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# Human YKL39 (chitinase 3-like protein 2), an osteoarthritis-associated gene, enhances proliferation and type II collagen expression in ATDC5 cells

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ABSTRACT

Human YKL39 (chitinase 3-like protein 2/CHI3L2) is a secreted 39 kDa protein produced by articular chondrocytes and synoviocytes. Recent studies showed that hYKL-39 expression is increased in osteoarthritic articular chondrocytes suggesting the involvement of hYKL-39 in the progression of osteoarthritis (OA). However little is known regarding the molecular function of hYKL-39 in joint homeostasis. Sequence analyses indicated that hYKL-39 has significant identity with the human chitotorisidase family molecules, although it is considered that hYKL-39 has no enzymatic activity since it lacks putative chitinase catalytic motif. In this study, to examine the molecular function of hYKL-39 in chondrocytes, we overexpressed hYKL-39 in ATDC5 cells. Here we report that hYKL-39 enhances colony forming activity, cell proliferation, and type II collagen expression in these cells. These data suggest that hYKL-39 is a novel growth and differentiation factor involved in cartilage homeostasis.



## 1. Introduction

Osteoarthritis (OA) is a group of diseases and mechanical abnormalities involving degradation of articular cartilage and the subchondral bone. It is reported that OA affects 27 million people in the US (2005) and it is estimated that 80% of the US population will have radiographic evidence of OA by age 65 [1]. These statics indicate that the inhibition of cartilage loss and the promotion of cartilage repair are important issues to address, provided increases in life expectancy. Since OA is a multifactorial disease with factors such as aging, obesity, and joint instability, understanding the molecular pathophysiology of OA is important to develop more effective treatments. In this regard, many researchers have tried to identify the genes with altered expression patterns in OA cartilage [2].

hYKL-39 was discovered as a novel 39 kDa protein from the conditioned medium of human articular cartilage chondrocytes in primary culture [3]. It is currently recognized as a biochemical marker for the progression of osteoarthritis in humans [4]. However, the biological significance of hYKL-39 in cartilage homeostasis has not been appropriately defined.

hYKL-39 is closely related to other human proteins such as hYKL-40 (CHI3L1 [5]), chitotriosidase [6], AMCase (acid mammalian chitinase) [7], Oviductin [8], and SI-CLP (stabilin-1 interacting chitinase-like protein) [9]. All 6 proteins share significant sequence identity with bacterial chitinases and belong to the Glyco_18 domain-containing protein family [10]. Among these, only chitotriosidase and AMCase contain the putative chtinase catalytic motif, FDGxDxDxE, and are reported to have chitin hydrolytic activities. Since chitin is a major component of a diverse range of organisms such as insects, fungus, bacteria, nematodes, crustacean, and plants, it is considered that these two proteins are involved in the innate immune system [7,11]. In contrast, the other 4 proteins including hYKL-39 are reported to have no chitin hydrolytic activity and are classified as chitinase-like proteins (CLPs) or chi-lectins. Oviductin is exclusively expressed and secreted by oviductal epithelium and was reported to enhance in vitro fertilization rates in humans [12]. However, the specific function of Oviductin in this process remains to be established since no abnormality in the fertilization process was observed in mice null for Oviductin. SI-CLP was reported to be a binding protein for stabilin-1, a scavenger receptor expressed by macrophages and is considered to be involved in host defense and inflammatory reactions. Both hYKL-39 and hYKL-40 are reported to be expressed in joint tissues [4]. hYKL-40 is reported to be involved in the process of cell proliferation and extracellular matrix protein expression in articular chondrocytes [13]. Similar to hYKL-40, recent studies reported that

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hYKL-39 regulates cell proliferation in human embryonic kidney 293 cells and U373 human glioma cells [14]. However the molecular function of hYKL-39 in chondrocytes and synoviocytes has not been tested.

Since the expression of hYKL-39 but not that of hYKL-40 is greatly increased in osteoarthritic cartilage [4], we aimed to examine the role of hYKL-39 in the process of cell proliferation and differentiation of chondrocytes. Here we report that forced expression of hYKL-39 enhanced cell proliferation and type II collagen expression in mouse chondrogenic ATDC5 cells. These data suggest that hYKL-39 may be a novel growth and differentiation factor for articular cartilage chondrocytes and may regulate joint homeostasis in adults.

## 2. Materials and methods

### 2.1. Cell culture

ATDC5 cells were purchased from Riken Cell Bank (Tsukuba, Japan) and maintained in DMEM/F12 medium (1:1) under 5% $CO_2$ supplemented with 5% FBS and 100 units/ml penicillin, 100 μg/ml streptomycin, and 250 ng/ml amphotericin B (Invitrogen, CA). To induce chondrocytic differentiation, cells were seeded on 6-well plates and incubated for 2 weeks in the growth medium supplemented with 10 μg/ml human insulin (Sigma–Aldrich, MO).

