Evaluation of Biochemical Changes in Skin-Derived Mesenchymal Stem Cells during *In Vitro* Neurodifferentiation by FT-IR Analysis

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Abstract. Recently FT-IR analysis has been employed to study changes in molecular signatures during embryonic stem cell differentiation. We were interested to find out whether FT-IR spectroscopy could be applied to analyze changes in human skin mesenchymal stem cell (S-MSC) biochemical profile during *in vitro* neurodifferentiation. S-MSCs were propagated in serum-free medium with EGF and FGF-2 during six weeks. Neural progenitor cell line ReNcell CX (Millipore) was used as a reference cell line. Samples were collected each week and analyzed for neural marker nestin, tubulin β III, GFAP, and CD271 expression. FT-IR analysis was carried out using microplate reader HTS-XT (Bruker, Germany). Despite the immunophenotype similarity, FT-IR spectroscopy revealed distinct profiles for S-MSC culture and ReNcell CX cells. FT-IR spectra analyses showed changes of protein and lipid concentration during neurodifferentiation and different carbohydrate composition in ReNcell CX and S-MSCs. It was possible to discriminate between S-MSC cultures at different time points during neurodifferentiation. The results of this study demonstrate that FT-IR spectroscopy is more sensitive than conventional immunophenotyping analysis and it has a great potential for the monitoring of the stem cell differentiation status.

Keywords: FT-IR, skin mesenchymal stem cells, neurodifferentiation

1. Introduction

Many clinical and preclinical studies have demonstrated the ability of adult stem cells to treat various conditions, for example, cardiovascular diseases, autoimmunity disorders, and neurodegenerative diseases [1]. However, safety is the major concern for the stem cell therapeutic application because of the risk to introduce cells of the unknown phenotype [2]. Conventional cell characterization involves cell phenotype analysis that is based on cell surface and intracellular marker expression and the disadvantage of this approach is lack of a broad view on the cell biochemical profile. Fourier transform infrared (FT-IR) spectroscopy provides fast and label-free analysis of the macromolecular composition of cell

Figure 1: Comparison of neural lineage marker nestin, tubulin β III, GFAP, and CD271 expression after propagation in neurodifferentiation medium for two weeks in S-MSCs (a) and ReNcell CX cell line (b).

population which could be useful to monitor cell differentiation status [3–6]. Here, we use S-MSC neurodifferentiation as a model for FT-IR spectra analysis to validate the ability of the method to discriminate between differentiated and undifferentiated stem cells.

The aim of this study was to use FT-IR spectroscopy to follow the biochemical changes in human S-MSCs during *in vitro* neurodifferentiation process.

2. Materials and Methods

2.1. Cell Culture

Human skin samples were obtained from postsurgery materials in accordance with Latvian Central Ethics Committee authorized approval. Patients signed informed consent.

S-MSC cultures were obtained as described earlier [7, 8]. Cells were propagated in cell growth media DMEM-F12 (3 : 1) containing penicillin and streptomycin (100 u/mL and 100 μ g/mL), supplemented with 10% of fetal calf serum. ReNcell CX cells (Millipore) were grown in tissue culture flasks precoated with laminin (20 μ g/mL) in DMEM-F12 (3 : 1) supplemented with B27 (2%), FGF (20 ng/mL), and EGF (20 ng/mL).

2.2. Neurodifferentiation

S-MSCs 3×10^5 were transferred into T75 flasks (Sarstedt) and allowed to adhere in serum-supplemented medium for 24 hours. Then, cell culture medium was changed to serum-free neurodifferentiation medium DMEM-F12 (3 : 1) containing FGF-2 (20 ng/mL), EGF (20 ng/mL), and B27 (2%). Cells were harvested each week during six-week period (w0, w1, w2, w3, w6) for subsequent morphology, immunophenotype, and FT-IR analysis.

2.3. Immunophenotyping

For immunofluorescence analysis, cells were grown in 4-well chamber slides (Nunc) in neurodifferentiation media. After each week, specimens were rinsed with PBS and fixed/permeabilized with ice-cold acetone for 2 min at -20°C . After fixation slides were air dried and blocked with 5% BSA in PBS for 45 min. Cells were subsequently incubated overnight with primary antibodies against nestin (clone196908), Tubulin β III (clone TuJ 1), GFAP (Clone 273807; all from R&D Systems). Next, cells were rinsed with PBS and incubated with secondary anti-mouse Ig-Alexa Fluor 488 antibody for 1 h in the dark. Specimens were counterstained with DAPI, mounted with Fluoromount (Dako), and analyzed under the microscope (Leica DMI4000 B). Image overlay was performed using Image-Pro Express software. Cells were stained with PE-conjugated CD271 antibody (Clone C40-1457) and analyzed on FACSCalibur flow cytometer using CellQuest software (BDBiosciences).

2.4. FT-IR Analysis

The amount of cells in the sample, 1×10^6 cells, was determined according to the absorption spectra intensity 0.35–1.25 to fulfill Lambert-Buger-Beer Law endowing that the intensity of band

Figure 2: Normalized FT-IR spectra of S-MSC cultures propagated in neurodifferentiation medium at day 1 (w0), week 1 (w1), week 2 (w2), week 3 (w3), and week 6 (w6) in comparison with ReNcell CX cell line spectra and the HCA—vector normalization, Ward's algorithm in the region 1775–997 cm^{-1} .

is proportional to the concentration. 3–10 μL of sample were dropped on a silicon plate and dried at room temperature. FT-IR analysis was carried out using microplate reader HTS-XT (Bruker, Germany) over the range 4000–600 cm^{-1} in absorption mode and data processed using OPUS 6.5. Spectra were evaluated by vector normalization, 2nd derivative, hierarchical cluster analysis (HCA) and quantitative analysis [9].

