INTRODUCTION
Aging leads to a decline in the functional competence of human bodies as well as to degenerative changes in connective tissues such as tendons. Aging tendons lose the integrity of their matrix and mechanical strength. However, the precise cellular mechanisms responsible for the detrimental effects of aging on tendons are unknown. We recently identified tendon stem cells (TSCs) in rabbits and mice [1, 2]. TSCs possess multi-differentiation potential; namely, they can not only differentiate into tenocytes under normal physiological conditions but also non-tenocytes (e.g. adipocytes, chondrocytes, and osteocytes) under aberrant conditions. Aging could be one such aberrant condition for tendons. Therefore, we aimed in this study to test the hypothesis that with aging, the number of TSCs decreases and TSCs tend to differentiate toward non-tenocytes.

METHODS
TSCs were isolated from the patellar tendons of mice in four age groups (2.5, 5, 9, and 24 months) and cultured using a published protocol [1]. TSC proliferation for each of the four age groups was determined by measuring cell population doubling time (PDT). The stemness of the TSCs was examined through the immunostaining of stem cell markers nucleostemin, Oct-4, Sca-1, and Nanog. In addition, the expression of tenocyte related genes collagen I and tenomodulin and non-tenocyte related genes LPL, Sox-9, and Runx-2 was measured using quantitative real time RT-PCR (qRT-PCR). LPL, Sox-9, and Runx-2 are specific markers for fatty tissue, cartilage tissue, and bony tissue, respectively.

For statistical data analysis, one-way ANOVA was used, followed by Fisher’s PLSD test for multiple comparisons. P < 0.05 between two groups was considered to be significantly different.

RESULTS
The proliferation of mouse TSCs markedly decreased in an age-dependent manner, as evidenced by rising PDT values (Fig. 1). Moreover, the extent of the stem cell marker expression by TSCs (nucleostemin, Oct-4, and Sca-1) also decreased in an age-dependent manner (Fig. 2). Finally, qRT-PCR analysis showed that the expression of tenocyte-related genes (collagen type I and tenomodulin) was down-regulated with increasing mouse age (Fig. 3), whereas the expression of non-tenocyte-related genes (LPL, Sox-9, and Runx-2) was markedly upregulated with mouse age (Fig. 4).

DISCUSSION
This study showed that aging has detrimental effects on mouse TSCs in terms of decreased cell proliferation and reduced stemness of TSCs, as demonstrated by increased PDT, downregulation of stem cell and tenocyte marker expression, and upregulation of non-tenocyte marker expression. These findings are consistent with those of a previous study on rat TSCs [3]. Since TSCs play a critical role in tendons’ maintenance and repair, these data may explain why aging people have problems repairing injured tendons and tend to develop tendinopathy characterized by lipid deposition, proteoglycan accumulation, and calcification, likely due to aging TSCs differentiating into non-tenocytes.

SIGNIFICANCE
The detrimental effects of aging on tendon stem cells must be considered when devising treatment regimens for tendon injuries of aging patients in clinics.

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REFERENCES