### 2.2. Establishment of hYKL-39 overexpressing ATDC5 cells

Human total RNA was extracted from synovial membranes using TRIZOL reagent (Ambion, CA) according to the manufactures information. Isolated total RNA was further purified using RNeasy mini prep kit (QIAGEN, Germany). Complementary DNA was prepared using Transcriptor First Strand cDNA synthesis kit (Roche, Germany) and the coding sequence for human hYKL-39 (accession number U49835) was amplified by PCR using Tth DNA polymerase (TAKARA, Japan). Primers for hYKL-39 were 5′-atggaccagaagtctctctg-3′ (sense) and 5′-gtacttcagggcaacaagtc-3′ (antisense). A full-length cDNA fragment encoding hYKL-39 was subcloned into pENTER-D-TOPO (Invitrogen, CA) and sequenced for verification. Lentivirus carrying hYKL-39 cDNA was constructed using the ViraPower Lentiviral Expression System (Invitrogen, CA). To establish stably hYKL-39 overexpressing cells, hYKL-39-Lentivirus particles were infected into ATDC5 cells and maintained in the growth medium supplemented with Blasticidin (2 μg/ml) for two weeks. We employed a Lentivirus carrying beta-galactosidase (LacZ) cDNA as an experimental control. Q-PCR experiments indicated hYKL-39 gene expression only in the transformed cells (Fig. 1A).

### 2.3. Colony formation assay

One hundred or 1000 of stably transformed cells were seeded per 10 cm dish and maintained in the normal growth medium for 14 days. Cells were fixed with buffered 10% formalin solution and stained with crystal violet according to Sakaguchi et al. [15]. Colonies larger than 1 mm in diameter were counted.

### 2.4. Cell proliferation assay (MTT assay)

hYKL-39- or LacZ-overexpressing ATDC5 cells were seeded onto 96 well plates at the concentration of 100 cells/well. Cell proliferation rate was monitored at day 1, 3, 5, 7, and 10 using the Cell Proliferation Kit I (Roche, Germany) according to the manufacturer's protocol.

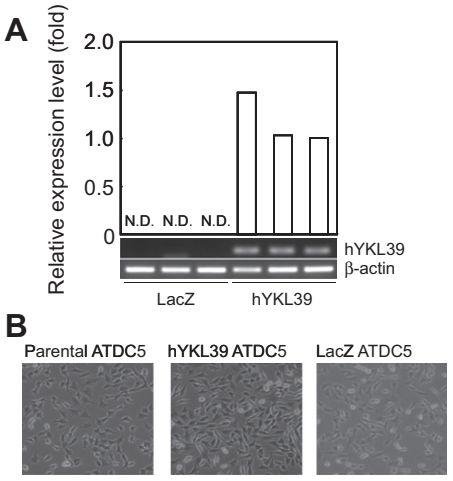


**Fig. 1.** Stable overexpression of hYKL-39 in ATDC5 cells Lentivirus carrying hYKL-39 or beta-galactosidase (LacZ) cDNA was infected into ATDC5 cells. After the infection, cells were maintained in the presence of Blastcidin for two weeks to establish stable transformants. (A) Q-PCR analyses confirmed the overexpression of hYKL-39 gene in the transformants. N.D.: not detected. (B) Morphology of the parental ATDC5 cells, YKL39-overexpressing cells, and LacZ-overexpressing cells. Overexpression of hYKL-39 did not significantly alter the appearance of ATDC5 cells.

### 2.5. Cell cycle analysis

hYKL-39- or LacZ-overexpressing ATDC5 cells were seeded onto 6 well plates at the concentration of $10^6$ cells/well. On the next day, medium was pulse labeled with BrdU (final concentration 10 μM) and stained using APC BrdU flow kit (BD Pharmingen, CA) according to the manufacturer's protocol. Populations in G0/G1, S, and G2/M phases were measured by flowcytometry (FACS Calibur, BD Pharmingen, CA).

### 2.6. In vitro chondrocytic differentiation of ATDC5 cells

hYKL-39- or LacZ-overexpressing ATDC5 cells were seeded at a density of 6000 cells/$cm^2$. At day 3, medium was replaced with fresh medium supplemented by 10 μg/ml of human insulin (Sigma–Aldrich, MO) [16]. Cells were maintained for an additional 14 days to allow for the chondrocytic differentiation.

### 2.7. Gene expression analysis

Total RNA was isolated using RNAeasy kit (Qiagen, Germany). Complementary DNA was synthesized using Transcriptor first strand cDNA synthesis kit (Roche, Germany) and relative mRNA amount was quantified by LightCycler® 480 Real-Time PCR System (Roche, Germany) using FastStart TaqMan®Probe Master kit (Roche, Germany). Combinations of PCR primers and taqmann probes were as follows. Relative amounts of mRNA were calculated and standardized as previously described [17,18].

