

## Histochemical analysis of self-organizing 3D spheroid-like cultures of adipose-derived mesenchymal stem cells

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Growth of the cells within cell culture *in vitro* is regulated by contact inhibition. When the cells form monolayer and contact with neighboring cells, they stop proliferation, migration and remain quiescent. According to published data adipose-derived mesenchymal stem cells (AD-MSc) do not follow this rule, grow above each other, form foci. Interestingly, in our culture late passage cells organized in 3D spheroid-like structures *in vitro*. The aim of the project was to study cell within these spheroids.

AD-MSc were isolated from rat visceral adipose tissue by collagenase digestion, cultivated in standard conditions. AD-MSc monolayer culture was Ki-67+,  $\alpha$ -SMA+, desmin-low, CK19-. Some spheroids, formed by AD-MSc of the passage 12 were fixed in formalin. Paraffin slices were stained histochemically with hematoxylin-eosin and immunohistochemically with antibodies against Ki-67 (proliferation),  $\alpha$ -SMA (migration), desmin, CK19 (epithelial cell marker). Remaining spheroids were placed in a 6-well plate to check viability and possibility of cells for explantational outgrowth.

Histochemical staining demonstrated fusion of cells with formation of syncytium-like structures. On the periphery cells were polygonal, round, had epithelial morphology. Groups of cells organized in line inside spheroid remained elongated AD-MSc shape. Most of the cells did not proliferate. There were no migrating  $\alpha$ -SMA+ cells. Polygonal cells on the periphery and a few cells inside the spheroid were desmin+. Interestingly, peripheral and elongated shape cells expressed epithelial marker CK-19+. Probably, within spheroid there was spontaneous mesenchymal-epithelial transdifferentiation. When spheroids were placed in a new culture dish we observed explantational outgrowth of spindle-shaped AD-MSc suggesting that cells within spheroids were alive, able to outgrow and revert through epithelial-mesenchymal transdifferentiation back to

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