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Regulation of adipose-derived adult stem cells differentiating into chondrocytes with the use of rhBMP-2

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Background

Adipose tissue has been demonstrated to contain a population of progenitor cells that can differentiate into bone and cartilage. Studies have suggested that adipose-derived adult stem (ADAS) cells can be induced to differentiate into chondrocytes by transforming growth factor-β (TGF-β). In this study, we examined whether bone morphogenetic protein-2 (BMP-2), as a member of the TGF-β superfamily, could regulate ADAS cells to differentiate into a chondrolineage.

Methods

ADAS cells were isolated and induced by rhBMP-2. These cells were cultured in pellets for 2 weeks, and the chondrogenic phenotype was observed in vitro *and* in vivo. *ADAS cells cultured without BMP-2 were used as controls.*

Results

After 2 weeks of culture, the differentiated ADAS cells reacted positively to Alcian blue and collagen II, and the content of collagen II protein was obviously up-regulated at day 14. Glycosaminoglycan (GAG) content gradually increased from day 2 to day 14 ($P < 0.05$). However, H&E staining and collagen II expression were weak, and there was a little collagen II protein and GAG detected in the control group. Additionally, the pellets of ADAS cells induced by rhBMP-2 were transplanted into BALB/C nude mice and formed cartilage lacuna at week 8 in vivo.

Discussion

These data demonstrate that rhBMP-2 induce ADAS cells to differentiate into chondrocytes in vitro *and* in vivo. *This is useful for basic and clinical studies aimed at repairing cartilage damage. But in a control group, ADAS cells tended towards differentiation into chondrocytes, which was affected by ITS. We will be exploring the mechanism further.*

Keywords

adipose tissue, adult stem cell, chondrocyte, chondrogenesis, recombinant human bone morphogenetic protein-2.

Introduction

As one of main cartilage types, hyaline (articular) cartilage plays an important role during the development of the skeletal system [1]. Articular cartilage functions not only as a frictionless articulating surface in diarthrodial joints but also as shock absorber, loaded with several times the body weight. However, when damaged by aging, developmental disorders, trauma, excessive weight or a combination of these, articular cartilage has only a very limited capacity to renew its original structure. The difficulty in self-repair of cartilage seems to be a result of non-dividing chondrocytes, insufficiency of progenitor cells, slow cell metabolism rate and a paucity of blood supply [2]. Thus articular cartilage repair represents a major challenge in the field of orthopedics. Lesions of cartilage tissue require effective therapeutic strategies. Various methods have been designed to repair cartilage damage, for example abrasion, drilling and microfracture [3–8]. Unfortunately, currently available therapies are not satisfactory, as there is no well-established method for restoring normal function. An alternative to the conventional treatment, however, is cell-based cartilage tissue engineering.

Because terminally differentiated chondrocytes are of limited use for tissue-engineered cartilage, multipotential

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adult stem cells have been explored to provide an alternative cell source [9]. In recent years, chondrocyte progenitor cells have been identified in various tissues, including BM [10–12], trabecular bone [13,14], periosteum [15–17], muscle [18] and synovial membrane [19,20]. Promising chondrocyte progenitor cells are easily isolated, expanded and abundant in resources [21]. Moreover, progenitor cells possess the potential for differentiation into chondrocytes. The present study was mainly focused on BM-derived adult stem (BMDAS) cells as a source of chondrogenic progenitor cells for cartilage tissue engineering. But the use of BMDAS cells raises two major issues: donor-site morbidity and loss of multidifferentiation potential after the first confluence during the *in vitro* expansion [22]. In addition, the increase of passage number *in vitro* often leads to decreasing proliferative capacity of adult stem cells, which will influence the damaged cartilage regeneration *in vivo*. Recent studies on stem cells have indicated that adult stem cells derived from adipose tissue is an attractive cell source that has the capability of multipotentiality to differentiate into osteogenic, chondrogenic, myogenic, neurogenic, hematopoietic or endothelial cells [23–31]. Because adipose tissue is obtained as lipo-aspirate from the patient in a less invasive manner and provides a large quantity of autologous cells, the use of adipose-derived adult stem (ADAS) cells for tissue engineering is an alternative way of providing seed cells [32]. In addition, these cells also show a high rate of proliferation and low senescence rate even when harvested from an adult, and do not cause immune rejection [33,34]. Thus it seems that adipose tissue is a very promising tissue source for chondrocyte progenitor cells and could greatly promote the development and usefulness of cartilage tissue engineering.

Bone morphogenetic proteins (BMP) are members of the transforming growth factor-β (TGF-β) superfamily, and the activity of BMP was first identified in the 1960s [35]. Studies have demonstrated that BMP are multifunctional growth factors and regulate many key physiologic processes, including embryonic development and cellular functions [36]. Currently more than 47 members of the BMP family have been identified. Among these members, BMP-2 is one of the most potent inducers of chondrogenesis, which not only induces undifferentiated stem cells to differentiate into chondrocytes but also stimulates committed chondrogenic cells to mature [37]. Results of previous studies have suggested that murine C3H10T1/2 cells are induced to undergo chondrogenic differentiation through high-density micromass culture and BMP-2 treatment, and the induction of chondrogenesis is dependent upon BMP-2 dose, treatment time and initial cell-plating density of the micromass cultures [38]. In a similar cell pellet culture system, BMP-2 promoted human mesenchymal stromal cells (hMSC) to differentiate into chondrocytes by increasing gene expression of collagen II over 14-day culture periods compared with control treatments [39]. In other studies on MSC, Sekiya *et al.* [40] demonstrated that BMP-2 was more effective than BMP-4 and BMP-6 in obtaining large amounts of cartilage-rich proteoglycans in *in vitro* cartilage formation. In an experiment for repairing a cartilage defect, 10 μg BMP-2 could induce muscle-derived stem cells to express collagen II mRNA at day 4 after transplantation, a mature cartilage mass was formed 5 weeks after transplantation, and the defect was repaired and restored to normal morphologic condition within 6 months of transplantation [40].

With the understanding that BMP-2 has a profound effect on stem cells, we hypothesized that BMP-2 could induce chondrogenic differentiation of ADAS cells. In this study, we investigated the chondrogenesis of ADAS cells from rabbit in pellet culture following the use of BMP-2 *in vitro* and *in vivo*.

Methods

Cell culture

ADAS cells were isolated from cervical adipose tissue of adult rabbits (age ≥ 4 months), using a modification of a method reported previously [41]. In brief, the tissue obtained was washed with equal volumes of PBS to remove red cells. The adipose tissue was minced finely using surgical scissors, and the extracellular matrix was digested for 1 h at 37°C with 0.15% collagenase (type I; Sigma, St Louis, MO, USA) in PBS. Once digested, enzyme activity was neutralized with culture medium containing DMEM (Gibco, Paisley, UK), 10% FBS (Sijiqing Biological Engineering Materials Co., Hangzhou, China), penicillin 100 U/mL and streptomycin 100 μg/mL. The samples were filtered through a 500-μm mesh filter to remove tissue debris. The cell suspension was centrifuged at 800 ***g*** for 10 min to obtain a pellet, and the pellet was resuspended in culture medium. The cells were seeded in 25-cm^2 flasks (Corning-Costar, Acton, MA, USA) at density of 4×10^5 cells/cm^2 and incubated at