YKL-39: 5′-ACAACAGCCCTCTGAGCAA-3′ (sense) and 5′-AGTACCCCACAGCATATTCCA-3′ (antisense), probe #87.

Actin-beta: 5′-GGATGCAGAAGGAGA-3′ (sense) and 5′-CCACCGATCCACACAGAGTA-3′ (antisense), probe #63.

Jumonji: 5′-GGCTGAAGCTGATGTACCG-3′ (sense) and 5′-CTTTGCCACATATCTGGTTGAC-3′ (antisense), probe #68.

Cyclin D1: 5′-GAGATTGTGCCATCCATGC-3′ (sense) and 5′-CTCCTCTTCGCACTTCTGCT-3′ (antisense), probe #67.

Type II collagen: 5′-CCCACTTACCAGTGTGTTTCG-3′ (sense) and 5′-GGGCTCCAATGATGTAGAGATG-3′ (antisense), probe #62.

Integrin alpha-1 (Itga1): 5′-GATGGGGACGTCAACATTCT-3′ (sense) and 5′-TGTGGTTAAGACGCTACCAAAG-3′ (antisense), probe #108.

Integrin alpha-v (itgav) 5′-GGTGTGGATCGAGCTGTCTT-3′ (sense) and 5′-CAAGGCCAGCATTTACAGTG-3′ (antisense), probe #21.

Integrin bata-1 (itgb1) 5′-CAACCACAACAGCTGCTTCTAA-3′ (sense) and 5′-TCAGCCCTCTTGAATTTTAATGT-3′ (antisense), probe #2.

Integrin bata-3 (itgb3) 5′-GTGGGAGGGCAGTCCTCTA-3′ (sense) and 5′-CAGGATATCAGGACCCTTGG-3′ (antisense), probe #31.

Discoidin domain receptor family, member 1 (DDR1) 5′-CTCCACCCCATTCTGCAC-3′ (sense) and 5′-CAGAAGGAGGCGGTA GGC-3′ (antisense), probe #108.

Discoidin domain receptor family, member 2 (DDR2) 5′-CGAAAGCTTCCAGAGTTTGC-3′ (sense) and 5′-GCTTCACAACACC-ACTGCAC-3′ (antisense), probe #55.

### 2.8. Statistical analysis

Statistical analyses were performed using Mann-Whitney's *U* test and *p* values less than 0.05 were considered as significant.

## 3. Results

### 3.1. Overexpression of hYKL-39 did not alter cell morphology, but increased colony-forming ability and proliferation of ATDC5 cells

To analyze the molecular functions of hYKL-39 in the proliferation and differentiation of chondrocytes, we established hYKL-39-overexpressing ATDC5 cell lines (Fig. 1A). We did not observe any morphological alteration in hYKL-39-overexpressing cells, when compared to that of parental ATDC5 cells or LacZ-overexpressing cells (Fig. 1B).

MTT assay revealed that overexpression of hYKL-39 enhanced cell proliferation by around three-fold (Fig. 2A). To further analyze the molecular function of hYKL-39 in the regulation of cell proliferation, we performed cell cycle analyses. As shown in Fig. 2B, we found the % population of S-phase cells was greatly increased in hYKL-39 overexpressing cells. Q-PCR analyses indicated that expression of Cyclin D1 [19,20], whose expression level is known to increase transiently in late G1 phase, was significantly increased in hYKL-39 overexpressing cells (Fig. 2 left panel). Furthermore, mRNA expression of Jumonji [21], which is a transcriptional repressor for Cyclin D1, was significantly decreased in these cells (Fig. 2C right panel).

In addition, we found that the overexpression of hYKL-39 increased colony-forming ability (Fig. 3). As shown in Fig. 3A and B, around 10% of the cells seeded could attach and form colonies in LacZ-ATDC5 cells (9.0 ± 3.9 colonies/100 cells seeded and 95.5 ± 57.3 colonies/1000 cells seeded). However, overexpression of hYKL-39 increased colony formation by three-fold (31.4 ± 7.8 colonies/100 cells, Fig. 3A upper panels and Fig. 3B left panel). These results were reproducible since we observed 321.3 ± 25.1 colonies from dishes plated with 1000 hYKL-39-ATDC5 cells (Fig. 3A lower panels and Fig. 3B right panel). Since interactions between extra-cellular matrix proteins and cell adhesion molecules such as DDR (Discoidin Domain Receptor) and integrins regulate cell adhesion and colony formation [22,23], we analyzed mRNA expression of these molecules in hYKL-39-overexpressing ATDC5 cells (Fig. 3C). Q-PCR analyses indicated that DDR1 expression is significantly increased in the hYKL-39 overexpressing cells while integrin beta-1 and beta-3 expression was significantly decreased. In contrast, we did not observe any alteration in the expression of DDR2, alpha-1, and alpha-v integrins between LacZ cells and hYKL-39 overexpressing cells (Fig. 3C).

