COMPARISON OF OSTEOGENIC POTENTIAL AT DIFFERENT AGES BETWEEN ADULT HUMAN ADIPOSE DERIVED AND BONE MARROW STEM CELLS FROM SAME DONORS

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Introduction: Bone marrow derived stem cell (BMSC) has been a golden standard of adult stem cell therapy. However, the decreased abilities in proliferation and osteo-differentiation with aging were found in BMSC, accordingly, they might make BMSC therapy not practical for senile. Adipose-derived stem cell (ADSC) possesses good proliferation in spite of aging. However, the osteogenesis capacity of hADSC in senile with osteoporosis is not reported yet. Therefore, we are interested in the difference of the osteo-differentiation between hBMSC and hADSC from same donors. The mineralization of two stem cells from identical donors in different ages was determined by using Alizarin Red staining.

Materials and Methods: Procurement of samples: The mesenchymal stem cells were isolated and cultured from the patients received hip surgery. During surgery, cutaneous fatty tissue from operation wound (5 g) and bone marrow fluid from iliac crest (5 mL) were obtained for stem cell isolation in culture. A younger sample was obtained from a male donor, 36 y/o, with fracture(TA 36 y/o); two elder ones were obtained from a female donor with osteoarthritis (OA, 64 y/o) and a male donor with femoral neck fracture due to osteoporosis (OP, 68 y/o). Cell culture: Both hADSC and hBMSC were cultured in modified MCDB 153 medium referred as K-NAC medium, containing Keratinocyte-SFM, supplemented with N-acetyl-L-cysteine (2 mM) and L-ascorbic acid 2-phosphate (0.2 mM). Induction of osteogenic differentiation: The differentiation capacity of mesenchymal stem cells at 5th passage was analyzed toward the osteogenic pathway. Until 80% of confluence, the stem cells were cultured with osteogenic medium (OM) to induce differentiation. The mRNA expressions of genes related to osteogenesis were evaluated by real-time PCR. The ALP activity was evaluated by chemiluminescent method and mineralization was assayed by Alizarin Red staining.

Results: Because of the extracellular matrix formation, the samples will hold the red color formed from Alizarin Red-Calcium complex on the dishes. By the staining, it indicates hADSC from younger donor performed highly mineralization ability but 1-week slower than hBMSC from the same donor. (Figure 1a) In comparison with younger one, hBMSC from elder one with OA showed less potential after osteo-induction. Interestingly, hADSC in this case showed stronger osteo-induction. (Figure 1b) In OP case, the induction of hADSC still demonstrated some mineralization potential since day 10 and good mineralization was noted on day 22. The preparation of hBMSC has not been done because of poor proliferation in this case. (Figure 1c) The expression of osteogenesis-related mRNA of hBMSC and hADSC were analyzed during osteo-induction. In this study, the expression of bmp2, cbfa1 and ALP of hBMSC were detectable from day 8. The expression of bmp2 in hADSC case was illustrated on day 15, and about 1-week latter than hBMSC. By the way, the expressions of cbfa1 and ALP presented from the 2nd day after osteogenic induction in ADSC. (Figure 2) Before induction, ADSC expresses lower alkaline phosphatase (ALP) activity than BMSC does. After osteo-induction, the activity is significantly increased with time and was 100 fold increased in ADSC and BMSC after 21 and 14-day induction, respectively. (Figure 3)

Discussion: Although biologic difference exists between adipose-derived and bone marrow stem cells, the osteogenic capacity of ADSC appears to be less than BMSC and only slightly affected by age. This suggests that ADSC may be a particularly useful cellular source for orthopaedic regeneration in an osteoporosis population.

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Figure 1. Alizarin Red staining of stem cells after osteo-induction. Donors are: (a) TA, 36 y/o; (b) OA, 64 y/o; (c) OP, 68 y/o

Figure 2. The mRNA expression of adult mesenchymal stem cells after osteo-induction. (a) hBMSCs (b) hADSCs

The analysis of ALP activity