Human chitinase 3-like protein 2 (YKL39) is a novel secreted protein which supports cell proliferation and chondrocytic differentiation of ATDC5.

INTRODUCTION:
Human chitinase 3-like protein 2 (YKL39) is a 39kDa protein produced by articular chondrocytes and synovioctyes [1]. Recent studies showed that YKL39 expression is increased in osteoarthritic articular chondrocytes [2], suggesting that this molecule is involved in the pathogenesis of osteoarthritis (OA). However, molecular functions of YKL39 in articular cartilage homeostasis and in the pathogenesis of OA have not yet been clarified. Molecular and biochemical analyses indicated that YKL39 is a secreted protein and has significant sequence similarity with human chitotriosidase and bacterial chitinase. However, it is considered that YKL39 has no chitinase activity due to the absence of glutamate residue which is essential for the activity of chitinase [1]. This study aimed at elucidating the role of YKL39 in the joint homeostasis and in the pathogenesis of osteoarthritis (OA). For this purpose, we overexpressed YKL39 in ATDC5 cells and found that cell proliferation and chondrogenic differentiation was significantly accelerated in these cells.

METHODS:
Establishment of YKL39 overexpressing ATDC5 cells: Human YKL39 cDNA was obtained by RT-PCR from synovial tissue. Lentivirus carrying YKL39 cDNA was constructed using ViraPower Lentiviral Expression System (Invitrogen, CA). To establish overexpressing cells, YKL39-Lentivirus was infected into ATDC5 cells and cells were treated with Blasticidin (2μg/ml) for two weeks. We employed LacZ-Lentivirus as an experimental control. Q-PCR experiments indicated YKL39 gene expression in the overexpressing cells (data not shown).

Colonies formation assay (CFU): One hundred or one thousand of YKL39 overexpressing cells or LacZ overexpressing cells were seeded onto 60cm² dishes and maintained in the complete medium (alpha-MEM, 10%FBS, 1% Penicillin and Streptomycin) for 14 days. Colonies were fixed and stained with Crystal Violet for visualization [3].

Quantitative PCR: Total RNA was prepared using RNAeasy kit (Qiagen). To prepare cDNA, Transcriptor first strand cDNA synthesis kit (Roche) was employed. LightCycler® 480 Real-Time PCR System (Roche) were used for the analyses. All the Q-PCR experiments were performed more than three times. The results were presented as mean ± SD. Statistical analyses were performed using Mann-Whitney’s U test and p values <0.05 were considered significant.

Cell proliferation (MTT) assay and cell cycle analysis (Flow cytometry): One hundred cells were seeded onto 96-well plates and proliferation rate was measured using Cell Proliferation (MTT) Kit (Roche). For the cell cycle analysis, cells were pulse labeled with BrdU using APC BrdU flow kit (BD Pharmingen) and % populations in G0/G1, S, and G2/M phases were measured by Flow cytometry (FACS Calibur). Total RNA was also prepared and expressions of cyclin D1 and Junmonji were analyzed by Q-PCR.

In vitro differentiation assay: ATDC5 cells were seeded at a density of 6000 cells/cm². After 3 days, medium was replaced with 1:1 mixture of Dulbecco’s modified Eagle’s medium and Ham’s F-12 medium (Gibco, Taarstrup, Denmark) supplemented with 5% fetal bovine serum and 10 mg/ml of human insulin (Sigma-Aldrich) [4]. Cells were maintained for additional 14 days and mRNA expression levels of type II collagen were analyzed by Q-PCR.

RESULTS:
As shown in Fig1, we found that overexpression of YKL39 greatly enhanced colony forming capacity of ATDC5 cells. To elucidate if this was due to the acceleration of cell cycle progression, we performed MTT assay and cell cycle analysis. As shown in Fig1 (right panel), MTT assay revealed that overexpression of YKL39 in ATDC5 cells enhanced cell proliferation by 3-fold. Cell cycle analyses indicated that the % population of S-phase cells was greatly increased in YKL39 overexpressing cells (YKL39 cells 40.7 %, LacZ cells 24.6 %). Q-PCR experiments showed that expression of cyclin D1, which is shown to increase during G1 to S transition, was significantly increased in YKL39 overexpressing cells (Fig2 middle panel). In contrast, the expression of Jumonji, which is a negative transcriptional regulator for CDK4, was significantly decreased in the same cells (Fig2 left panel). These data suggest that YKL-39 functions as a novel growth factor for ATDC5 cells.

To examine if YKL-39 affects the chondrocytic differentiation of ATDC5 cells, we analyzed chondrocyte differentiation-related gene expression, such as type II collagen, in these cells. As shown in Fig2 (right panel), we found that type II collagen expression was greatly increased in the YKL-39 overexpressing cells. These data strongly suggest that YKL-39 also acts as a novel cytokine which supports chondrocytic differentiation of ATDC5 cells.

CONCLUSION:
An osteoarthritis-associated gene, YKL39, enhances cell proliferation and chondrocytic differentiation of ATDC5.

REFERENCES: