INTRODUCTION
The degenerative pathomechanism of intervertebral disc remains unclear. However, numerous studies have mentioned the importance of cellular pathobiology in the disc tissue degradation, based on gene-quantitation analyses such as real-time RT-PCR. An essential step to quantify target gene expression is normalization to account for varying amount of RNA isolated from different samples, and house-keeping genes (HKGs) have generally been used as internal references; because it is often assumed that the expression levels of HKGs are constant across many forms of experimental conditions. Nevertheless, it is controversial whether all the so-called HKGs are appropriate for intervertebral disc research, because only a few reports have described the feasibility of common HKGs as endogenous controls. Our objective was, using the rat tail static compression loading-induced disc degeneration model we validated, to clarify the feasibility of common HKGs in the disc cells under mechanical loading conditions.

METHODS
This experiment was approved by the Animal Care and Use Committee of our institute. Forty-eight 12-week-old male Sprague-Dawley rats were equipped with an Ilizarov-type device with springs between the 8th and 10th coccygeal (C) vertebrae and statically loaded for 0, 7, 28, or 56 days at 1.3 MPa (Fig. 1A-C). Loaded (C8-9 and C9-10) and unloaded (C11-12 and C12-13) discs were harvested.

RESULTS
Radiography: Radiographs were taken and the disc height index (DHI) of each disc was calculated (Fig. 1). MRI: T2-weighted MRIs were taken and classified into 5 grades using the Pfirrmann classification. Movements of discs were detected in the loaded discs. Histology: Sagittal disc sections were stained with hematoxylin and eosin and safranin-O, and graded using the histological grades established by Masuda et al. mRNA quantification: High-throughput mRNA expressions of common five HKGs [ß-actin, ß-glucuronidase, ß-2 microglobulin, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and lactate dehydrogenase A (LDHA)] in C9-10 loaded and C12-13 unloaded disc nucleus pulposus (NP) tissues were calculated using real-time RT-PCR. These expression levels in C9-10 were relatively presented to C12-13 unloaded disc (each n=6). Immunofluorescence: Immunofluorescence analyses for ß-actin and GAPDH and the positive cell count analysis was performed to detect their protein-distributions (each n=6). Statistical analyses: Two-way ANOVA and the Turkey-Kramer post-hoc test were used to assess time and gene effects. P-values were set at 0.05.

DISCUSSION
Progressive disc height loss in radiography, lower NP intensities on T2-weighted MRI, and histological degeneration with ECM alterations firmly validate this model for disc degeneration research. High-throughput HKG data exhibited a drastic decrease of ß-actin from the early stages at the mRNA and later protein-distributional levels. Mechanical stress might cause cell deformation with the breakdown of cytoskeleton such as ß-actin. In this static compression loading research for intervertebral disc cells, ß-actin, compared to GAPDH, is potentially recognized to be unfitted as an endogenous control.

REFERENCES