Preliminary Study for the Evaluation of the Muscle-Derived Stem Cell Metabolism using MR Spectroscopy

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Introduction

Stem cells have a characteristic that produces the clone cell prior to differentiate other cells at constant condition. However, recent study shows that materials generated during stem cells differentiation were unknown and presumed to be fat tissues. Hence, the aim of this study is to measure the metabolism change when Muscle Derived Stem Cells (MDSCs) are differentiated to adipocyte with ^{1}H NMR Spectroscopy.

Materials & Methods

A. Prepared Specimens: Muscle Derived Stem Cells (# of cells: $1*10^7$/ml) were used as subjects. The MDSCs of high concentration being depressed to fibrin gel were cultured in 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. The subjects were divided into three groups under the following conditions: *Group1*- DMEM+fibrin gel (reference), *Group2*- DMEM+fibrin+ undifferentiated MDSCs (cultured for 1day, and 1week), *Group3*- DMEM+fibrin+ differentiated MDSCs (only cultured for 1week) as displayed in Table 1. The 3D cultured MDSCs were filled in 5mm NMR tube.

B. Data Acquisition: MRI/MRS data were acquired via a 14.1T NMR/MRI system (Biospin, Bruker, Germany). Images were obtained using GRE sequence with the following parameters: TR/TE=100/6ms; Flip Angle=30°; FOV=2cm; matrix=128*128. 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; NEX=512.

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

Table 1. Grouping of Experimental Specimens

	Group 1	Group 2	Group 3
Specimens	Fibrin gel	(1) 1day cultured (2) 1week cultured MDSCs	1 week cultured MDSCs
Adipogenic	×	×	O
Characteristics	Fibrin gel	Fibrin gel + Undifferentiated MDSCs	Fibrin gel + Differentiated MDSCs
# of Subject	3	3/4	5

Results

Figure 1 compares the MR spectra of the three groups of subjects and analyzes the newly formed metabolite peaks during the differentiation of the MDSCs. In the result, the common peaks at 3.7/3.5/1.8/1.22/0.8ppm have been detected at each spectrum. Group 3, cultured MDSCs for 1 week into adipocyte, reveals a new peak at 2.6ppm that is considered as an energy metabolite substance, citrate. It is well known that the citrate is an intermediate in the TCA (Krebs) Cycle. Lipid peak concentration at 0.8~1.8ppm also increases in group 3. Table 2 shows the summary of the local metabolite peak variation during the MDCs' differentiation. Magnified spectra of Figure 1 are shown in Figure 2 to depict the small spectral peaks in detail. No significant metabolite peak variations were found except for the peak at 2.6ppm.

Table 2. Quantity comparison of each Group peaks

Group	3.5 ppm	2.6 ppm	1.8 ppm	1.2 ppm	0.8 ppm
1	1.00		0.12	0.06	0.18
2-(1)	1.00		0.14	0.12	0.16
2-(2)	1.00		0.15	0.16	0.10
3	1.00	0.42	0.14	0.29	0.16

Discussion & Conclusion

It is observed that ^{1}H MR spectral peak intensity at 2.6ppm after seven days of differentiation (group 3) from MDSCs to adipocyte increases but not in group 1(one day) and group 2(7 days with no-differentiation). This new metabolite peak result from the differentiation of MDSCs is inferred as a citrate. Citrate is a key component of the energy metabolite cycle in mitochondria. For further work, the quantitative study of metabolite change along the differentiation of various stem cells will be performed.

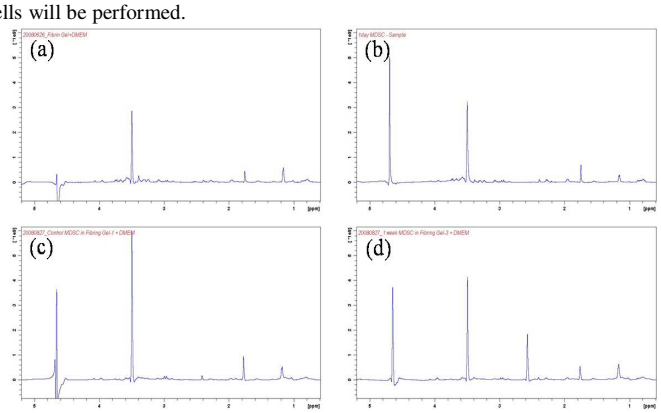

Figure 1. MR Spectrum. (a)Group1; fibrin gel, (b) Group2; fibrin+undifferentiated MDSCs: cultured 1day, (c) Group2; 1week, (d)Group3; fibrin+differentiated MDSCs: cultured 1week

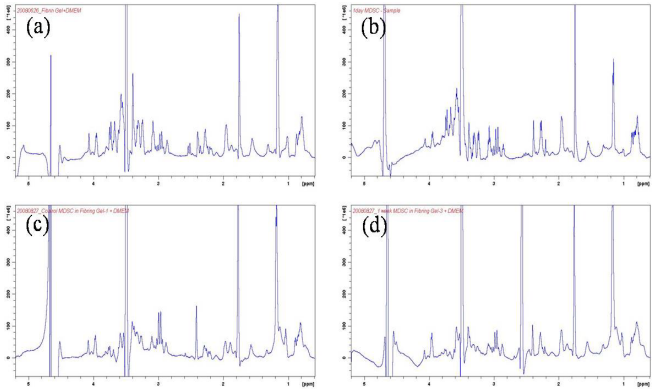

Figure 2. Magnified Spectrum (a)Group1; fibrin gel, (b) Group2; fibrin+undifferentiated MDSCs: cultured 1day (c) Group2; 1week, (d)Group3; fibrin+differentiated MDSCs: cultured 1week

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References

[1] Chang H. Lee, et al., 28th IEEE EMBS International Conference, pp775-778, 2006
[2] Patricia A. Zuk, et al., Molecular Biology of the Cell, Vol.13, pp4279-4295, 2002
[3] Kristine G. Gaustad, et al., BBRC, Vol.314, pp420-427, 2004
[4] S. Kabli et al., *ISMRM2008,* 113, [2] S. Yang et al, *ISMRM2008*, 115