Proton MR Spectroscopy Measurements for Metabolomic Changes during Adipogenic Differentiation of Muscle Derived Stem Cells

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Introduction

The stem cells have the unique properties of pluripotency/multipotency and they are able to differentiate into a diverse range of specialized cell types. The unknown materials generated during stem cells differentiation were presumed to be another cell. The aim of this study is to measure and establish the metabolite change when the Muscle Derived Stem Cells (MDSCs) were differentiated into adipocyte using the ^{1}H MR Spectroscopy. In order to measure the MRS metabolite change in detail, the liquefied MDSCs was used.

Materials and Methods

A. Sample Preparation The MDSCs of which seeding density was $5x10^6$cell/mL were used as subjects of this study. The high concentration of MDSCs which were **depressed** to fibrin gel were cultured in the three-dimensional (3D) culture system and differentiated into adipocyte using the adipogenic media. The 3D culture system could produce more cellular signals than the common cell culture systems. Following the 3D culture, the fibrin gel and MDSCs were liquefied using the papain lysis buffer. The subjects were divided into four groups according to the sample preparation, as shown by Table 1. The Adipogenic MDSCs in group 4 were differentiated for 14day. The liquefied samples were filled in 5mm NMR tube (500µl).

Table 1. Group of Liquefied Samples

Group	Adipogenic Media	Fibrin gel	Papain buffer	MDSCs	
				Non Adipogenic	Adipogenic
1	O	X	X	X	X
2	O	O	O	X	X
3	O	O	O	O	X
4	O	O	O	X	O

*** Items of this table shows the added materials**

B. Data Acquisition The MRI/MRS data were acquired via a 14.1T NMR/MRI system (Biospin, Bruker, Germany). The spectrums were obtained using the PRESS pulse sequence with the following parameters: TR/TE=3000/6.46ms; acquisition size=16000; voxel size=3×3×3mm^3; NEX=128.

C. Data analysis The acquired data were analyzed by the NMR spectrum processing software (TopSpin 2.1, Bruker, Germany) after the phase/baseline correction, peaks picking and integration.

Results & Discussion

Figure 1 shows ^{1}H MR Spectra of adipogenic media (group 1), papain digested fibrin (group 2), papain digested MDSCs (group 3) and adipogenic MDSCs (group 4). Figure 2 depicts magnified MR spectra of the group 3 and groups 4 of the subjects and newly formed metabolite peaks during the adipogenesis of the MDSCs marked by asterisk symbols were shown in Figure 2(b). This shows the increasing peak intensities at 0.89, 1.24, 1.9, 2.48 and 3.0~3.1ppm from liquefied MDSCs differentiated into adipose (group 4). The peaks appeared at 0.89, 1.24 and 1.9ppm are mobile peaks which were generated during the differentiation process. Besides, the peak at 2.48ppm is produced by the glutamic acid (glutamine) which is an important substance for cell metabolism. It affects the cycle of citric acid on mitochondria relating to amino acid degradation. Hence, we confirmed that the differentiation of MDSCs into adipose increases the vitality of mitochondria. Also, the peaks between 3.0 and 3.1 ppm are creatine which is related to energy metabolism. The creatin has the ATP which could be dissolved into ADP and causes the increase of protein synthesis.

Conclusion

It could be observed that the ^{1}H MR spectral peak intensity increases at 0.89/1.24/1.9/2.48/3.0 ~3.1ppm after 14 days of differentiation (group 4) from MDSCs into adipocyte, while not detecting other group. Besides, we could notice that the intensity of adipose peak increases following the differentiation process. We concluded that the various substances which are related to cell metabolism such as; glutamine, and creatine could activate the differentiation process. Future work requires quantitative analysis of metabolite peak changes on the differentiation of various stem cells.

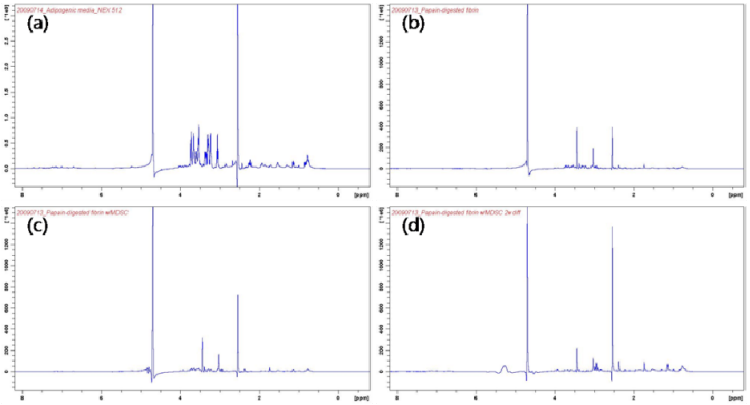

Figure 1. MR Spectrum: (a) Group1-Adipogenic Media, (b) Group2- Papain digested fibrin gel,(c) Group3-Papain digested MDSCs, (d) Group4-Papain digested adipogenic MDSCs

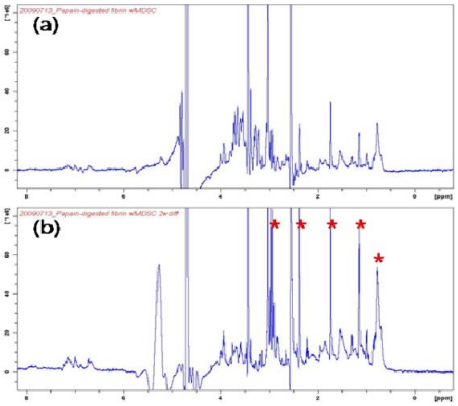

Figure 2. Magnified MR Spectrum (×20): (a) Group3-Papain digested MDSCs, (b) Group4-Papain digested adipogenic MDSCs. Asterisk symbols (*) are newly formed metabolite peaks

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