Verification of metabolite peak change during chondrogenesis of human mesenchymal stem cells using Proton NMR

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Introduction

Embryonic stem cells have pluripotency property but most of researchers use adult stem cells because of secure quantity. Human mesenchymal stem cells (hMSCs) capable of differentiating to osteocytic, chondrocytic and adipocytic under appropriate stimulation are typically used. Past few years, several studies have been conducted on characteristics of its differentiation. This paper is intended to investigate on metabolite changes of chondrogenic hMSCs using ^{1}H NMR to confirm the differentiation.

Materials and Methods

A. Cell Preparation The hMSCs at 100% confluency in 175cm^2 culture flasks were differentiated to chondrocyte with chondrogenic media containing of DMEM high-glucose with ITS+ Premix(BD, Ltd), ascorbic acid, dexamethasone and growth factor TGF β-3 during 2week(14 days). Harvested cells were washed 3 times using 0.9% saline D_2O for removing the media signal effect. Cells were centrifuged at 1,500rpm for 3minutes and then the cell pellet was filled in 5mm NMR tube (2x10^{6}cells/500μl). The subjects were divided into four groups according to the differentiation period. NMR data were acquired at 4days, 11days and 14days after the onset of the chondrogenic differentiation of hMSC. The degree of chondrogenesis was biologically assessed by alcian blue staining at 10 days

B. Data Acquisition The NMR spectrum data were acquired via a 500MHz NMR spectrometer(Unity-Inova 500, Varian, USA). The spectrums were obtained using the PRESAT one-pulse sequence with the following parameters: Relaxation delay = 20ms, 45° pulse, number of transients(nt)=512, acquisition time(at) =1.998sec, solvent = D_2O.

C. Data analysis The acquired data were analyzed by the NMR spectrum processing software, ACD/NMR manager and jMRUI. Peak picking/fitting and integration was performed to find out the change of the cell metabolites after phase/baseline correction

Results & Discussion

Figure 1 shows the result of alcian blue staining of hMSCs and chondrogenic hMSC. Chondrogenic hMSC were more thickly stained than non-induced group. It means the degree of chondrocyte was increased. Acquired NMR spectrum data was shown in Figure 2. The higher intensity peaks such as 2.17-1.20ppm, 3.64-3.68ppm and 2.82ppm detected from hMSCs and chondrogenic hMSCs have no significant changes, authors chose the peak at 2.82ppm considered as fatty acid(=CH-CH$_2$-CH=) to be used as a reference for peak normalization. It was proved that peaks at 3.78ppm, 3.56ppm, 3.23ppm, 2.94ppm-2.97ppm, 1.49ppm and 1.48ppm in all of the chondrogenic hMSCs spectrums (4day~11days) are proportional to differentiation period. The peak at 3.78ppm seems to be α-glucose cell metabolite activated by leading on conversion of glycerol to glucose. In 14days spectrum, a decrease of fatty acid peaks at 1.29-1.32ppm and acetate peak at 1.92ppm were observed. And the creatine peak at 3.04ppm (arrow in Figure 2) disappeared in spectrum immediately after chondrogenesis.

Conclusions

Peak intensities of ^{1}H MR spectrum tend to increase at 1.33/1.48/1.49/2.94-2.97/3.23/3.56/ 3.78 ppm after the onset of chondrogenic differentiation from hMSCs. On the other hand, several lipid and fatty acid peaks were reduced during the differentiation process. In this study, the possibilities are shown that the various metabolites related to chondrogenesis such as; lipid, fatty acid, creatine and lactate can be measured by MR spectroscopy technique. Further study on the relationship between cell metabolism and MR peak intensity changes during the stem-cell differentiation and its quantitative analysis includig other cell types is in working progress.

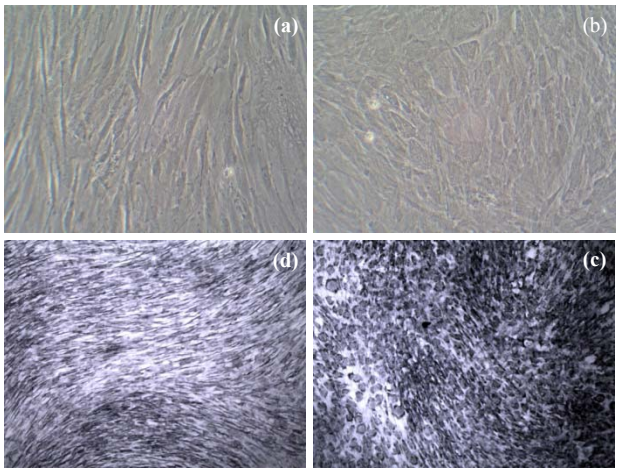

Figure 1. **Microscopy pictures of hMSC and 10days chondrogenic hMSC**. (a) non stained hMSC, (b) Alcian blue stained hMSC (c) non stained chondrognic hMSC (d) Alcian blue stained chondrogenic hMSC for 10days

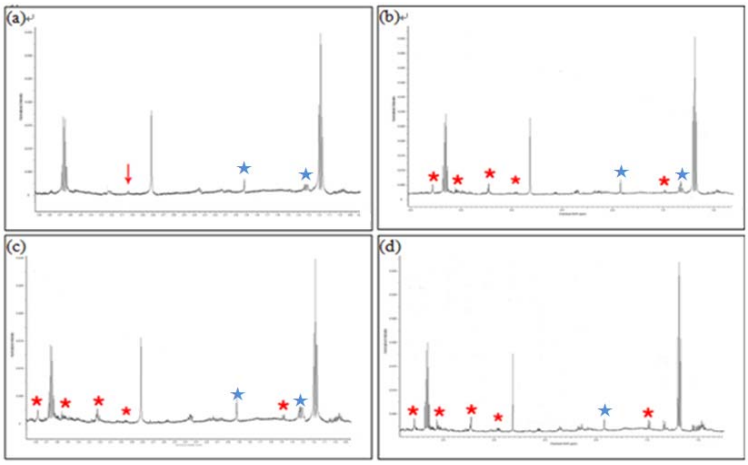

Figure 2. Acquired MR Spectrum (x25): (a) non-induced hMSCs, (b) 4days chondrogenesis, (c) 11days chondrogenesis, (d) 14days chondrogenesis. Asterisk symbols are increased metabolite peaks. Blue pentagram symbols are decreased metabolite peaks. An arrow is creatine peak.

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