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Adipose tissue derived stem cells proliferation ability in obese patients with early stage of type 2 diabetes

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Introduction

Adipose tissue derived stem cells (ADSCs) emerge as applicable and promising biological material for cell therapy of obesity, diabetes and associated complications. ADSCs are able to differentiate into brown adipose tissue or insulin-producing β-cells. On the other hand, some studies revealed lower ability of survival, regeneration and differentiation of ADSCs isolated from patients with long term type 2 diabetes with cardiovascular complications. It appears, therefore, important to define the group of diabetic patients that would still potentially benefit from autologous ADSCs treatment.

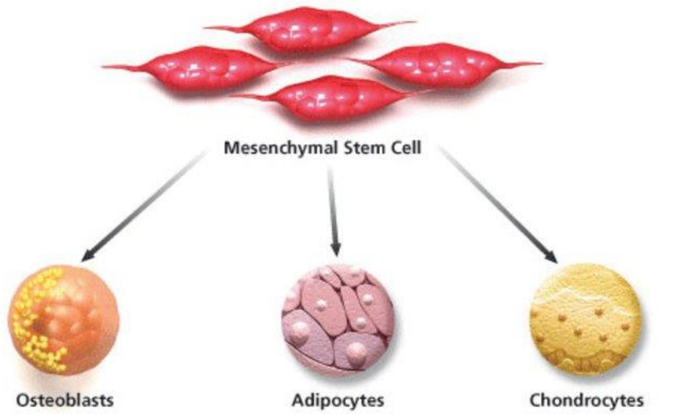

Figure 1. Differentiation potential of mesenchymal stem cells (MSC). They are population of multipotent stem cells that can be found in adipose tissue, bone marrow, Wharton's jelly, whole blood. MSC have ability to differentiate into e.g. osteoblasts, adipocytes, chondrocytes, cardiomyocytes. (http://www.sigmaaldrich.com/content/dam/sigma-aldrich/product0/013/mesenchymal-stem-cell_457.tif/_jcr_content/renditions/mesenchymal-stem-cell_457-large.jpg)

Aim

The aim of our study was to assess the proliferation ability of ADSCs obtained from obese patients at early stage of type 2 diabetes with no cardiovascular complications.

Material

Peripheral adipose tissue was acquired by lipoaspiration from 9 obese patients with type 2 diabetes (3M and 6F; aged 45.3±12 years, diabetes duration 4.8±3.3 years, BMI median 39, HOMA-IR median 9.6) treated with metformin, and from 11 healthy control patients (4M and 7F; aged 41.4±16 years, BMI median 26, HOMA-IR median 1.3).

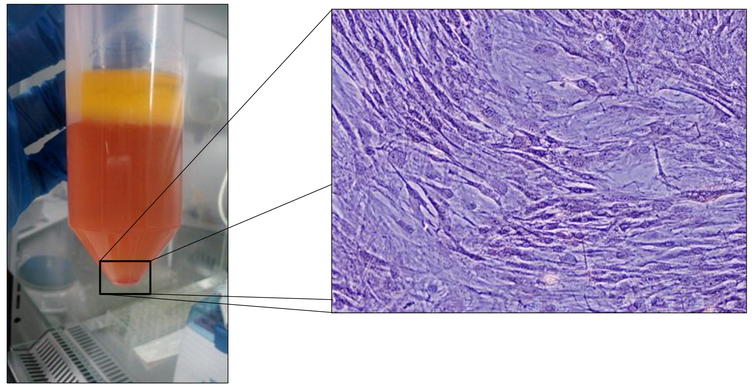

Figure 2. Isolation of adipose derived stem cells (ADSC). ADSC were isolated from aspirated adipose tissue after digestion with colagenase type 1. The homogenized and digested tissue was centrifuged to obtain the stromal vascular fraction (SVF). After that, SVF was transfered into culture dishes. Adherent, fibroblast-like shaped cells were passaged into new dishes and intentified by characteristic features.

Methods

The phenotype of ADSCs from each patient was confirmed by flow cytometry with the use of antibodies targeted against CD90, CD105, CD73 (positive markers). The cocktail of antibodies targeted against CD45, CD34, CD11b, CD79α and HLA-DR was used for negative markers analysis to fully confirm the identity of cells. Proliferation ability of ADSCs was measured by LDH, WST-1 and Sulforhodamine B (SRB) assays after 72h cultivation and also assessed by gene expression analysis of histone H3 and Ki67 with the use of RT-qPCR. For statistical analysis Mann-Whitney U or t-test were used (α=0.05).

