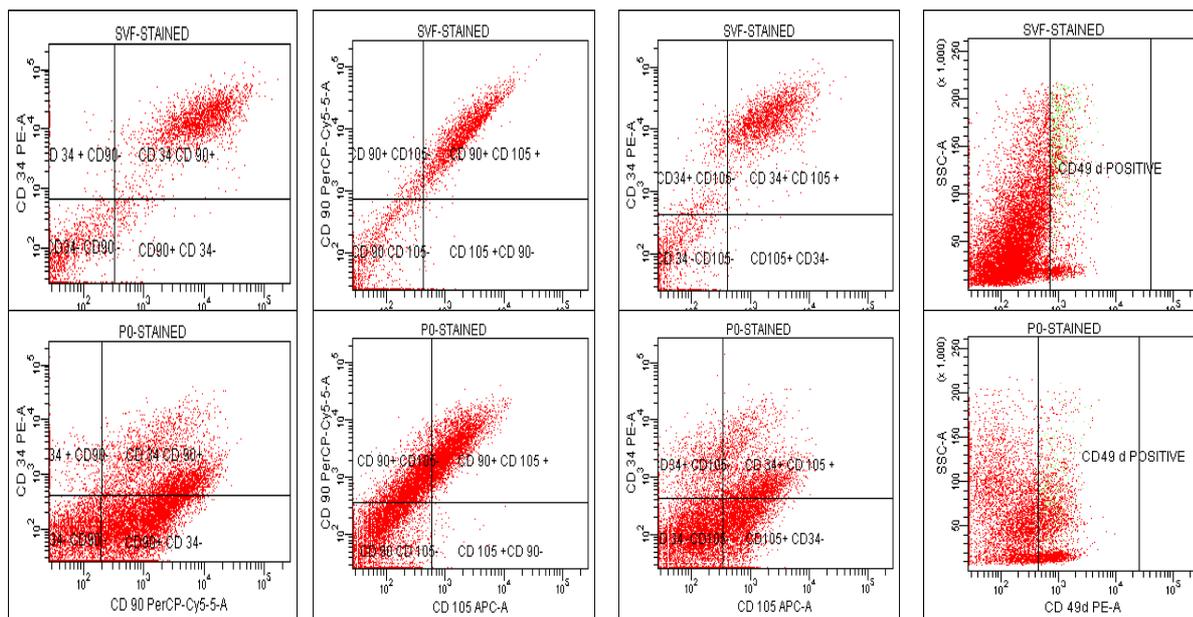
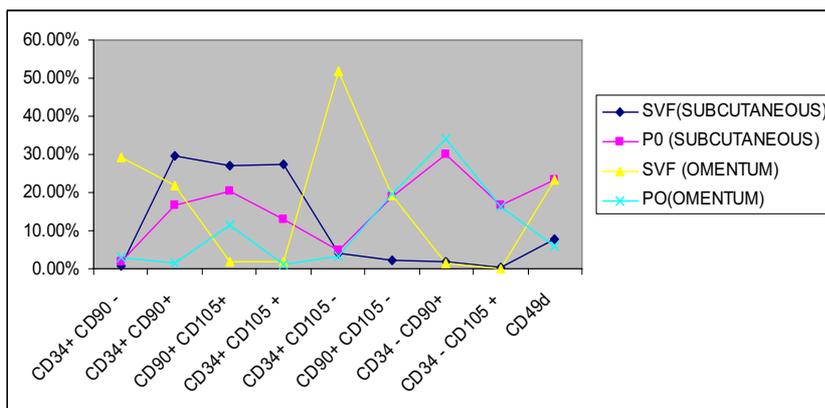


Figure 1. Flowcytometric enumeration of HSC and MSC in SVF and P0 cells of subcutaneous fat

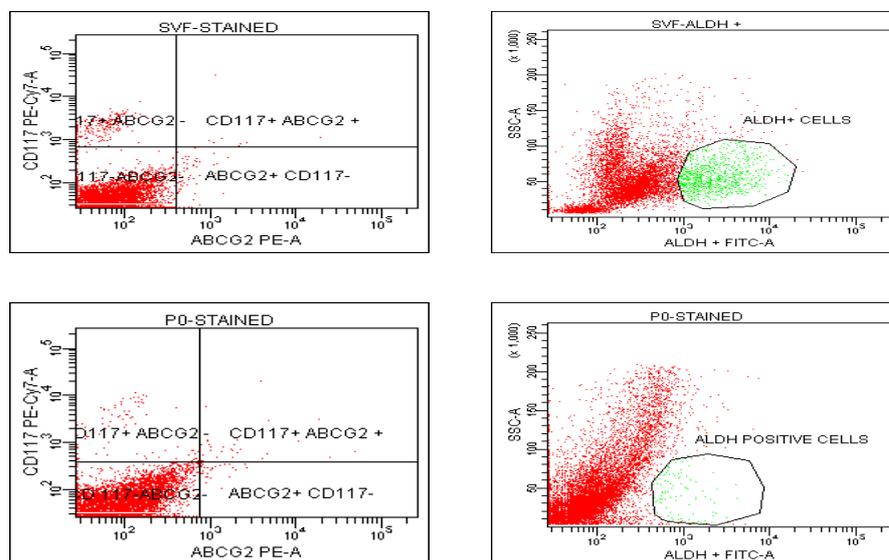


Figure 2. Flowcytometric enumeration of side population cells of SVF and P0 cells of subcutaneous fat

