Preliminary study on MR spectroscopy measurements for metabolomic change during adipogenic differentiation of human mesenchymal stem cell

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Introduction

Stem cells have the unique properties of pluripotency/multipotency and they are able to differentiate into a diverse range of specialized cell types. Unknown materials generated during the differentiation of the stem cells were presumed to be another cell. The purpose of this study is to compare the metabolite changes between the pellet samples and the hydrogelation lysed samples of the human mesenchymal stem cells (hMSC) which are differentiated to adipose using magnetic resonance spectroscopy (MRS) along the passing time.

Materials and Methods

A. Sample Preparation Adipogenic differentiation of hMSCs was processed for 4 cycles using adipogenic induction medium and adipogenic maintenance medium (1cycle: adipogenic induction medium - 3 days + adipogenic maintenance medium - 1 day). The adipogenic differentiation rate of hMSCs was confirmed by Oil Red O staining as shown in Figure 1. Two type samples, one was hydrogelation lysed cell samples prepared by using 2.5% viscosity alginic acid and solubilization solution (55mM sodium citrate, 150mM NaCl). The other was cell pellet washed by D_2O saline (99% D_2O+0.9% NaCl) and minimized H_2O signal. All samples were filled in 5mm NMR tube with external reference, 45.8mM 3-(trimethylsilyl)-1-propanesulfonic acid, sodium salt (DSS, Sigma).

B. Data Acquisition 14.1T NMR micro-imaging machine (Bruker, Germany) was used to obtain the spectrum from the samples with Point Resolved Spectroscopy (PRESS; volume selected) pulse sequence and zqpr pulse (total volume) sequence.

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

The adipose MR peaks were increased at both hydrogelation lysed sample (Figure 2) and cell pellet sample (Figure 3). Especially, lipid signals of methyl group (-CH_3) and methylene group (-CH_2) were noticeably increased. For the samples of lysis cells no signal increment was found except lipids (Table 1). On the other hand, the other metabolite variations in the cell pellet samples were observed such as isoleucine, alanine, leucine, lysine, methionine, glutamate, glutamine, choline, phosphocholine, myo-inositol and creatine etc. as shown in Table 2. In case of methionine signal, peak 16 in Figure 3(b), it was not observed during 1~3 cycles, but we could find it at 4 cycle when completed adipogenic differentiation

Conclusion

In this study, we confirmed that MR spectral peaks related to various lipid metabolites were increased when hMSCs were differentiated to adipose. During adipogenic differentiation process, authors also observed that lipid and other amino-acids related to energy metabolism were increased from the pellet of the cell samples. On the basis of this results, confirming the cell metabolites in the pellet will be helpful to find the basis standard of in-vivo cell metabolite measurements.

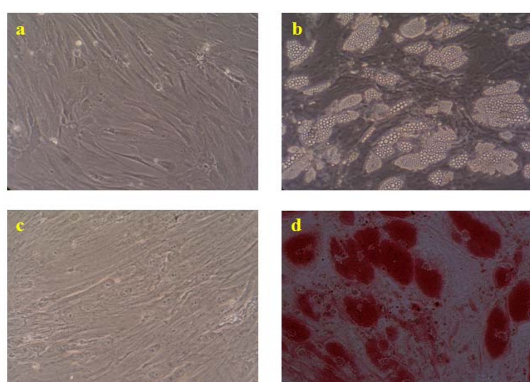

Figure 1. Morphological change and Oil Red O staining during adipogenic differentiation of hMSC. (a) non-differentiated hMSC, (b) morphology change were treated with adipogenic media, (c) Oil Red O staining: non-differentiated hMSC (×100), (d) Oil Red O staining: adipogenic differentiated hMSC (×200)

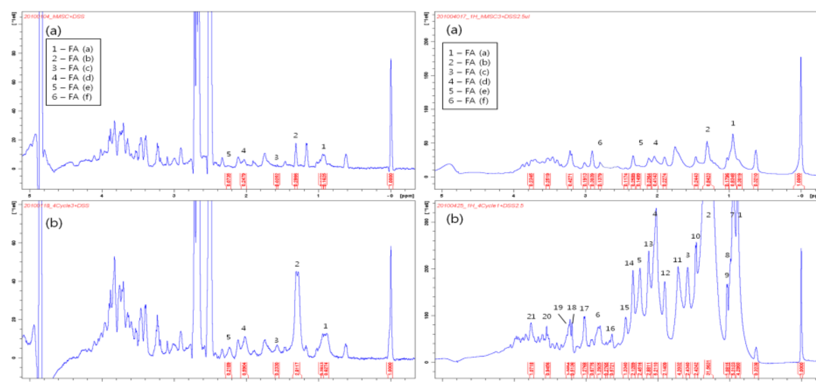

Figure 2. MR spectrum of lysed sample with DSS sample: (a) non-differentiated hMSC (300μℓ) + DSS (10μℓ), (b) adipogenic 4cycle(300μℓ) + DSS(10μℓ)

Figure 3. MR spectrum of pellet sample with DSS sample: (a) non-differentiated hMSC + DSS(2.5μℓ), (b) adipogenic 4cycle + DSS(2.5μℓ)

Table 1. Major Metabolites in Adipogenic hMSC: Lysed Samples†

	Metabolite	Chemical Group	Chemical Shift(ppm)
1	Fatty acids (a)	-C$\boldsymbol{H}_3$	0.85-0.92
2	Fatty acids (b)	-(C$\boldsymbol{H}_2$)$_n$-	1.26-1.32
3	Fatty acids (c)	-OOC-CH_2-C$\boldsymbol{H}_2$+-O-CH_2-C$\boldsymbol{H}_2$-+-CH_2-C$\boldsymbol{H}$-$(CH_3)_2$	1.54-1.63
4	Fatty acids (d)	=CH-C$\boldsymbol{H}_2$-CH_2+=CH-C$\boldsymbol{H}_2$-CH_3	1.99-2.10
5	Fatty acids (e)	-COO-C$\boldsymbol{H}_2$-CH_2-	2.20-2.26
6††	Fatty acids (f)	=CH-C$\boldsymbol{H}_2$-CH=	2.81-2.82

† Italic characters indicate protons assigned at chemical shift
†† Measured only at pellet experiment (see Figure 3)

Table 2. Major Metabolite in Adipogenic hMSC: Pellet sample

	Metabolite	Group	Chemical shift (ppm)
7	Isoleucine	δCH_3	0.94-0.95
8	Valine	γCH_3	0.98
9	Isoleucine	γCH_3	1.00-1.03
10	Alanine	βCH_3	1.47
11	Leucine	βCH_2+γCH	1.71-1.72
12	Lysine	γCH_3	1.90
13	Methionine	εCH_3	2.11-2.12
14	Glutamate	γCH_2	2.33-2.35
15	Glutamine	γCH_2	2.43-2.45
16	Methionine	γCH_2	2.63
17	Creatine	CH3	3.01-3.02
18	Choline	-N^+-$(CH_3)_3$	3.19
19	Phosphocholine	-N^+-$(CH_3)_3$	3.22
20	Myo-inositol	-CH-	3.54-3.55
21	Glutamine	αCH	3.76

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References

[1] C. Shi, X. Wang , S. Wu et al, J Tissue Eng Regen Med 2008; 2: 482–490.
[2] Chang H. Lee, et al., 28th IEEE EMBS International Conference, 2006; 775-778
[3] W.S. Turner, C. Seagle, J.A. Galanko et al,Stem Cells 2008; 26: 1547-1555
[4] I.F. Duarte, J. Marques, A.F. Ladeirinha et al, Anal. Chem. 2009; 81: 5023–5032