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The lncRNA, Nespas, Is Associated with Osteoarthritis Progression and Serves as a Potential New Prognostic Biomarker

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Abstract

Introduction. In this article, we explored the hypothesis that the long noncoding RNA, Nespas, promotes osteoarthritis (OA) by supporting abnormal lipid metabolism in human chondrocytes. *Materials and Methods*. Human articular chondrocytes from osteoarthritis patients were used and expression level of Nespas were determined by real-time polymerase chain reaction. Introduction of Nespas and Nespas-associated genes/miRNAs were performed by using a lentiviral system. The effect of Nespas on mitochondrial function was determined by staining mitochondria and analyzing mitopotential and mitochondrial genes. Moreover, to identify the responsible molecules in Nespas-induced pathogenesis, profiling of peroxisomal genes and miRNAs were applied and interactome analysis was performed. *Results*. Highly elevated levels of Nespas and Acyl-CoA synthetase 6 (ACSL6) were observed in OA patients. Both Nespas overexpression and ACSL6 upregulation into human chondrocytes induced typical OA characteristics, such as downregulation of type II collagen; upregulation of type I collagen, metalloproteinase 13, and caspase-1 and -3; and dysfunction of mitochondria and peroxisome. Co-expression of Nespas and ACSL6 siRNA reduced caspase-1 and -3 levels. Moreover, Nespas overexpression significantly suppressed levels of miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p, and these miRs are known to potentially target ACSL6 according to ingenuity pathway analysis. We also confirmed that these miRs were significantly suppressed in human OA chondrocytes. Overexpression of miR-291a-3p, -196a-5p, -23a-3p, -24-3p, or let-7a-5p in the presence of Nespas suppressed levels of ACSL6, caspase-1 and -3. *Discussion*. Overall, we suggest that elevated Nespas level in OA are associated with OA pathogenesis by suppressing miRs targeting ACSL6 and subsequent ACSL6 upregulation.

Keywords

Nespas, ACSL6, osteoarthritis, human articular chondrocytes

Introduction

Osteoarthritis (OA) is a multifactorial disease characterized by the destruction of articular cartilage due to genetic, developmental, biochemical, and biomechanical factors.[1-3] OA is characterized by articular cartilage destruction due to an imbalance in homeostasis of extracellular matrix components, mainly type II collagen and the proteoglycan aggrecan, due to the action of matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase domain with thrombospondin motifs family (ADAMTSs) proteins. Despite its high prevalence, OA pathogenesis is poorly understood because of complex underlying networks. Lately, it was found that alterations in noncoding genes can contribute to OA pathogenesis.[4-6]

Noncoding RNAs (ncRNAs), a large portion of human transcriptome compared to protein-coding genes, are currently known to play crucial roles both in basic biology and in major pathologies such as cancer[7] by modulating structural scaffolds, modifying chromatin, and regulating gene expression at both the transcriptional and posttranscriptional levels. These ncRNAs can be broadly divided into short ncRNAs (typically up to 200 nucleotides in length) including tRNAs, snRNAs, snoRNAs, and miRNA and long ncRNAs (lncRNAs; from 200 nucleotides up to ~100 kb). Particularly, it is well known that microRNAs undertake