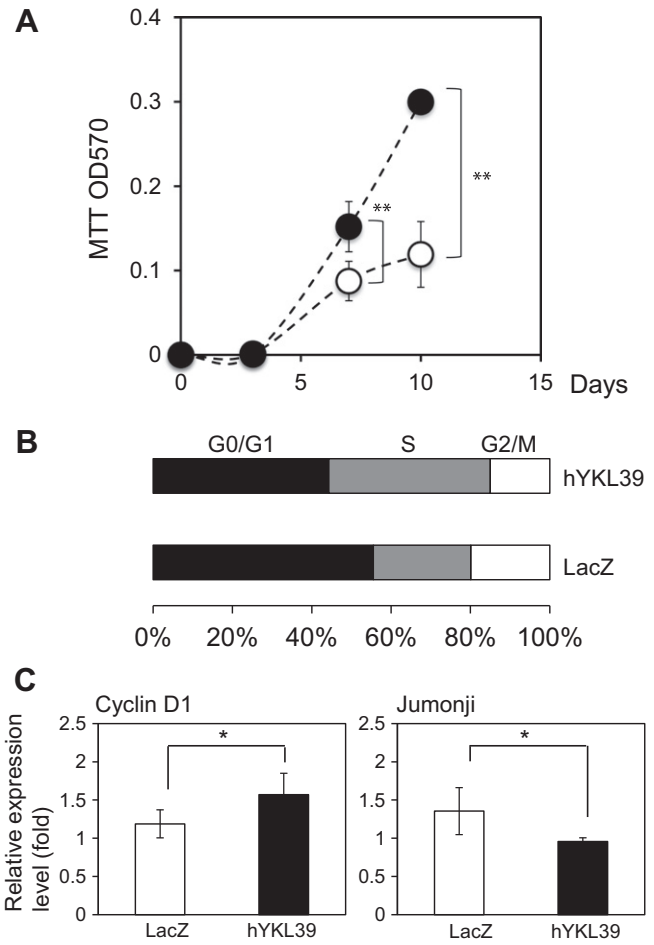


**Fig. 2.** YKL39 enhances cellular proliferation of ATDC5 cells by promoting G1/S transition (A) Overexpression of hYKL-39 increased cell proliferation of ATDC5 cells. 100 cells were seeded on 96 well-dishes and maintained in the normal growth medium. Cell proliferation rate was monitored by MTT assay. Closed circle: hYKL-39-overexpressing cells, open circle: LacZ-overexpressing cells. Difference is statistically significant, $^{**}p < 0.01$ (B) Cell cycle analyses revealed that the population of S-phase cells is increased in the hYKL-39 overexpressing cells. Ten thousand cells were analyzed by flowcytometry and % population of each cell cycle was indicated. (C) Relative expression of Cyclin D1 and Jumonji in hYKL-39-overexpressing cells. Overexpression of hYKL-39 enhances Cyclin D1 and suppresses Jumonji in ATDC5 cells. Difference is statistically significant, $^{*}p < 0.05$.

### 3.2. Overexpression of hYKL-39 increases type II collagen expression in ATDC5 cells

To examine if the overexpression of hYKL–39 enhances chondrocyte differentiation-related genes in ATDC5 cells, we treated control and hYKL-39 overexpressed ATDC5 cells with chondrocytic differentiation medium for 2 weeks and analyzed type II collagen gene expression in these cells by Q-PCR. As shown in Fig. 4, we found that overexpression of hYKL-39 significantly enhanced type II collagen gene expression in ATDC5 cells.

## 4. Discussion

Chitin is a long-chain biopolymer of *N*-acetylglucosamine (GlcNAc) similar to the structure of cellulose and a component of a diverse range of organisms such as insects, fungus, bacteria, nematodes, crustacean, and plants. Since chitin is a component of

