INTRODUCTION: Bone marrow stromal cells (BMSCs) are pluripotent progenitor cells that have the ability to differentiate into various kinds of tissue cells. Recent studies have reported that BMSCs act as feeder cells for some types of cells in vitro. Tendon and ligament fibroblasts supposed to have some interaction with BMSCs in a living body. In a tissue engineering technique, tissue regeneration is achieved by culturing isolated cells on scaffold. Therefore, it is important to increase the number of isolated cells for tissue regeneration. Utilizing the BMSCs as feeder cells in a co-culture system with targeting cells seems to be one of the promising alternatives to cytokines or growth factors.

In the present study, we hypothesized that BMSCs would have a feeder effect on fibroblast proliferation and viability through some soluble factors. To test this hypothesis, rat fibroblasts were co-cultured with BMSCs in micro-porous membrane-separated culture systems. The objectives of this study were 1) to determine the feeder effects of BMSCs on the tendon fibroblasts in terms of proliferation, synthesis of ECMs, and ability of migration and adhesion, 2) to clarify the mechanism affecting fibroblast behavior, and 3) to clarify the animal specificity of BMSCs on fibroblasts behavior in the current co-culture system.

METHODS: 1. Membrane-separated co-culture study. Fibroblasts from WKHA rats (1 × 10^5 cells) were seeded in BD Falcon™ Multiwell Insert Systems with a micro-porous membrane (BD Biosciences, San Jose, CA USA) with feeder cells of BMSCs from WKHA rats (5 × 10^5 cells: B-F group) or fibroblasts (5 × 10^5 cells: Control). To test the proliferation of the co-cultured fibroblasts, the number of fibroblasts was counted at day 3, 7, 10, and 14 after seeding in each experimental group. (n = 10 in each time period). For the evaluation of ECM synthesis, the mass of co-cultured fibroblasts was stained for type I and type III collagen at day 14 after seeding. Quantitative real-time PCR was performed with gene-specific primer of type I and type III collagen at day 7 after seeding (n = 10). To test the migration and adhesion ability of co-cultured fibroblasts, in vitro wound healing assay and cell adhesion assay were performed according to the protocol12 with a little modifications (n = 10 in each assay).

2. Conditioned medium culture study. To examine the effects of soluble factors, a conditioned medium was collected from the B-F group and the control. To clarify the influence of signals from fibroblasts, the culture medium was also collected from BMSCs (5 × 10^5 cells) of WKHA rats without feeding cells of fibroblasts (B group). Fibroblasts (1 × 10^5 cells) were cultured in the conditioned medium without feeder cells. The number of fibroblasts was counted at day 3, 7, 10, and 14 after seeding in all experimental groups (n = 6 in each time period).

3. Animal specificity of feeder effect study. Fibroblasts were co-cultured with allogeneic (ACI rat, Allo group), xenogeneic (Japanese white rabbit, Xeno group), and autogeneic BMSCs. The proliferation of co-cultured fibroblast was studied the same method as membrane-separated co-culture study (n = 10 in each time period).

RESULTS: 1. Membrane-separated co-culture study. The number of fibroblasts significantly increased with time in the B-F group compared to the control (Fig. 1). Immunohistochemical evaluation showed that there were more ECM products in the B-F group compared to the control (Fig. 2). Quantitative real-time PCR showed no difference between two groups in terms of mRNA expression of type I and type III collagen. Fibroblasts co-cultured with BMSCs had higher ability of migration and adhesion compared to the control (Fig. 3, 4).

2. Conditioned medium study. In the B-F group and the B group, the number of fibroblasts significantly increased compared to the control (Fig. 5).

3. Animal specificity of feeder effect study. The number of fibroblast was significantly higher in groups, which co-cultured with not only autogeneic BMSCs but also allogeneic or xenogeneic BMSCs, compared to the control (Fig. 6). Furthermore, xenogeneic BMSCs had highest feeder effect. On the other hand, allogeneic BMSCs had least effect.

DISSCUSSION: The present results indicate that BMSCs have the influence on the proliferation, migration and adhesion but little on the synthesis of ECMs of tendon fibroblasts as a feeder cell. Even the second use of a culture medium from BMSCs significantly increased the number of fibroblasts. Based on these results, the mechanism of the feeder effects is considered to be affected from a certain signal of soluble factors of BMSCs without cross-talk between these two types of cells. In the living body, various factors affect the viability of the tendon fibroblasts. The cell amplification with use of co-culture method considered to be physiological in terms of the use of exogenous growth factors. In addition, this study suggests that the effects are available even if the feeder cells are originated from other species. The feeder effect was highest in co-cultured with xenogeneic BMSCs. The reason of this result may be the amount of some soluble factors secreted by BMSCs, from large size of animals.

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