AGE RELATED EFFECTS ON CHONDROGENIC DIFFERENTIATION OF RAT BONE MARROW STROMAL CELLS

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Introduction

Endogenous repair of full thickness cartilage defects involves de novo chondrogenesis by pluripotent bone marrow stromal cells (MSCs) (1,2). This process is less effective in adults than in skeletally immature individuals, suggesting age- or maturation-related declines in MSC functions (3). Based on this we hypothesized that the chondrogenic potential of MSCs declines markedly with skeletal maturation. To test this we compared cartilage-related gene expression and extracellular matrix formation by MSCs from immature, adult and old rats cultured under chondrogenic conditions.

Methods

Bone marrow from femurs and tibias was harvested from skeletally immature rats (1-week old), mature rats (12 weeks-old) and aged rats (1-year old) with IRB approval. Marrow from 6 animals per age group were plated in serum-containing growth medium (DMEM, and 10% FBS), with IRB approval. Marrow from 6 animals per age group were plated in serum-containing growth medium (DMEM, and 10% FBS), with IRB approval. MSC cultures were fed with serum-containing growth medium (DMEM, and 10% fetal bovine serum). After 3 days, non-adherent cells were washed out of the cultures. MSCs were trypsinized and re-plated at a density of 5 × 10^5 cells/dish (for RNA extraction) or 2.5 × 10^6 cells/dish (for in vitro cartilage formation). These first passaged cultures were fed with serum-free chondrogenic medium as described (4). After 3-5 days the high-density monolayer MSCs spontaneously formed aggregates. For RNA extraction, the cells were cultured in monolayer for 7 days and then total RNA was extracted. For in vitro cartilage formation experiments, the cells were cultured for 3 weeks. Cell aggregates were harvested and frozen sections were prepared. Cartilage extracellular matrix gene expression and chondrocyte differentiation related gene expression were examined by RT-PCR, microarray hybridization (Affymetrix GeneChip® Rat Genome 230 2.0 Array), and by histologic analysis.

Results

Young MSCs differred from mature and old MSCs in their response to chondrogenic induction. RT-PCR showed that RNA levels for type II collagen, aggrecan and link protein were expressed at higher levels in 1 week cells than in 12 week or 1 year cells. However, expression of the transcription factor Sox9 was similar in all three age groups (Figure 1). Microarray analysis revealed a similar pattern of expression for these genes. The microarrays also indicated that cartilage oligomeric matrix protein, cartilage homeo protein 1, collagen type IX, and chondroitin sulfate proteoglycan 6 were expressed at higher levels in 1-week old cells than in 12-week old or 1-year old cell. In contrast, collagen type XI expression was similar in all three groups (Table). Aggregates formed by 1-week old cells stained strongly with safranin-O indicating extensive cartilage matrix formation. However, aggregates from 12 week old and 1 year old cells stained only weakly, if at all by safranin-O, indicating poor cartilage matrix formation (Figure 2). Immunofluorescence staining for collagen type II, aggrecan, and link protein showed that these proteins were abundant in 1-week old aggregates but nearly absent in aggregatess of older cells (data not shown).

<table>
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<tr>
<th>Gene Description</th>
<th>Probe Set</th>
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<tr>
<td>Collagen type II alpha 1</td>
<td>1371226_at</td>
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<tr>
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<td>700 366 230</td>
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</table>

Table Microarray Analysis of Cartilage Related Gene Expression

Figure 2 Matrix Production in MSC Cultures. Safranin-O stained aggregates from 1 week old (1W), 12 week old (12W), and 1 year old (1Y) MSC cultures. The red staining in the 1 week old culture indicates a proteoglycan rich matrix.

Discussion

Our data showed that the expression of cartilage matrix genes and other chondrocyte differentiation related genes in 1-week old MSCs in chondrogenic medium was much higher than that in 12-weeks and 1-year old rat MSCs. Cartilage formed in vitro was observed only in 1-week old rat MSCs. These findings indicate an age-related decline of the chondrogenic potential of rat MSCs.

Key words bone marrow stromal cells (MSCs), rat, chondrogenic differentiation

References


Acknowledgements

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Figure 1 Agarose gel electrophoresis of PCR Products. GAPDH control, Collagen type II (COL2), aggrecan (AGG), cartilage link protein (LNK); 1 week-old MSCs (1w), 12 week-old MSCs (12w), 1 year-old MSCs (1y).

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