Characteristic features of MSC	
cell culture	adherence to plastic
differentiation potential	osteoblasts, adipocytes and chondroblasts
≥ 95% population of cell with expression surface markers	CD90, CD73, CD105
≤ 2 % population of cell with lack of surface expression	CD45, CD34, CD14 or CD11b, CD79a, CD19, HLA-DR

Outcomes

Whereas WST-1-assesed cell viability and LDH activity of ADSC obtained from patients with diabetes did not differ from controls (Fig. 3 and 4), the expressions of histone H3 and Ki67 were significantly upregulated in ADSCs from diabetics (both $p<0.05$; Fig. 5). In addition, the SRB assay revealed higher density of cellular protein content in the diabetic group ($p<0.05$; Fig. 6).

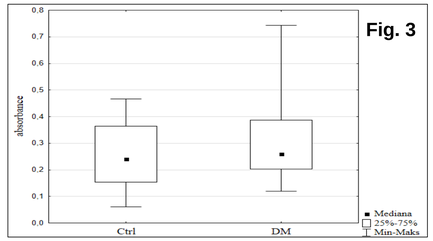

Figure 3. Cell viability of ADSC of patients with type 2 diabetes (DM) compared to the control cells (Ctrl) estimated with the WST-1 assay. The bars represent the median with the 25th and 75th quartiles and the minimum and maximum of absorbance. $p>0.05$; Mann Whitney U test.

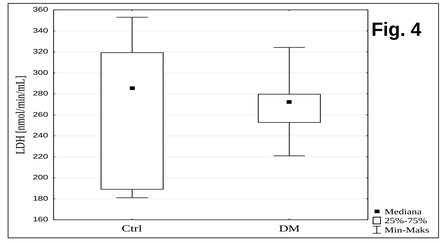

Figure 4. Cell LDH activity of ADSC of patients with DM compared to the control cells (Ctrl) estimated with the LDH assay. The bars represent the median with the 25th and 75th quartiles and the minimum and maximum of absorbance. $p>0.05$; Mann Whitney U test.

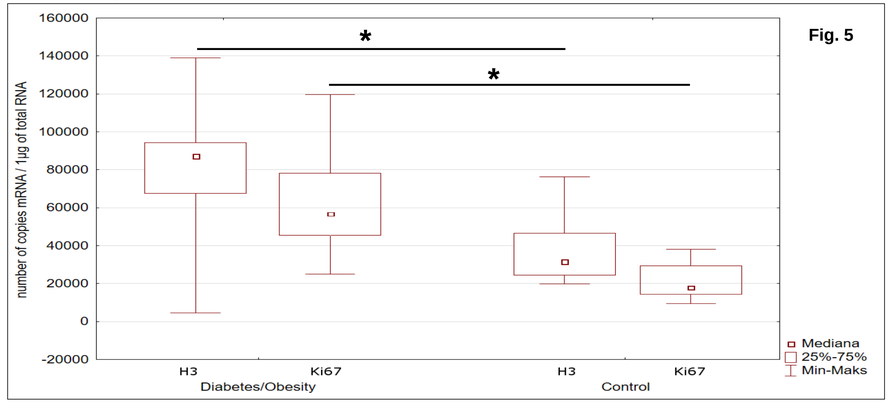

Figure 5. The mRNA levels of histone H3 and Ki67 in the ADSC of patients with type 2 diabetes mellitus compared to the control group. The bars represent the median with the 25th and 75th quartiles and the minimum and maximum of the copy numbers per 1 µg of total RNA. *$p<0.05$; Mann Whitney U test.

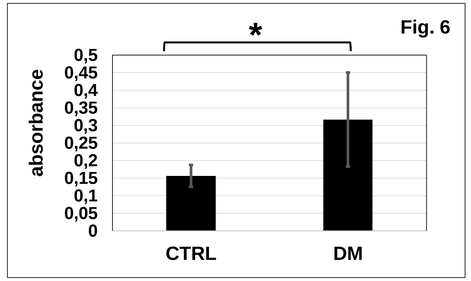

Figure 6. Total cell protein content in ADSC of patients with DM compared to the control cells (Ctrl) estimated with the SRB assay. The bars represent means ± standard deviations. *$p<0.05$; t-test

Conclusion

Our results revealed higher proliferation ability of ADSCs obtained from obese patients with short term type 2 diabetes than from healthy subjects. While intriguing and promising, this outcome requires further, larger studies to elucidate its nature and verify its clinical significance.