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their biological functions by suppressing their target genes.[8,9] Previous studies[10-12] identified several miRNAs involved in OA pathogenesis. It has been shown that miR-22 regulates PPARα and BMP-7 in OA chondrocytes, so inhibiting miR-22 stimulated BMP-7 and PPARα expression, whose expression is frequently downregulated in OA cartilage. MicroRNA-140 is one of the major miRNAs relevant for OA[2] and is highly suppressed in OA cartilage, and ADAMTS5 is one potential key target.[13] According to Jones *et al*.,[14] miR-9, miR-98, and miR-146 are involved in the IL-1β-induced dedifferentiation of human articular chondrocytes and modulation of miR-9 affects MMP-13 secretion.[14] However, the vast majority of ncRNAs are lncRNAs that are greater than 200 bases in size,[15] and investigating lncRNAs is a challenging complexity due to poor cross-species sequence conservation. LncRNAs are involved in several biological processes including cell proliferation,[16,17] differentiation,[18,19] and migration.[20,21] To date, the role of lncRNAs in tumor growth, carcinogenesis, and metastasis has been reported in different malignancies,[22,23] and lately, lincRNAs have gotten an increasing amount of attention in several different inflammatory diseases. For example, BACE1-AS plays a role in Alzheimer disease[24]; CDKN2B-AS1 in Coronary Atherosclerosis[25]; IGF2-AS, MEG3, and PVT1 in diabetes[26]; and ANCR in bone disease.[27] However, there have been no investigations regarding the involvement of lncRNAs in OA pathogenesis. Some miRs are processed from lncRNAs, indicating that lncRNAs are just as important as miRNAs. One well-known OA-related miRNA, microRNA-675, is also processed from the lncRNA H19[28] and is known to regulate Col2a1 and Sox9 expression and affect cartilage homeostasis.[29,30]

For the aforementioned reasons, we analyzed lncRNA profiles with human OA chondrocytes to identify new factors involved in OA pathogenesis. Here, we report Nespas as a novel factor involved in OA pathogenesis by dysregulation of peroxisomal integrity and function.

Methods

Cell Culture

The study was approved by the appropriate institutional review board (Wonkwang University Ethics Committees). Normal human chondrocytes were obtained from biopsies of normal patients (n = 20, age = 45.6) with no history of joint disease. Small slices of cartilage (n = 20, age = 72.5) isolated from a healthy area (non-OA) or a severely damaged area (OA) were sequentially digested with 0.06% collagenase (Sigma, St Louis, MO) and seeded at a density of 1.5×10^4 cells cm^{-2} in chondrocyte growth medium (Cell Application, San Diego, CA) with 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin (Gibco Invitrogen, Gaithersburg, MD).

Western Blotting

Cell lysate (30 μg) were electrophoresed and transferred to nitrocellulose membranes (Schleicher and Schuell, Keene, Germany). The membranes were individually probed with antibodies specific for MMP-2 and -13 (1:1000 dilution, Millipore, Darmstadt, Germany). The blots were developed using a peroxidase-conjugated secondary antibody (1:5000 dilution), and the immunoreactive proteins were visualized with an ECL system (Amersham, Buckinghamshire, UK).

Bead Culture of Human Articular Chondrocytes

Normal human chondrocytes, non-OA chondrocytes, and OA chondrocytes were encapsulated in alginate beads. Briefly, the chondrocytes were resuspended at a density of 4×10^6 cells mL^{-1} in sterile alginate solution (1.25% alginate in 150 mM NaCl). The cell suspension was carefully dropped into a 102 mM $CaCl_2$ solution. The beads were allowed to polymerize for 10 minutes, were washed twice with phosphate-buffered saline, and then transferred to Dulbecco's modified Eagle medium/F12 (1:1) medium supplemented with 10% fetal bovine serum, 25 μg mL^{-1} ascorbic acid, and 10 μg mL^{-1} gentamycin. The beads were cultured at 37°C in a humidified atmosphere of 5% CO_2 for up to 3 days.

Lentiviral Infection

Lentiviral production vectors for Nespas and a negative control lentivirus were transfected with Third Generation Packaging Mix from Applied Biological Materials Inc. (ABM; Richmond, Canada) into Lenti-X 293 cells (Clontech, Mountain View, CA) using Lentifectin (ABM) in Opti-MEM I medium (Invitrogen, Carlsbad, CA). The supernatant was collected and lentiviral particles were concentrated using Lenti-X Concentrator (Clontech) and stored at −80°C. Chondrocytes cultured in alginate bead or monolayer-cultured were infected with lentivirus supernatant for 3 days in a humidified incubator at 37°C with 5% CO_2 in the presence of 5 ng/mL polybrene.

