INTRODUCTION:
Development of long bones is initiated by chondrocyte differentiation and maturation, which is followed by degradation of hypertrophic chondrocytes, invasion of blood vessels, and differentiation of migrated mesenchymal progenitor cells toward osteoblasts, each of which processes are tightly coordinated. Recently, Schnurri(Shn)-2 and -3, members of large zinc-finger proteins, were shown to be necessary in this cooperation by chondrocytes and osteoblasts in vivo, that combined loss of Shn2 and Shn3 in mice resulted in dysregulated bone development characterized by delayed chondrocyte maturation and accelerated osteoblast differentiation[1]. Although Shn3 inhibits osteoblast differentiation through interaction with Runx2 protein to lead it to proteasomal degradation[2], the molecular mechanisms of which Shn2 and Shn3 affect chondrocyte differentiation are unclear. To address this question, we employed short-interfering RNA (siRNA) to knock-down Shn2 gene in chondrogenic progenitor cells, and evaluated its possible roles in chondrocyte differentiation.

METHODS:
Mouse chondrogenic progenitor cells ATDC5 cells were maintained in DMEM/F12(1:1) culture medium containing 5% fetal bovine serum(FBS). FBS was replaced (serum free) with ITS-plus supplement during the differentiation assays. Chondrocyte differentiation was induced by bone morphogenetic protein (BMP)-2 or -6 on the next day of siRNA transfection (day0). Differentiation of chondrocyte was assessed by quantification of mRNA for marker genes, such as Sox5, Sox6, Col2a1, Col11a2, Ppr, Ihh, Col10a1, Mmp13, by real-time reverse transcription polymerase chain reaction. The status of BMP signaling was indirectly monitored by the expression of Id1 gene. Osteoblast differentiation was studied using MC3T3-E1 cells.

RESULTS:
To confirm the functional validity of loss of Shn3 by siRNA for Shn3(siShn3), we transfected the siRNA into MC3T3-E1 osteoblasts, first. Giving the osteoblast differentiation was enhanced in Shn3 KO osteoblasts[3], and Shn3 heterozygous mice (worth 50% knock-down) showed mildly increased bone volume[4], knock-down of Shn3 in osteoblasts should result in enhanced differentiation. The efficiency of knock-down by siShn3 was confirmed to be 70% decrease in Shn3 mRNA expression in MC3T3-E1. Indeed, during the BMP-2-induced osteoblast differentiation, siShn3-transfected MC3T3-E1 cells showed mildly accelerated osteoblast differentiation. The efficiency of Shn3 knock-down in ATDC5 by siShn3 was 80% reduction. In BMP-2-induced ATDC5 cells, treatment of siShn3 led the cells to show mildly decreased expression of both proliferating chondrocyte marker Col2a1 and maturation marker Col10a1, on day2. On day4, siShn3-transfected cells showed substantial suppression not only in expression of both Col2a1 and Col10a1, but also in mRNA level of Sox9, the master regulator of chondrogenesis.

DISCUSSION:
We showed knock-down of Shn3 in ATDC5 cells inhibited the chondrogenic differentiation in vitro. This result suggests that endogenous Shn3 promotes chondrocyte differentiation from the early stage of chondrogenesis. Because chondrogenic defect was not mentioned in Shn3 KO mice[3], and chondrogenesis was delayed in Shn2/3 double KO mice[3], basal expression level of Shn2 might be low in ATDC5 cells in vitro. Although Shn3 is not linked to BMP-Smad signaling so far, Shn3 may alter BMP signaling in chondrocytes because the chondrogenic marker genes were up-regulated upon BMP treatment, which were further down-regulated by siShn3. The possible relationship of Shn3 and BMP signaling in chondrocytes is under investigation.

REFERENCES: