FT-IR analysis of dermal stem cell macromolecular profile during neurodifferentiation

[2]M. Grube, [1, 3] V. Parfejevs, [2]M. Gavare, [3]L. Cappiello, [1]R. Muceniece, [1]U.Riekstina

[1]Faculty of Medicine, University of Latvia, Riga, Latvia
[2]Institute of Microbiology and Biotechnology, University of Latvia, Riga, Latvia
[3]Faculty of Biology, University of Latvia, Riga, Latvia

Dermal stem cells are promising candidates for regenerative medicine because they are able to differentiate into neuronal, smooth muscle, melanocyte, chondrocyte, adipocyte, osteoblasts and Schwan cells in vitro [1, 2]. Synchrotron and global FTIR microspectroscopy is shown to be a valuable tool for studies of different stem cells [3]. The aim of this study was to show the potential of FTIR spectroscopy for human dermal stem cell discrimination during neurodifferentiation *in vitro* using HTS-XT microplate reader.

Dermal cells were differentiated using serum-free medium DMEM/F12 (3:1) with EGF and FGF-2 during 6 weeks. Neural progenitor cell line ReNcell CX (*Millipore*) was propagated in serum-free medium with EGF and FGF-2 on laminin coating. Samples were collected each week. Expression of neural markers nestin, tubulin bIII, GFAP, p75 was analysed by immunocytochemical and flow cytometry methods. FTIR spectra were collected using microplate reader HTS-XT (Bruker, Germany).

FTIR spectra of 1-6 week old dermal cell cultures and ReNcell CX cell line revealed distinct profiles. Spectra showed changes of Amid I (1651 cm^{-1}), Amid II (1154 cm^{-1}) and lipid band intensities, the minimum between Amid I and Amid II bands, and the carbohydrate composition. In the spectra of 6th week dermal cells small band at 1054 cm^{-1} assigned to glycogen was well pronounced while not detected in ReNcell CX spectra. The 2nd derivative spectra indicated changes between ReNcell CX line and dermal cells in several regions. The spectra profile of ReNcell CX in 1005-950 cm^{-1} region significantly differed from any of dermal samples and thus allowed to discriminate the samples during the neurodifferentiation. Quantitative analysis of cell macromolecular components also showed differences in the composition of ReNcell CX and dermal cells. During neurodifferentiation the concentration of proteins decreased - this may indicate the change in the overall protein conformational state within the dermal cell, and the concentration of lipids increased.

Expression of nestin, tubulin bIII, GFAP and p75 were observed in differentiated dermal cells and ReNcell CX cell line. Even though neural marker expression pattern was similar, we found remarkable differences between dermal cell and neural progenitor cell FTIR profile in protein, lipid and carbohydrate concentrations and composition. Therefore FTIR molecular signature analysis along with the traditional immunophenotyping methods provides additional information about the cell differentiation status when dermal stem cells are considered as potential cell therapeutics for neurological disorders.

References

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