The Constitutive Expression of Type X Collagen in Mesenchymal Stem Cells from Osteoarthritis Patients is Reproduced in a Rabbit Model of Osteoarthritis

INTRODUCTION

We previously showed that a major drawback of current cartilage and intervertebral disc tissue engineering is that human mesenchymal stem cells (MSCs) from osteoarthritis (OA) patients express type X collagen [1], a marker of late-stage chondrocyte hypertrophy (associated with endochondral ossification). It was not clear whether our observations with type X collagen message expression were a universal phenomenon with human bone marrow stem cells or whether they are related to variables such as age, gender, site, disease status (osteoarthritis/rheumatoid arthritis), or drug therapy. In the present study, we investigated whether the expression of type X collagen can be reproduced in MSCs from rabbits in a surgical instability model of osteoarthritis (anterior cruciate ligament transection (ACLT)).

MATERIALS AND METHODS

Rabbit Experimental Osteoarthritis model: - All the procedures were approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Montreal. Fifteen skeletally mature female New Zealand White rabbits were used in this experiment. Five (5) rabbits served as controls and the remaining 10 had a surgical transection of the anterior cruciate ligament (ACLT) performed under general anesthesia as described previously [2]. Briefly, the left ACL was exposed by a medial parapatellar skin incision, the patella was subluxated laterally, and the knee was placed in full flexion. The anterior cruciate ligament was transected, and the incision was sutured in a routine manner. Post-operative analgesics were administered for 72h. Animals were humanely euthanized (control and operated animals) at week 12. The macroscopic articular cartilage changes were then immediately graded as described below:

1: Intact surface: surface normal in appearance and does not retain India ink
2: 0 mm < Fibrillation ≤ 8 mm
3: 4 mm < Fibrillation ≤ 8 mm
4: 8 mm ≤ Fibrillation
5: 0 mm < Ulceration ≤ 2 mm
6: 2 mm < Ulceration ≤ 5 mm
7: 5 mm < Ulceration

NB: The measurements represent the length of the lesion.

Source of stem cells – Bone marrow aspirates were aseptically harvested from the proximal femur and the tibia by making an incision at the neck of the femur and injecting DMEM which was then aspirated with the bone marrow. The tibia was approached distally again by making an incision at the neck of the femur and injecting DMEM which was then aspirated with a medial parapatellar skin incision, the patella was subluxated laterally, and the knee was placed in full flexion. The anterior cruciate ligament was transected, and the incision was sutured in a routine manner. Post-operative analgesics were administered for 72h. Animals were humanely euthanized (control and operated animals) at week 12. The macroscopic articular cartilage changes were then immediately graded as described below:

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Gene expression - Total RNA was extracted using Trizol (Invitrogen). Reverse transcription reaction was performed using SuperScript II RNase H-RRT (Invitrogen) and then amplified by PCR using gene-specific primers for types I and X collagen. The housekeeping gene GAPDH was used as an internal control and served to normalize the results. PCR products were visualized by ethidium bromide staining on 2% agarose gels.

RESULTS

One sample from each group was eliminated because no cells attached after 72 hours in culture, giving a total of n=4 controls and n=9 ACLT animals. Macroscopic cartilage grades are illustrated in Figure 1. Typical fibrillation and erosion of the articular cartilage, hallmarks of osteoarthritis, were observed in the ACLT joints but not the control joints.

DISCUSSION

The present study suggests that the expression of type X collagen in OA is a universal phenomenon that is due to the disease process itself and not other environmental factors. Finding ways to suppress this expression of type X collagen is important for future tissue engineering strategies to succeed in OA patients. It is tempting to suggest that type X collagen may play a role in the bone marrow stromal microenvironment, as mice deficient in type X collagen show defects in hematopoiesis. Further studies are however necessary to better understand the effect of OA on hypertrophy.

REFERENCES


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Figure 1: Macroscopic articular cartilage lesions of the proximal femur observed in the rabbits. (A) Intact cartilage surface, (B) fibrillation (enhanced uptake of India ink between arrows), (C) complete ulceration between the arrows on the femoral condyle. (D) Quantification of the composite cartilage surface macroscopic score reflecting the total disease burden (sum of compartment scores) in the control and OA (ACLT) joints.

Figure 2: Expression of type X collagen mRNA in MSCs from experimental OA rabbits.