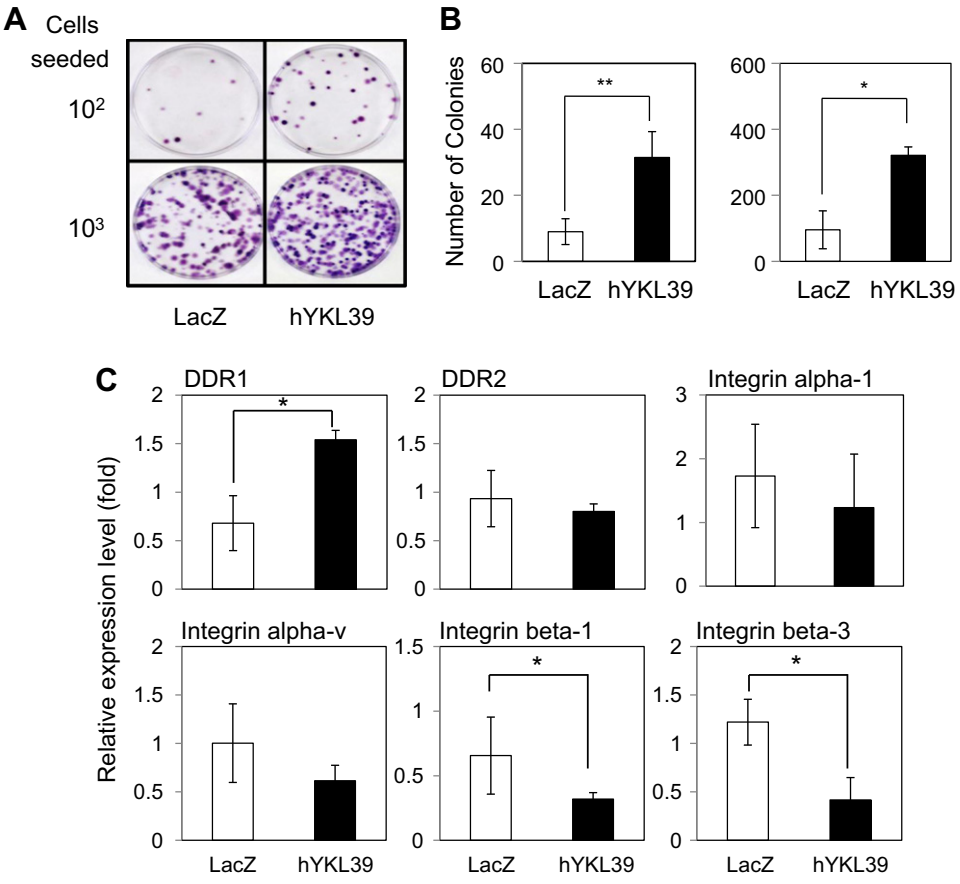


**Fig. 3.** Overexpression of hYKL-39 enhances colony-forming activity of ATDC5 cells (A and B) Overexpression of hYKL-39 increased colony-forming activity by three-fold in ATDC5 cells. One hundred (upper panels) or 1000 cells (lower panels) were seeded on 10 cm dishes and maintained in the normal growth medium for 14 days. Dishes were stained with crystal violet and colonies with more than 1 mm in diameter were counted. Nine independent experiments were performed and results were shown in mean +/- SD. $^{*}p < 0.05$, $^{**}p < 0.01$. (C) Q-PCR analyses of cell surface receptor molecules for extracellular matrices. Closed column: hYKL-39-overexpressing cells, open column: LacZ-overexpressing cells. $^{*}p < 0.05$.

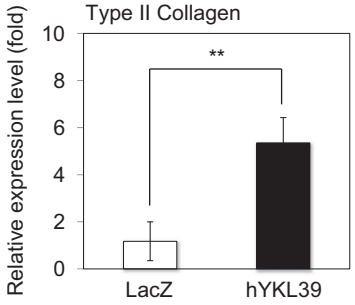


**Fig. 4.** Overexpression of hYKL-39 enhances type II collagen expression in ATDC5 cells Six thousand cells/cm$^2$ of hYKL-39- and LacZ-overexpressing ATDC5 cells were maintained in the growth medium supplemented with 10 μg/ml of insulin for 14 days. Type II collagen mRNA expression was measured by Q-PCR. $^{**}p < 0.01$.

exoskeletons and cell walls in these organisms, chitinases are considered to have important roles in the growth and the process of tissue regeneration of these organisms [24].

In humans, six chitinase family molecules have been identified, however, due to the lack of chitin and chitin biosynthesis these molecules are considered to have altered physiological functions. Furthermore, four family members including hYKL-39 are reported to lack chitinase activities and are classified as chi-lectins. Molecular functions of these four molecules are considered to be totally independent from those of chitinases in non-primates [6].

hYKL-40, which has the highest sequence identity with hYKL-39 [6], is the most analyzed molecule among the human chi-lectins [25–27]. hYKL-40 was originally discovered as a heparin-binding protein secreted from bovine breast tissue during the massive tissue involution that follows the cessation of lactation [5]. YKL-40 was subsequently discovered in the conditioned medium of human synoviocytes [28], chondrocytes [3,29], and MG-63 osteosarcoma cells [30]. Up-regulation of hYKL-40 expression was reported in porcine vascular smooth muscle cells undergoing a differentiation transition [31], in human airway epithelial cell line BEAS-2B under hypoxic condition [32], and in murine mammary tumors initiated by *neu*/*ras* oncogenes [33]. Areshkov et al. reported that hYKL-40 enhanced ERK phosphorylation and $^{3}H$-thymidine incorporation in human embryonic kidney 293 cells and U373 human glioma cells [14]. These data suggest that hYKL-40 modulate cellular function by controlling the MAPK/ERK signaling pathway. However the molecular mechanisms behind how hYKL-40 promotes ERK phosphorylation and subsequently increases cellular proliferation still need to be determined.