Real-Time Polymerase Chain Reaction (RT-PCR)

The quantitative RT-PCR was carried out in triplicate for each individual gene of each sample using a SYBR Green detection system on an ABI StepOnePlus machine (Applied Biosystems, Foster City, CA). Forty cycles of the following PCR protocol were run: 95°C for 10 seconds for denaturation, 55°C for 1 minute for annealing, and 72°C for 1 minute for extension. Quantification of the PCR signals was achieved by comparing the cycle threshold value (Ct) of the gene of interest with the Ct value of the reference gene, GAPDH. The oligonucleotides used as primers are listed in Supplementary Table S1.

Peroxisomal Gene Profiling

RNAs isolated using Trizol (Invitrogen) were reverse-transcribed using a PrimeScript 1st strand cDNA Synthesis Kit (#2680A; TaKaRa, Otsu, Shiga, Japan) and oligo dT primers according to the manufacturer's recommendation. The relative abundance of each gene was determined byRT-PCR with specific primers for 92 peroxisomal genes. Expression level of *GAPDH* (5′-GATCATCAGCAATGCCTCCT-3′ forward, 5′-TGTGGTCATGAGTCC TTCCA-3′ reverse) was used as a reference gene. Gene expression data were analyzed and visualized using GenEx (Weihenstephan, Germany).

miRNA Profiling

MicroRNA expression profiling was performed using a miScript System (miRNA PCR Array miFinder, Qiagen, Hilden, Germany). The reverse transcription (RT) reaction was performed using 1 μL miScript Reverse Transcriptase Mix, 4 μL 5× miScript RT buffer, and 15 μL RNase-free water at 37°C for 60 minutes, and the resulting RT product was diluted to 100 μL. Quantitative RT-PCR was performed using 10 μL SYBR Green PCR Master Mix, 2 μL miScript universal primer, 2 μL specific primer, 1 μL cDNA, and 5 μL RNase-free water for 45 cycles at 94°C for 15 seconds, 55°C for 30 seconds, and 70°C for 30 seconds using an ABI StepOnePlus PCR system (Applied Biosystems) Each reaction was run in triplicate and performed at least twice. MiRNA expression data were normalized to the expression levels of RNU6. Gene expression data was analyzed and visualized using GenEx (Weihenstephan, Germany).

Staining of Mitochondria and Peroxisomes

To visualize mitochondria in living cells, 50 nM MitoTracker CMXRos (No. M-7512, Molecular Probes, Eugene, OR) was incubated with cells for 30 minutes before confocal microscopy. Images were captured with a FluoView FV1000 (Olympus, Tokyo, Japan) confocal microscope.

To visualize peroxisomes, cells grown on coverslips were washed, incubated with an antibody toward PMP70 (Thermo Scientific, Pittsburgh, PA) for 1 hour, and then incubated for 1 hour with rhodamine (TRITC)-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) at room temperature.

Annexin V and Mitopotential Assay

Cellular apoptosis was assessed using the Muse cell analyzer (Merck Millipore, Billerica, MA). Briefly, human osteoarthritic chondrocyte cells infected with control or siACSL4 lentivirus were collected, centrifuged at 300 × *g* for 5 minutes, and resuspended in 100 μL of Annexin V and dead cell detection reagent (#MCH100105; Merck Millipore, Billerica, MA) in phosphate-buffered saline containing 1% bovine serum albumin at room temperature for 20 minutes. The percentage of live, early apoptotic, late apoptotic, and dead cells were analyzed in accordance with the Millipore guidelines.

The mitochondrial depolarization state of treated cells was assessed using a Muse cell analyzer (Merck Millipore, Darmstadt, Germany) using a mitopotential assay kit (EMD Merk Millipore, Billerica, MA). Briefly, both floating and adherent treated cells were collected, centrifuged at 300 × *g* for 5 minutes, and then a 100 μL aliquot of cell suspension was first added to 95 μL of diluted Muse MitoPotential dye, and after 20 minutes at room temperature, 5 μL of 7-AAD reagent dye was added. After 5 minutes, the percentage of live, depolarized, and dead cells in the cell suspensions was measured immediately using a Muse cell analyzer (EMD Merk Millipore).

