EFFECT OF EXOGENOUS CARTILAGE PROTEOGLYCAN ON CHONDROGENESIS OF BONE MARROW MESENCHYMAL STEM CELLS

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INTRODUCTION

Bone marrow contains mesenchymal stem cells (MSCs) capable of differentiating into cells of numerous tissue lineages including chondrocytes [1]. In addition to their multipotency, MSCs can be acquired by bone marrow aspiration without permanently damaging tissues, efficiently expanded in monolayers by serial passages without altering their differentiation potential, and may be a suitable autogenous cell source for articular cartilage repair. To make the multiplipotential MSCS, differentiate into chondrogenic lineage stimulation with cell growth factors is required [2]. It is known that during chondrogenesis there are increases of proteoglycans (PGs), such as versican and aggrecan which contain chondroitin sulfate chains [3]. The purpose of this study was to investigate the effect of exogenous proteoglycan from salmon nasal cartilage on chondrogenic differentiation of MSCs, and to clarify that PGs work for chondrogenesis acceleration instead of cell growth factors.

METHODS

Isolation and cultures of MSCs

To isolate MSCs, bone marrow aspirates were taken from the femurs of Japanese white rabbits. Nucleated cells were isolated with a density gradient (Ficoll-paque) and resuspended in complete culture medium consisting of αMEM, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 250 ng/ml amphotericin B, and 2 mM L-glutamine and incubated at 37°C in a humidified atmosphere containing 95% air and 5% CO₂. After 24 h, non-adherent cells were discarded, and adherent cells were thoroughly washed twice with phosphate-buffered saline (PBS). The confluent cells were transferred to the next passage using 0.25% trypsin and 1mM EDTA for up to four passages.

Pellet culture

A micromass culture system was used for chondrogenic differentiation. Approximately 2.5×10³ MSCs was placed in a 15-ml polypropylene tube and centrifuged at 450g for 10 min. The pellets were cultured, at 37°C with 5% CO₂, in 500 μl chondrogenic medium that contained 10⁻² M dexamethasone in high-glucose DMEM supplemented with 50 μg/ml ascorbate-2-phosphate, 40 μg/ml proline, 100 μg/ml pyruvate, 6.25 μg/ml insulin, 6.25 μg/ml transferrin, and 6.25 ng/ml selenium acid, with or without 500 ng/ml recombinant human bone morphogenetic protein-2 (BMP-2) and 10 ng/ml recombinant human transforming growth factor-β3 (TGF-β3), and with or without 100 μg/ml PG from salmon nasal cartilage. The medium was replaced every 3-4 days for 21 days.

Histology

After 21 days of culture, pellets were harvested and fixed in 10% buffer formalin overnight at room temperature. The fixed pellets were embedded in paraffin, cut into 5 μm-thick sections, and stained with hematoxylin-eosin (H-E) and alcian blue. RNA isolation and real-time PCR

Pellets were digested with 3 mg/ml collagenase, and 1 mg/ml hyaluronidase for about 3 h at 37°C to remove matrix proteins. Total RNA was extracted by using an RNeasy micro kit (Qiagen) and cDNA was synthesized by reverse transcription. Gene expression levels of type I/II/X collagen and aggrecan mRNA of MSCs was inhibited by direct action of PG. Another reason may be that chondrogenic differentiation of MSCs was inhibited by direct action of PG. Another reason may be that PG may have interacted with growth factors in the culture medium. We found that PG affects MSCs in some way and, further studies are needed to clarify this mechanism of function.

REFERENCES


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