

## CHARACTERIZATION OF CELL SURFACE MARKERS FOUND ON THE STROMAL VASCULAR FRACTION OF OBESE HUMAN LIPOSUCTION DONORS

### Hypothesis / aims of study

Cells within the stromal vascular fraction (SVF) are a reservoir of adipose derived stem cells with potent regenerative properties. The SVF therefore, is a promising option for therapeutic intervention in stress urinary incontinence (SUI). Little is known about the effect comorbid factors associated with SUI, (such as obesity and/or morbid obesity) have on the effectiveness of this stem cell population. Obesity reduces the speed of tissue regeneration and an association between obesity and increased stress incontinence has been previously demonstrated [1].

Thus, we hypothesize cells of the SVF, obtained from obese or morbidly obese donors will have different immunophenotypic cell subpopulation distribution than observed in non obese individuals.

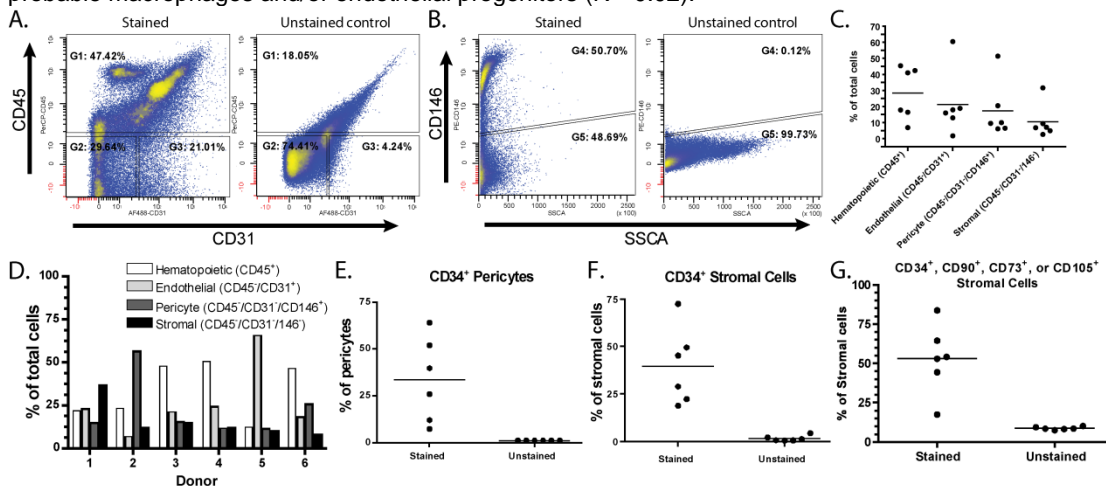
Aims: To: 1) determine the cell subpopulation makeup by multicolour cell surface immunostaining flow cytometry of the SVF obtained from obese and morbidly obese individuals and 2) assess specifically distribution of cells with mesenchymal cell surface markers obtained from the SVF of obese and morbidly obese donors.

### Study design, materials and methods

After obtaining donor's informed consent we obtained fat tissue obese and/or morbidly obese donors (n=6) (with BMI scores > 25). After maceration, fat tissue was incubated with collagenase to release cells from extracellular matrix. Once separated from the fat tissue cells were stained with fluorescent conjugated antibodies against CD31, CD34, CD45, CD73, CD90, CD105, and CD176 and analysed on a BD LSR II Flow Cytometer. The software Winlist™, by Verity Software House©, was used to sort individual cell data on the basis of the fluorescence intensity of known cell surface markers to determine the frequency of specific cell sub-populations.

### Results

We observed a broad distribution of immunophenotypic sub populations within the SVF obtained from obese donors. Flow cytometry data were gated (see Fig. 1A,B) to identify hematopoietic, endothelial, pericyte, and possible stromal cell populations. Endothelial cell populations were the most variant, while the stromal population was the least variable (Fig. 1C). The standard deviation of single marker positive cell frequency observed in our sample set was larger than observed in a previous study [2]. However, no significant trend in the frequency of single positive marker cells was observable between our obese population and non-obese donors from previously published immunophenotypic characterizations of SVF [2]. Linear regression analysis identified a modest ( $R^2=0.75$ ) linear association of BMI with an increase in the frequency of cells with the protein tyrosine phosphatase, receptor type C, CD45<sup>+</sup>, and a very slight ( $R^2=0.2$ ) declining trend associated with the stem cell marker CD34<sup>+</sup> marker. Further analysis of the CD45<sup>+</sup> cell subpopulation revealed a strong increasing linear association between BMI and probable endothelial progenitor cells of hematopoietic origin (CD45<sup>+</sup>/CD31<sup>+</sup>/CD90<sup>+</sup>) ( $R^2=0.90$ ), a modest linear association between BMI and a subset of activated T-cells (CD45<sup>+</sup>/CD31<sup>+</sup>/CD146<sup>+</sup>) ( $R^2=0.46$ ) but not no linear association with (CD45<sup>+</sup>/CD31<sup>+</sup>/CD105<sup>+</sup>) probable macrophages and/or endothelial progenitors ( $R^2=0.02$ ).



**Figure 1.** **A.** Gating for hematopoietic lineage positive (CD45<sup>+</sup>), endothelial cells (CD45<sup>+</sup>/CD31<sup>+</sup>), and possible pericytes and probable stromal cells (CD45<sup>+</sup>/CD31<sup>+</sup>). **B.** Gating based on CD146 staining to differentiate pericyte (CD45<sup>+</sup>/CD31<sup>+</sup>/CD146<sup>+</sup>) cell population (from potential stromal cells (CD45<sup>+</sup>/CD31<sup>+</sup>/CD146<sup>-</sup>)). **C.** The mean percent and range of hematopoietic lineage, endothelial, pericyte, and potential stromal cells observed for each of the 6 donors. **D.** The percentage of each immunophenotypic cell type observed as a percent of total cells from each donor. The percent of CD34<sup>+</sup> cells present in either the **E.** pericyte or **F.** the probable stromal cell population. **G.** The percentage of the probable stromal cell fraction that is positive for any MSC marker (CD34, CD90, CD73, or CD105).

### Interpretation of results

Finding increased variance but not in the average frequency of SVF cell immunophenotypic subpopulations is an expected result and consistent with our hypothesis that obesity alters the cell subpopulation of the SVF. Similarly our observation of an increased frequency of CD45<sup>+</sup> cells, activated T-cells, and probable endothelial progenitor cells of hematopoietic origin with BMI but not with macrophages and a decreasing trend with BMI and CD34<sup>+</sup> cells potentially indicates increased inflammatory potential and a reduction of the adipose derived stem cell reservoir.

### Concluding message

Variations in the stem cell populations of obese donors represent a risk factor when using SVF for SUI intervention. While the SVF derived from the obese and morbidly obese patient is potentially of limited effectiveness and present a risk of increased inflammatory response, the cells of stromal origin were the least variable ranging from between 10 and 25 % of the SVF for all donors. Understanding the cell subpopulations of SVF associated with obesity allows us to determine if these inter-donor differences alter efficacy or indicate elimination of specific SVF cell subpopulations prior to SVF stem cell therapy in the treatment SUI.

### References

1. Dwyer, P.L., E.T.C. Lee, and D.M. Hay, Obesity and urinary incontinence in women. BJOG: An International Journal of Obstetrics & Gynaecology, 1988. 95(1): p. 91-96.
2. Baglioni, S., et al., Characterization of human adult stem-cell populations isolated from visceral and subcutaneous adipose tissue. The FASEB Journal, 2009. 23(10): p. 3494-3505.

### Disclosures

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