The 1.28 ppm biomarker: not specific for neural progenitor cells, but also in the mesenchymal stem cells and differentiated adipocytes measured by NMR spectroscopy

Z-F. xu[1], C-Y. shen[2], L-P. wu[2], Y-Y. xiao[3], Y-W. chen[3], and R-H. wu[3]

[1]medical imging, the 2nd Affiliated Hospital, the Medical College of Shantou University, shantou, guangdong, China, People's Republic of, [2]Multidisciplinary Research Center of Shantou University, shantou, guangdong, China, People's Republic of, [3]the 2nd Affiliated Hospital, the Medical College of Shantou University

BACKGROUNDS: NMR-visible mobile lipid (ML), the 1.28 parts per million (ppm) biomarker, which was thought to be arisen from mobile lipid droplets formed in the cells[1], has been considered as unique for the neural progenitor cells (NPCs)[2].Scientists are trying to explore the specific biomarker of the stem cells, as a promising work to trace the cells after transplantation by MR spectroscopy.

OBJECTIVE: The aim of the present study was to determine whether the 1.28 ppm biomarker also resides in other kinds of stem cells and non-stem cells, and to approach this biomarker changes with adipogenic differentiation，meanwhile to study the relationship of the 1.28 ppm biomarker with mobile lipid droplets.

METHODS: The 1H NMR spectroscopy of the CE109 cells, mesenchymal stem cells (MSCs) and its adipogenic cells were performed in the study(Fig.1). When the CE109 cells and MSCs grew up to80-85% confluence, at passage 4, they were used for spectroscopy analysis with perchloric acid (PCA) extraction,and measured by in vitro 9.4T NMR spectroscopy.After about two weeks adipogenic differentiation[3], the Red-O staining and RT-PCR were needed for the lipid droplets demonstration and adipogenic differentiation identification(Fig.1,2).

RESULTS: Our 1H NMR experiments demonstrated that the 1.28 ppm biomarker was observed in human MSCs without growth-arrested, but absent in the EC109 cells. After adipogenic differentiation induced for 2 weeks, the 1.28 ppm biomarker climbed, remarkably, with the mobile lipid droplets generation(Fig.1,3). Concentrations of 1.28 ppm biomarker in MSCs group vs adipogenic differentiation group were as follows: (0.66±0.17)n mmol/L vs (1.18±0.07)n mmol/L ($P < 0.01$)(Fig.4). Here, we also find that mobile lipid droplets are not the origin contributing to 1.28 ppm biomarker for the MSCs.

CONCLUSION: Our results don't support the hypothesis that 1.28 ppm biomarker is specific for the NPCs, because it also in the MSCs and the cells adipogenic differentiation induced. Moreover, it indicates that it is valuable for monitoring the MSCs differentiation after cell transplantation, according to the 1.28 ppm biomarker changes. These, however, require more further experiment evidences to confirm.

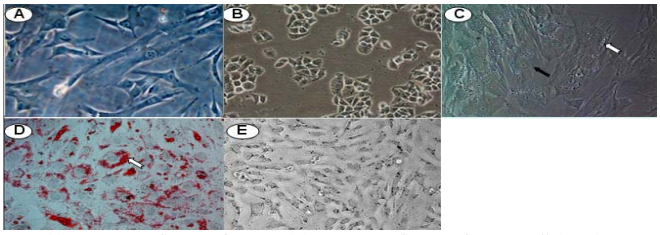

Fig.1 (A) 80-85% confluence of MSCs . (B) 80-85% confluence of EC109 cells(x100) (C) The MSCs adipogenic differentiation induced at 14 days, the cells shape transformed into ovoid/round morphology (dark arrowhead). Large numbers and big size of the mobile lipid droplets (white arrowhead). (D) Adipogenic differentiation with red-O stain, the mobile lipid droplets combined with the stain appeared red (white arrowhead). (E) The MSCs used for NMR spectroscopy with red-O stain, no mobile lipid droplets was observed.

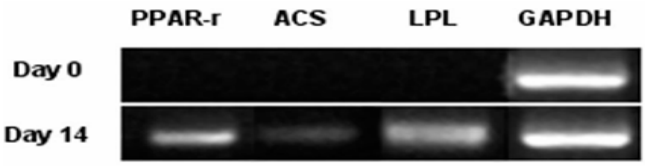

Fig. 2. Reverse Transcription-Polymerase Chain Reaction (RT-PCR). After adipogenic differentiation induced for 14 days, the mRNA expression, such as PPAR-r, ACS,and LPL are visible, which is absence in the MSCs without differentiation.

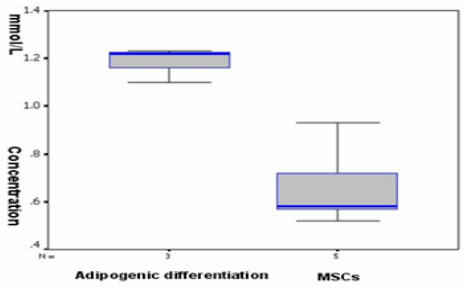

Fig.4. The concentration of the 1.28ppm biomarker for the MSCs and its adipognic differentiation. Concentrations of 1.28 ppm biomarker in MSCs group and adipogenic differentiation group was (0.66±0.17)n mmol/L(N=5) and (1.18±0.07)n mmol/L (N=3) ($P < 0.01$). (n: represents the number of CH2 group contributing to the 1.28 ppm biomarker).

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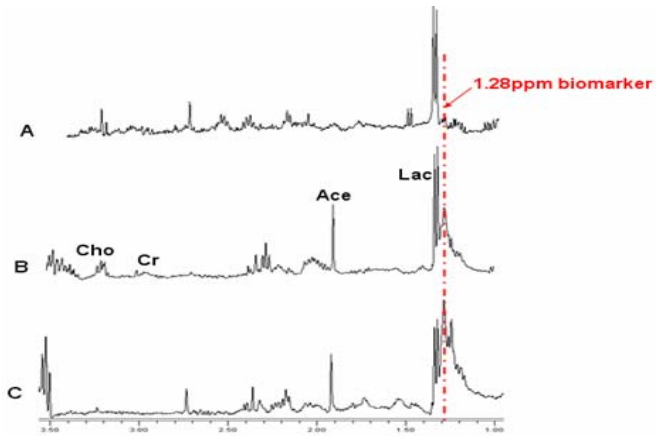

Fig.3. 1.28 ppm biomarker is dominant in MSCs and adipogenic differentiation induced, which demonstrates 1.28 ppm signals is not specific for NPCs.
(A) 1H-NMR spectra of EC109 cells at 4 passages in culture. The 1.28 ppm signal (dotted line) is clearly absent.(n=2) (B) The 1.28 ppm peak is dominant in the MSCs spetrum, at 4 passages. Moreover, assigned signals are observed: choline containing compounds (3.21-3.24 ppm), creatine (3.05 ppm), acetate (1.91 ppm), succinate (2.41 ppm), lactate (1.31ppm), fatty acid methylene chain (1.28 ppm), etc.(n=5) (C) 1H-NMR spectra of MSCs after adipogenic differentiation for 14 days. The 1.28 ppm peak increased. Of course, we also can observe the fatty chain (1.54 ppm,-O-CH2-CH2-), etc.(n=3)