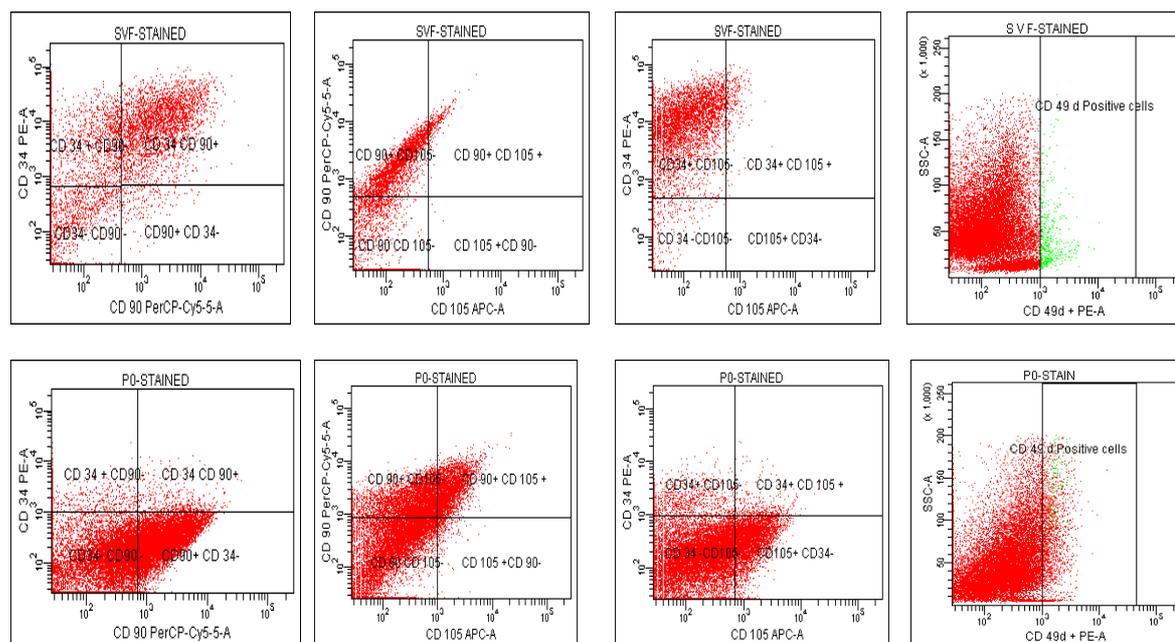


Figure 3. Flowcytometric enumeration of HSC and MSC in SVF and P0 cells of omentum fat

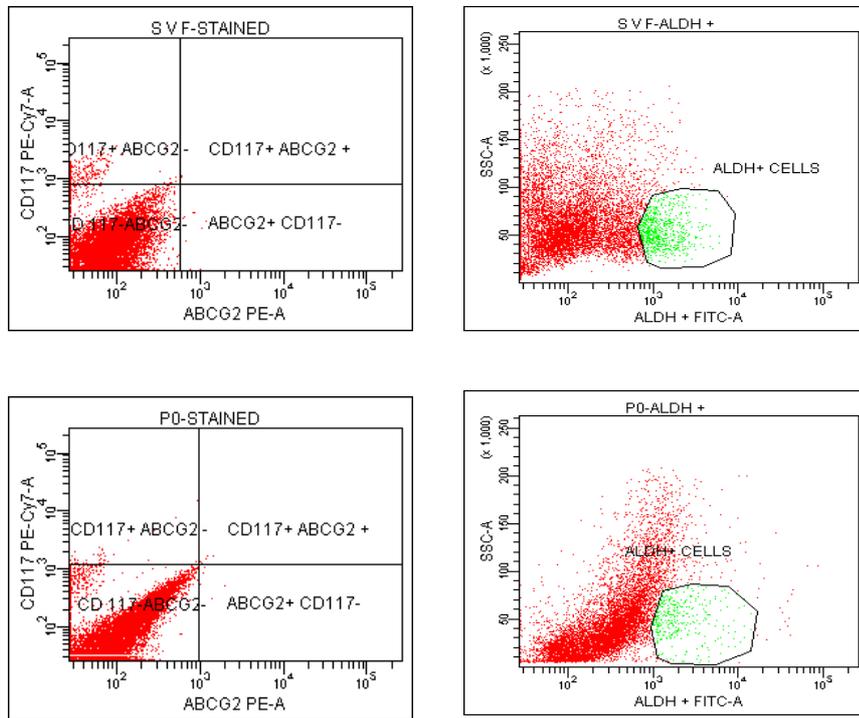


Figure 4. Flowcytometric enumeration of side population cells of SVF and P0 cells of omentum fat