The changes of the metabolite profile as human mesenchymal stem cells differentiate to adipocytes mesured by in vitro 9.4T MR spcetroscopy

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Backgrounds: Cellular-based therapies using Mesenchymal stem cells (MSCs) are being evaluated as promising treatment options for many diseases and injuries, and shown a great attractiveness in regenerative medicine. However, it remains challenging to develop clinical applications, for the major limitation in tracking transplanted stem cells and monitoring their physiological activities and functions in vivo. Nuclear magnetic resonance (NMR) spectroscopy is capable of acquiring and quantifying the cellular metabolites in tissue non-invasively, accordingly, it will be a perspective approach for monitoring the differentiation and functions of the stem cells in vivo, after transplantation.

Objective: In this study, we attempt to study the alteration of metabolite of MSCs undergoing adipogenic differentiation to targeted fat cells in vitro, using 9.4T high-resolution 1H NMR spectroscopy.

Methods: When the MSCs grew up to 80-85% confluence, at passage 4, they were used for adipogenic differentiation and spectroscopy analysis. The numbers of the both cell lines is enough for analysis（about 6-7×106）, then they were measured by in vitro 9.4T NMR spectroscopy with perchloric acid (PCA) extraction. The MSCs underwent adipogenic differentiation about two weeks, the cells differentiation was assessed using the Red-O staining and RT-PCR.

Results: Several major metabolites can be observed in the MR sepectroscopy that is before and after differentiation of MSCs , including choline, creatine, glutamate and myo-inositol, acetate and some fatty acids,etc (Ttable 1,Fig.3). Quantification of metabolite concentrations was performed , the levels of intracellular metabolites, such as choline, creatine, glumate and acetate all decreased, with the increased level of methionine , succinate and fatty acids after the MSCs differentiation 2 weeks. Intracellular choline, acetate, glumate and creatine reduced from 6.3 ± 0.68, 0.97 ± 0.23, 0.3 ± 0.05 and 0.1 ± 0.02mmol/L to 1.1 ± 0.06 ($p<0.01$), 0.45 ± 0.1 ($p<0.01$), 0.16 ± 0.08mmol/L ($p<0.05$) and non-detected, respectively. Inversely, the methionine, succinate increased from 0.03 ± 0.01, 0.11 ± 0.02mmol/L to 0.12 ± 0.05 ($p<0.01$) and 0.15±0.05mmol/L ($p>0.05$), in addition the fatty acides also increased remarkabley in naked eye, without statistical analysis. In addition, many lipid droplets formed in the MSCs and the mRNA expression such as PPAR-r, ACS, and LPL, using Red-O staining (Fig.1) and RT-PCR technology (Fig.2), when adipogenic differentiation induced for 2 weeks.

Conclusion: Mesenchymal stem cells are capable of differentiating to be target fat cells by drug induction. In our study, the changes of the intracellular metabolites after the MSCs adipogenic differentiation is obvious.It indicates that NMR spectroscopy will be a great promising approach for minotoring the physiological function and differentiation after MSCs transplanation.

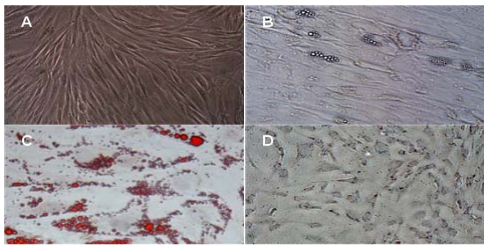

Fig.1.A is the human mesenchymal stem cells at passage 4. B and C demonstrate the MSCs after 2 weeks adipogenic differentiation without and with the red-o staining,respectively. D is the MSCs with red-o staining, without differentiation.

Metabolite	1H or spin	Chemical shift (ppm)
Valine (Val)	γ CH3	0.98 1.02
Fatty acids	- CH2 –(CH2)n –CH2-	1.28
Lactate (Lac)	- CH3	1.32
Fatty acids	(-CH2-CH3)	1.35
Fatty acids	(-CO-CH2-CH2-)	1.54
Acetate (Ace)	CH3	1.92
Glutamate (Glu)	β CH2 γ CH2	2.05 2.34
Methionine (Meth)	β CH2	2.19
Succinate (Suc)	(α, β CH2)	2.41
Fatty acids	- CH = CH-CH2 –CH = CH-	2.75
Creatine (Cr)	CH3	3.05
Choline (Cho)	N-(CH3)3	3.21
Phosphocholine (PC)	N-(CH3)3	3.22
Glyocerphosphocholine (GPC)	N-(CH3)3	3.23
Taurine (Tau)	N-CH2 S-CH2	3.27 3.43

Table.1.The intracellular metabolites and their chemical shif berfore and after differentiation of MSCs.(reference:B.SITTER,et al,2002 and Varanavasi Govindaraju, et al,2000.

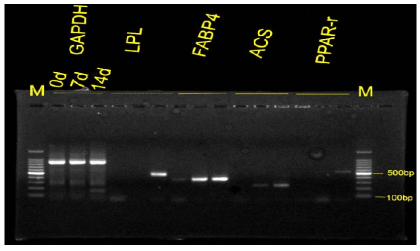

Fig.2. After adipogenic differentiation induced for 1and 2 weeks, the mRNA expr of adipocyte-specific genes, such as PPAR-r, ACS, and LPL are visible, wh absence in the MSCs, without differentiation.

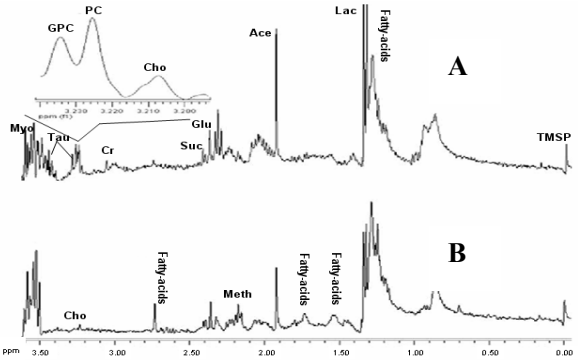

Fig.3. A and B demonstrate the NMR Spectroscopy of MSCs , before and after differentiation, Respectively.