3. Results and Discussion

S-MSCs were propagated in neurodifferentiation medium during six-week period and analyzed by immunophenotyping methods and FT-IR spectroscopy.

S-MSC cultures and ReNcell CX cells expressed neural progenitor marker nestin, glial marker GFAP, and neuronal marker tubulin βIII by immunofluorescence analysis (Figure 1). Differentiated S-MSCs as well as ReNcell CX were positive for neurotrophin receptor CD271 by flow cytometry analysis (Figure 1).

FT-IR spectra of S-MSCs *in vitro* at various differentiation stages and reference cell line ReNcell CX were evaluated. Despite similar expression of neural and glial lineage markers, S-MSCs and ReNcell CX showed distinct FT-IR spectra profiles (Figure 2).

FT-IR spectra showed changes of Amid I (1651 cm^{-1} , C=O stretching vibration and C–N groups) which is directly related to the backbone conformation and is generally considered to be characteristic of the α -helical structures), Amid II (1554 cm^{-1} , n-plane N–H bending vibration, stretching vibrations of C–N and C–C, this band is conformationally sensitive), and lipid band (1740 cm^{-1} , fatty esters) intensities, the depth of the minimum between Amid I and Amid II bands at 1591 cm^{-1} , and the carbohydrate composition ($1186\text{--}980\text{ cm}^{-1}$).

Quantitative analysis showed that during neurodifferentiation the content of proteins decreased from 68% dry weight (dw) in w0 sample to 62% dw in 6th week sample. The minimum between Amid I and Amid II bands increased and this may indicate the change in the overall protein content and α -, β -conformational states within S-MSCs. The content of lipids increased with the cell growth time from 3 to 9% dw. In the spectra of 6th week S-MSCs, a small band at 1054 cm^{-1} assigned to glycogen was well pronounced while not detected in the ReNcell CX spectrum.

The 2nd derivative spectra of all samples also indicated differences between ReNcell CX line and S-MSC samples over all region. In the region $1050\text{--}950\text{ cm}^{-1}$, ReNcell CX spectrum significantly differed from any S-MSC sample spectra. Remarkable differences were observed in protein and lipid regions of the 2nd derivative IR spectra. HCA of all sample spectra was performed and the dendrogram clearly showed distinct clusters of samples under study (Figure 2). Altogether, the data analysis of FT-IR spectra showed that S-MSC samples can be discriminated during the neurodifferentiation.

4. Conclusions

Our results clearly demonstrate that FT-IR spectroscopy could be used to monitor S-MSC differentiation status and it is more sensitive than the conventional immunophenotyping analysis. IR spectroscopy detected qualitative and quantitative changes in different macromolecular fractions—lipids, proteins, and carbohydrates during neurodifferentiation. HCA showed that starting from week two, S-MSC spectra cluster with the reference cell line ReNcell CX, possibly indicating the divergence towards neural phenotype. Thus, FT-IR spectroscopy seems to be quite sensitive and promising application for the stem-cell differentiation assessment in the future.

Acknowledgment

The authors would like to thank ESF funding 2009/0224/1DP/1.1.1.2.0/09/APIA/VIAA/055 for financial support.

References

- [1] M. Krampera, M. Franchini, G. Pizzolo, and G. Aprili, "Mesenchymal stem cells: from biology to clinical use," *Blood Transfusion*, vol. 5, no. 3, pp. 120–129, 2007.
- [2] N. Amariglio, A. Hirshberg, B. W. Scheithauer et al., "Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient," *PLoS Medicine*, vol. 6, no. 2, pp. 0221–0231, 2009.
- [3] F. C. Pascut, H. T. Goh, N. Welch, L. D. Buttery, C. Denning, and I. Notingher, "Noninvasive detection and imaging of molecular markers in live cardiomyocytes derived from human embryonic stem cells," *Biophysical Journal*, vol. 100, no. 1, pp. 251–259, 2011.

- [4] A. Downes, R. Mouras, and A. Elfick, "Optical spectroscopy for noninvasive monitoring of stem cell differentiation," *Journal of Biomedicine & Biotechnology*, vol. 2010, Article ID 101864, 10 pages, 2010.
- [5] P. Heraud, E. S. Ng, S. Caine et al., "Fourier transform infrared microspectroscopy identifies early lineage commitment in differentiating human embryonic stem cells," *Stem Cell Research*, vol. 4, no. 2, pp. 140–147, 2010.
- [6] D. Ami, T. Neri, A. Natalello et al., "Embryonic stem cell differentiation studied by FT-IR spectroscopy," *Biochimica et Biophysica Acta*, vol. 1783, no. 1, pp. 98–106, 2008.
- [7] U. Riekstina, I. Cakstina, V. Parfejevs et al., "Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis," *Stem Cell Reviews and Reports*, vol. 5, no. 4, pp. 378–386, 2010.
- [8] J. G. Toma, I. A. McKenzie, D. Bagli, and F. D. Miller, "Isolation and characterization of multipotent skin-derived precursors from human skin," *Stem Cells*, vol. 23, no. 6, pp. 727–737, 2005.
- [9] M. Grube, M. Bekers, D. Upite, and E. Kaminska, "IR-spectroscopic studies of *Zymomonas mobilis* and levan precipitate," *Vibrational Spectroscopy*, vol. 28, no. 2, pp. 277–285, 2002.