Introduction

For successful cell therapy, the choice of cell source is important. It is relatively easy and safe to obtain cells from synovium with minimal donor-site morbidity. Furthermore, recent studies have revealed that mesenchymal stromal cells (MSCs) form synovium could differentiate into chondrogenic cells in vitro (1-2). Therefore, they could be promising sources for cell-based cartilage repair if we can optimize the conditions for their chondrogenic differentiation.

FK506 (tacrolimus) is widely used as an immunosuppressive reagent that specifically suppresses T-cell activation. We have recently revealed that FK506 induces chondrogenic differentiation of clonal mouse embryonic carcinoma cells (ATDC5) (3). Therefore, FK506 might likewise enhance chondrogenic differentiation of MSCs. To test the hypothesis, we investigated the effect of FK506 on the chondrogenic differentiation of synovium-derived MSCs.

Material and methods

Isolation and Expansion of Mesenchymal Stromal Cells (MSCs):
MSCs were isolated from human synovium, harvested from three patients who underwent arthroscopic knee surgery in accordance with a protocol approved by the Institutional Ethical Committee. Synovium was digested with 4% collagenase for 2 hours and the cells were cultured in growth medium (HG-DMEM with 10%FBS and 1% Penicillin/Streptomycin). After 3 to 5 passages, the expanded MSCs were harvested and used for the following experiments.

In vitro Chondrogenesis:
Approximately 2 x 10^5 cells were centrifuged at 500g for 10 minutes. The pellet was cultured in chondrogenic culture medium (HG-DMEM with 10%FBS, supplemented with 10^{-6} M dexamethasone, 50 μg/ml ascorbate, 1-phosphate, 100 μg/ml sodium pyruvate, and ITS+ Premix) as described previously (4). To analyze the effect of FK506, these pellets cultured in chondrogenic culture medium supplemented with 0.1, 0.1, or 10μg/ml of FK506 for 3 weeks. Moreover, to evaluate the synergistic effect of FK506 with other growth factors, TGF-β1 (10ng/ml), rhBMP2 (100ng/ml), IGF1 (100ng/ml) were added to the culture medium, respectively. The size of pellets was evaluated by maximum cross sectional area (MCSA) calculated by WinROOF (MITANI Japan). Chondrogenic differentiation was assessed by Alcian blue staining and RT-PCR. Semi-quantitative RT-PCR was performed on RNA extracted from the pellet (n=3 for each group) using primer sets specific for human CollagenII, Sox9, aggrecan and a housekeeping gene glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). The assessments were in the linear range of the assay. Unpaired t-test was used with a significance level of p<0.05 for statistics.

Results

Pellets cultured in chondrogenic medium with FK506 were significantly larger than those without FK506 (Fig.1 left lane, Fig.2). Increase in size was most prominent at 1μg/ml (by 28.1%) and 10μg/ml (by 31.4%) (Fig. 1, 2). In accordance with pellet size analysis, FK506 significantly increased the expression of Sox9 and aggrecan without the presence of other growth factors, while FK506 significantly up-regulated gene expression of collagen II as well in combination with BMP-2 or TGF-β1 (Fig. 4, 5).

Discussion

This study demonstrated that FK506 enhances chondrogenic differentiation of synovium-derived MSCs. Moreover, FK506 synergistically enhances their chondrogenic differentiation with TGF-β1 or BMP2. FK506 is known to bind to FK506 binding proteins (FKBPs) and recent study revealed that FKBPPI2 binds to TGFβ-receptor (TGFβR) I to inhibit phosphorylation by TGFβRRII (5). Therefore, positive effect of FK506 on TGFβ-mediated chondrogenesis could be mediated by the manipulation of FKBP-TGFβR interaction. Currently, there is no substantial evidence to explain synergistic effect of FK506 on BMP-mediated chondrogenesis; however, present results suggest the involvement of FK506 in BMP signaling pathways for chondrogenesis. Although chondrogenic action of FK506 observed in this study was not prominent as compared with that of BMP2 or TGF-β1, FK506 could have an advantage in safety in clinical use because FK506 is available internationally as an immunosuppressant with FDA approval. With further optimization, FK506 could be potentially a unique chondrogenic reagent for clinical cell-based cartilage repair.

References