hYKL-39 is reported to be involved in articular cartilage homeostasis since its expression level is significantly increased in the osteoarthritic knee joint [4]. However, to date, molecular functions of hYKL-39 on chondrocytes and in the progression of knee osteoarthritis remain to be elucidated. One reason for that is because cellular receptor molecules as well as intracellular signaling pathways activated by hYKL-39 have not been identified. In this study, to elucidate the molecular functions of hYKL-39 on chondrocytes

we overexpressed hYKL-39 in ATDC5 cells and examined cellular responses in vitro. We showed that the forced expression of hYKL-39 increased cell proliferation, colony formation, and type II collagen gene expression in these cells. In addition, we showed that hYKL-39 regulated various cell cycle related genes and cell adhesion molecules in ATDC5 cells. These data suggest that hYKL-39 is a novel growth and differentiation factor for chondrocytic cells.

In parallel with hYKL-40, hYKL-39 is reported to induce ERK phosphorylation in both 293 and U373 cells [34]. However Areshkov et al. reported that hYKL-39 antagonized the effect of hYKL-40 and suppressed proliferation in these cells [14]. Since sustained phosphorylation of ERK was observed in the cells treated with recombinant hYKL-39 proteins, they discussed that distinguished regulation of ERK phosphorylation may alter the cellular responses between hYKL-39 and hYKL-40 [14]. In our study we found that cell proliferation was activated in hYKL-39 overexpressing cells. Our Q-PCR analyses revealed that this might be due to the up-regulation of a cell cycle regulator, Cyclin D1 and the down-regulation of Jumonji [20] in these cells. Since the expression of Cyclin D1 is also positively regulated by ERK signal pathway, we speculate that hYKL-39 enhanced cell proliferation at least partly via the ERK signal pathway in ATDC5 cells. Regarding the discrepancy between the results from 293 and U373 cells and ATDC5 cells, we speculate that the duration of ERK phosphorylation might be different in these cells. In the previous study, Areshkov et al. treated 293 and U373 cells with recombinant hYKL-39 proteins [14] while we created stable hYKL-39 overexpressing cell lines. One possible explanation for that discrepancy is that continuous activation of hYKL-39 signal pathways may alter the cellular responses which are observed in the transient activation. Further experiments are required to examine if the sustained of phosphorylation of ERK is observed in hYKL-39-overexpressing cells.

It is considered that both cell adhesion and proliferation play roles in the process of colony formation. Various extracellular matrix proteins such as collagens, fibronectin, and vitronectin and their receptors such as DDRs and integrins play important roles for cells to adhere [22,23,35]. In this study, we showed that overexpression of hYKL-39 enhanced colony formation in ATDC5 cells by around three-fold. Q-PCR experiments revealed that DDR1 [22], a cell surface receptor tyrosin kinase for collagens type I–VI and type VIII, was increased in the hYKL-39 overexpressing ATDC5 cells. Since the adhesion to type I collagen matrix of mesangial cells was significantly reduced in the absence of DDR1 [22], we speculate that increased colony forming ability in hYKL-39 overexpressing ATDC5 cells is due to the increased expression of DDR1. However, Curat et al. showed that cell proliferation was significantly increased in the mesangial cells from DDR1-null mice [22]. In addition, our Q-PCR analysis revealed that the expression of integrins beta-1 and beta-3, other receptors for collagens, were significantly suppressed in hYKL-39 overexpressing cells. Further analysis is needed to elucidate if the hYKL-39-DDR1 axis increased colony forming ability in ATDC5 cells. Kawai et al. showed that suppression of DDR2 expression or overexpression of kinase-deficient DDR2 protein inhibited proliferation of ATDC5 cells [23]. However we did not observe any alteration in DDR2 expression in hYKL-39 overexpressing cells by Q-PCR analysis.

In this study, we showed that overexpression of hYKL-39 significantly enhanced type II collagen expression in ATDC5 cells in the presence of insulin. Since Insulin/IGF signal is shown to induce PKB/Akt phosphorylation and promotes proteoglycans and type II collagen expression in ATDC5 cells [36], we speculate that hYKL-39 may also be involved in this signal pathway.

In summary, we showed that hYKL-39 enhanced Cyclin D1 and DDR1 expressions which are involved in cellular proliferation and colony formation in chondrocytic ATDC5 cells. We also showed that overexpression of hYKL-39 enhanced type II collagen expression. Although detailed molecular mechanisms still remain to be elucidated, these data strongly suggest that hYKL-39 may function as a novel growth and differentiation factor for the chondrocytes and chondrocyte progenitor cells. Since hYKL-39 is significantly increased in OA synovial cartilage, we expect that the identification of receptor molecules for hYKL-39 and analyses of intracellular signaling pathway may contribute to better understanding the pathogenesis of human OA.

