Bone marrow stromal cells of female Bag-1 heterozygous mice exhibit reduced osteogenic potential

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1. Introduction

Bag-1 (BCL-2-associated athanogene-1), the founding member of the Bag-family of co-chaperones, is a multifunctional protein which, by its ability to bind multiple partners, regulates transcription and molecular signalling crucial for cell proliferation, differentiation and apoptosis. Murine cells express two Bag-1 isoforms, Bag-1L (50 kDa) and Bag-1S (32 kDa), which are generated using alternate translation initiation sites in a single Bag-1 mRNA transcript. Expression of Bag-1 mRNA has been identified in several organs, with cartilaginous tissues showing highest expression in the developing mouse embryo. Furthermore, in long bones of postnatal mice, expression of Bag-1L and Bag-1S isoforms is detected in both chondrocytes and osteoblast-lineage cells. Significant apoptosis in the embryonic liver and brain, along with defective haematopoiesis and neuronal cell differentiation are the major causes of death in Bag-1- (null) mice between E12.5 and E13.5 of gestation, i.e., before the crucial stages of endochondral ossification characterised by vascular invasion of the mineralised hypertrophic cartilage matrix and deposition of bone by osteoblasts. Mice heterozygous for Bag-1 (Bag-1+) are viable, thereby allowing the analysis of the role of Bag-1 in bone development.

The study aims to elucidate the function of Bag-1 in osteoblast development by examining differences in osteogenic differentiation of bone marrow stromal cells (BMSCs) from Bag-1 heterozygous (+/-) and wild-type (+/+) mice.

2. Methods

a) Cellular DNA content by PicoGreen assay
b) Expression of osteoprogenitor genes by Real-time qPCR
c) Alkaline phosphatase (ALP) enzymatic activity
d) Osteocalcin (OCN) production by ELISA

Statistical analyses were performed using one way ANOVA with a post Tukey test and results were considered to be significantly different if P≤0.05 (probability of occurrence by random chance alone was less than 5%).

3. Results

Figure 4. Intracellular ALP enzyme activity (early osteoblast marker) was measured in day 28 cultures of BMSCs. Enzyme activity was normalised to the DNA content of the culture and ALP specific activity was calculated as nmol of pNPP (p-nitrophenol) released per hour per mg DNA. Results were expressed as mean ± SD, n = 3 mice in each group. ***P<0.001, **P<0.01.

Figure 5. Intracellular concentration of OCN (mature osteoblast marker) was measured in day 28 cultures of BMSCs. Concentration of OCN was normalised to the DNA content of the cells. Results were expressed as mean ± SD, n = 3 mice in each group. ***P<0.001, **P<0.01.

3. No differences in cell apoptosis were observed between the different groups of BMSC cultures

4. Conclusions

In female mice heterozygous for Bag-1, proliferation of BMSCs was enhanced at the expense of osteogenic differentiation. These studies indicate an important role for Bag-1 in osteoblast development and the need to understand the role of interacting factors modulating gender differences.

References


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