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Isolation and morphological characterization of IFP-derived stem-like cells: investigation on their potential role in osteoarthritis

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The extrasynovial adipose tissue Infrapatellar Fat Pad (IFP) is an emerging player in knee osteoarthritis (OA) [1,2]. While the role of constitutive adipocytes in secreting cytokines is known, little awareness on origin/function of the stem cells component exists. This study aims to isolate/characterize IFP-derived stem cells (IFP-dSCs) from OA patients investigating their role in disease development. IFP samples were processed to discard matrix and the adipocyte fraction. The resulting pellet was resuspended in proliferative medium and cultured routinely. IFP-dSCs morphology and ultrastructure were observed by optical microscope and Transmission Electron Microscope (TEM); expansion potential of cells was assessed by a population doubling level assay. IFP-dSCs vitality was assessed using a Apoptotic/Necrotic/Healthy Cells Detection Kit, while the metabolic activity of cell cultures was analysed by MTT assay. Flow cytometry analysis was performed to identify the presence of specific markers; in particular, IFP-dSCs were stained with antibodies against CD73/105/90/44/34/106, IL-6R/1R, VEGFR2. At last, IFP-dSCs plasticity was also assessed, evaluating their commitment towards the adipogenic, chondrogenic and endothelial lineages. IFP-dSCs isolation required 14 h; cells were fibroblast-like and typically spindle shaped at low density, showing a greater polygonal nucleus at high density. Semithin-sections stained with toluidine blue revealed vesicles in the cytoplasm, as confirmed by TEM analysis. These rounded formations of electron-dense material were in proximity of empty round vesicles and in some contact areas a partial fusion between the external membranes was appreciable. An exponential growth during the entire long-term expansion period was observed, with a replication time of 42.7 $\pm$ 3.8 h. From passage (P)8 to P20, cells performed 11.9 $\pm$ 0.9 population doublings. IFP-dSCs immunophenotype was positive for the investigated markers, suggesting a role in modulation of inflammation; interestingly, cells were 100% CD73+bright both at low and high P in colture. Preliminary data about plasticity revealed the ability in differentiating towards adipogenic and endothelial lineages. Further analysis will be required to assess chondrogenic commitment. Experimental evidence on IFP-dSCs seem to correlate histopathological features of IFP in OA (i.e. thickening of interlobular septa, increase in vascularization and innervation) with the stem cell component.

References

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Key words

Osteoarthritis, infrapatellar fat pad, adipose tissue, stem cells, immunomodulation, IFP histopathological features.

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