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## References

[1] C.f.D.C.a.P. (CDC), Prevalence and most common causes of disability among adults–United States, 2005, MMWR Morb. Mortal. Wkly. Rep. 58 (2009) 421–426.
[2] T. Aigner, J. Dudhia, Genomics of osteoarthritis, Curr. Opin. Rheumatol. 15 (2003) 634–640.
[3] B.E. Hakala, C. White, A.D. Recklies, Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family, J. Biol. Chem. 268 (1993) 25803–25810.
[4] E. Steck, S. Breit, S.J. Breusch, M. Axt, W. Richter, Enhanced expression of the human chitinase 3-like 2 gene (YKL-39) but not chitinase 3-like 1 gene (YKL-40) in osteoarthritic cartilage, Biochem. Biophys. Res. Commun. 299 (2002) 109–115.
[5] J.J. Rejman, W.L. Hurley, Isolation and characterization of a novel 39 kilodalton whey protein from bovine mammary secretions collected during the nonlactating period, Biochem. Biophys. Res. Commun. 150 (1988) 329–334.
[6] B. Hu, K. Trinh, W.F. Figueira, P.A. Price, Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family, J. Biol. Chem. 271 (1996) 19415–19420.
[7] R.G. Boot, E.F. Blommaart, E. Swart, K. Ghauharali-van der Vlugt, N. Bijl, C. Moe, A. Place, J.M. Aerts, Identification of a novel acidic mammalian chitinase distinct from chitotriosidase, J. Biol. Chem. 276 (2001) 6770–6778.
[8] E.B. Arias, H.G. Verhage, R.C. Jaffe, Complementary deoxyribonucleic acid cloning and molecular characterization of an estrogen-dependent human oviductal glycoprotein, Biol. Reprod. 51 (1994) 685–694.
[9] J. Kzhyshkowska, S. Mamidi, A. Gratchev, E. Kremmer, C. Schmuttermaier, L. Krusell, G. Haus, J. Utikal, K. Schledzewski, J. Scholtze, S. Goerdt, Novel stabilin-1 interacting chitinase-like protein (SI-CLP) is up-regulated in alternatively activated macrophages and secreted via lysosomal pathway, Blood 107 (2006) 3221–3228.
[10] J. Kzhyshkowska, A. Gratchev, S. Goerdt, Human chitinases and chitinase-like proteins as indicators for inflammation and cancer, Biomark Insights 2 (2007) 128–146.
[11] G.H. Renkema, R.G. Boot, A.O. Muijsers, W.E. Donker-Koopman, J.M. Aerts, Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins, J. Biol. Chem. 270 (1995) 2198–2202.
[12] S. Mugnier, M. Kervella, C. Douet, S. Canepa, G. Pascal, S. Deleuze, G. Duchamp, P. Monget, G. Goudet, The secretions of oviduct epithelial cells increase the equine in vitro fertilization rate: are osteopontin, atrial natriuretic peptide A and oviductin involved?, Reprod Biol. Endocrinol. 7 (2009) 129.
[13] B. Dozin, M. Malpeli, L. Camardella, R. Cancedda, A. Pietrangelo, Response of young, aged and osteoarthritic human articular chondrocytes to inflammatory cytokines: molecular and cellular aspects, Matrix Biol. 21 (2002) 449–459.
[14] P.O. Areshkov, S.S. Avdieiev, O.V. Balynska, D. Leroith, V.M. Kavsan, Two closely related human members of chitinase-like family, CHI3L1 and CHI3L2, activate ERK1/2 in 293 and U373 cells but have the different influence on cell proliferation, Int. J. Biol. Sci. 8 (2012) 39–48.
[15] Y. Sakaguchi, I. Sekiya, K. Yagishita, T. Muneta, Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source, Arthritis Rheum. 52 (2005) 2521–2529.
[16] L. Chen, T. Fink, X.Y. Zhang, P. Ebbesen, V. Zachar, Quantitative transcriptional profiling of ATDC5 mouse progenitor cells during chondrogenesis, Differentiation 73 (2005) 350–363.
[17] T. Niikura, D.J. Hak, A.H. Reddi, Global gene profiling reveals a downregulation of BMP gene expression in experimental atrophic nonunions compared to standard healing fractures, J. Orthop. Res. 24 (2006) 1463–1471.

