INTRODUCTION:
Rotator cuff repair is known to have a high failure rate. Little is known about the natural healing process of the rotator cuff repair site. It has previously been shown that bone marrow cells contribute to tendon regeneration. To improve the healing of the repaired tendon, we hypothesized that drilling procedure can induce bone marrow cells to repaired tendon site and accelerate the healing process.

Green fluorescent protein (GFP) has widely been used in vivo as a cell marker. We have showed the contribution of circulation-derived cells in early phase of healing process of tendon and skeletal muscle with GFP bone marrow chimeric rat (BMC rat) (ORS 2006, 2007, 2008, 2010). In present study, to follow bone marrow-derived cells, we created GFP bone marrow chimeric rat (BMC rat) model. The objective of this study was to follow GFP signal-positive bone marrow-derived cells using BMC rat after rotator cuff repair with drilling on the insertion site, and to elucidate the role of the cells in rotator cuff regeneration.

MATERIALS AND METHODS:
Creation of BMC rats:
This experimental procedure was approved by the Experimental Animal Center Committee at the authors’ institution. Sprague-Dawley (SD) rats (n=12) received 100Gy radiation from γ-ray generator. Bone marrow cells from GFP rats (n=12) were transplanted intravenously into irradiated SD rats. The GFP chimeric rates of peripheral blood were measured with FACS calibur at 4 weeks after injection.

Rotator cuff repair model:
Bilateral supraspinatus tendons of BMC rats were detached and repaired using the Mason–Allen method. Multiple drilling by 0.5-mm drill hole was performed before the tendon repair, on the one side of the greater tuberosity (drilling group). The tendon on the other side was repaired without drilling procedure (control group). At 2, 4 and 8 weeks after repair, the tendon insertion site were evaluated histologically by hematoxylin and eosin, and propidium iodide with confocal laser scanning microscope after tendon was repaired. All nuclei are focused on the insertion area of supraspinatus tendon and scanned by confocal laser scanning microscope after tendon was repaired. All nuclei were stained with propidium iodide (red) and merged with GFP signal on the left (green). GFP-positive cells have gradually increased at the insertion area on the drilling group, but few cells were observed in control group.

RESULTS:
The GFP chimeric rates at 4 weeks after injection was 46.4%. At 2, 4, 8 weeks after the rotator cuff repair with drilling performed group, GFP-positive cells were detected in the insertion area and gradually increased (Fig.1 B,C,H,I,N,O). While at the insertion site of non drilling group, GFP-positive cells was few even at the 8 weeks after the repair. (Fig.1 E,F,K,L,Q,R). At 4 and 8 weeks, the ratio of GFP-positive cells at the repair site and the ultimate force to failure of specimens was significantly higher in drilling performed group than control group (P<0.05). Repaired tendons contained fibroblast-shaped, GFP-positive cells which also stained by HSP47 (Fig. 4)

DISCUSSION:
Mesenchymal stem cells, which have an ability to differentiate into tendon cells exists in bone marrow. In this study, the GFP-positive cells were found in the insertion site of the drilling group, and gradually increased. The GFP-positive cells were found in collagen producing cells. Further more, ultimate force to failure was higher in drilling group at 4, 8 weeks after surgery. This result indicates that bone marrow-derived cells migrate into the repaired rotator cuff and differentiated to fibroblast and contributed to the rotator cuff healing process. The animal model which we used in this study was useful to analyze the recruitment of bone marrow-derived cells after rotator cuff repair.

SIGNIFICANCE:
This is the first report to clarify that the bone marrow-derived cells from the humeral bone infiltrate into the repaired rotator cuff and contribute to the postsurgical rotator cuff healing process.