[18] E. Willems, L. Leyns, J. Vandesompele, Standardization of real-time PCR gene expression data from independent biological replicates, Anal. Biochem. 379 (2008) 127–129.
[19] M. Kimata, T. Michigami, K. Tachikawa, T. Okada, T. Koshimizu, M. Yamazaki, M. Kogo, K. Ozono, Signaling of extracellular inorganic phosphate up-regulates Cyclin D1 expression in proliferating chondrocytes via the Na+/Pi cotransporter Pit-1 and Raf/MEK/ERK pathway, Bone 47 (2010) 938–947.
[20] M. Toyoda, H. Shirato, K. Nakajima, M. Kojima, M. Takahashi, M. Kubota, R. Suzuki-Migishima, Y. Motegi, M. Yokoyama, T. Takeuchi, Jumonji downregulates cardiac cell proliferation by repressing Cyclin D1 expression, Dev. Cell 5 (2003) 85–97.
[21] J. Jung, T.G. Kim, G.E. Lyons, H.R. Kim, Y. Lee, Jumonji regulates cardiomyocyte proliferation via interaction with retinoblastoma protein, J. Biol. Chem. 280 (2005) 30916–30923.
[22] C.A. Curat, W.F. Vogel, Discoidin domain receptor 1 controls growth and adhesion of mesangial cells, J. Am. Soc. Nephrol. 13 (2002) 2648–2656.
[23] I. Kawai, T. Hisaki, K. Sugiura, K. Naito, K. Kano, Discoidin domain receptor 2 (DDR2) regulates proliferation of endochondral cells in mice, Biochem. Biophys. Res. Commun. 427 (2012) 611–617.
[24] H.T. Tran, N. Barnich, E. Mizoguchi, Potential role of chitinases and chitin-binding proteins in host-microbial interactions during the development of intestinal inflammation, Histol. Histopathol. 26 (2011) 1453–1464.
[25] J.S. Johansen, B.V. Jensen, A. Roslind, D. Nielsen, P.A. Price, Serum YKL-40, a new prognostic biomarker in cancer patients?, Cancer Epidemiol Biomarkers Prev. 15 (2006) 194–202.
[26] C. Ober, Z. Tan, Y. Sun, J.D. Possick, L. Pan, R. Nicolae, S. Radford, R.R. Parry, A. Heinzmann, K.A. Deichmann, L.A. Lester, J.E. Gern, R.F. Lemanske, D.L. Nicolae, J.A. Elias, G.L. Chupp, Effect of variation in CHI3L1 on serum YKL-40 level, risk of asthma, and lung function, N. Engl. J. Med. 358 (2008) 1682–1691.
[27] J.S. Johansen, P. Christoffersen, S. MØller, P.A. Price, J.H. Henriksen, C. Garbarsch, F. Bendtsen, Serum YKL-40 is increased in patients with hepatic fibrosis, J. Hepatol. 32 (2000) 911–920.
[28] P. Nyirkos, E.E. Golds, Human synovial cells secrete a 39 kDa protein similar to a bovine mammary protein expressed during the non-lactating period, Biochem. J. 269 (1990) 265–268.
[29] J.S. Johansen, H.S. Jensen, P.A. Price, A new biochemical marker for joint injury. Analysis of YKL-40 in serum and synovial fluid, Br. J. Rheumatol. 32 (1993) 949–955.
[30] J.S. Johansen, M.K. Williamson, J.S. Rice, P.A. Price, Identification of proteins secreted by human osteoblastic cells in culture, J. Bone Miner. Res. 7 (1992) 501–512.
[31] L.M. Shackelton, D.M. Mann, A.J. Millis, Identification of a 38-kDa heparin-binding glycoprotein (gp38k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodeling, J. Biol. Chem. 270 (1995) 13076–13083.
[32] J.A. Park, J.M. Drazen, D.J. Tschumperlin, The chitinase-like protein YKL-40 is secreted by airway epithelial cells at base line and in response to compressive mechanical stress, J. Biol. Chem. 285 (2010) 29817–29825.
[33] B.W. Morrison, P. Leder, Neu and ras initiate murine mammary tumors that share genetic markers generally absent in c-myc and int-2-initiated tumors, Oncogene 9 (1994) 3417–3426.
[34] P.A. Areshkov, V.M. Kavsan, Chitinase 3-like protein 2 (CHI3L2, YKL-39) activates phosphorylation of extracellular signal-regulated kinases ERK1/ERK2 in human embryonic kidney (HEK293) and human glioblastoma (U87 MG) cells, Tsitol. Genet. 44 (2010) 3–9.
[35] M. Shimaya, T. Muneta, S. Ichinose, K. Tsuji, I. Sekiya, Magnesium enhances adherence and cartilage formation of synovial mesenchymal stem cells through integrins, Osteoarth. Cartil. 18 (2010) 1300–1309.
[36] W. Yin, J.I. Park, R.F. Loeser, Oxidative stress inhibits insulin-like growth factor-I induction of chondrocyte proteoglycan synthesis through differential regulation of phosphatidylinositol 3-Kinase-Akt and MEK-ERK MAPK signaling pathways, J. Biol. Chem. 284 (2009) 31972–31981.