Statistwical Analysis

Statistical analysis was performed using the SPSS program for Windows, Standard Version (version 18.0, SPSS Inc., Chicago, IL; http://www.SPSS.com). Student's *t* test was used to assess the differences between means. *P* values <0.05 were considered to be statistically significant.

Results

Nespas and ACSL6 Were Upregulated in OA Chondrocytes

In this study, we examined the expression level of Nespas in osteoarthritic chondrocytes to evaluate its involvement of OA pathogenesis. Compared to normal chondrocytes, the expression level of Nespas was significantly increased in most chondrocytes isolated from OA patients (**Fig. 1**).

Overexpression of Nespas Induced Typical OA Characteristics

To investigate the role of Nespas in OA pathogenesis, lentivirus particles containing a GFP-tagged Nespas expression construct were introduced into normal chondrocytes (**Fig. 2A**). Chondrocytes were bead-cultured and Nespas introduction was confirmed by observing the existence of GFP-positive cells (**Fig. 2A**, upper panel), and determining Nespas expression by RT-PCR (**Fig. 2A**, lower panel). On Nespas introduction, Nespas transcription was increased up to 4-fold compared to that in the control. Moreover, type I collagen expression was significantly increased whereas type II collagen expression was reduced by Nespas overexpression in normal chondrocytes (**Fig. 2A**, lower panel). To determine whether Nespas modulation was involved in OA pathogenesis, 2 major characteristics of OA pathogenesis,

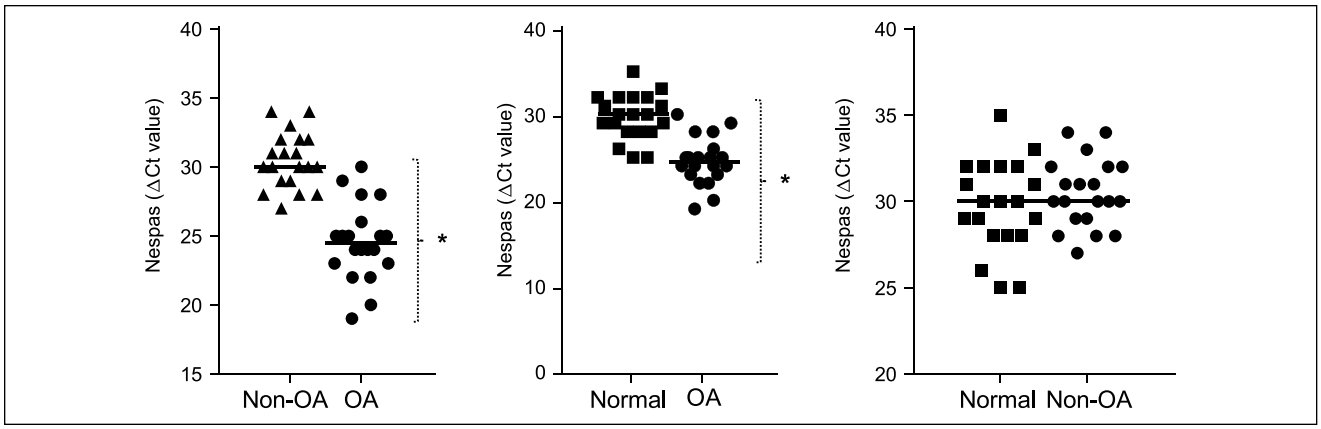


Figure 1. Upregulation of Nespas in human osteoarthritis (OA) chondrocytes. OA and non-OA chondrocytes were isolated from OA cartilage and normal chondrocytes were isolated from normal biopsy cartilage were cultured and the expression level of Nespas were analyzed by RT-PCR.

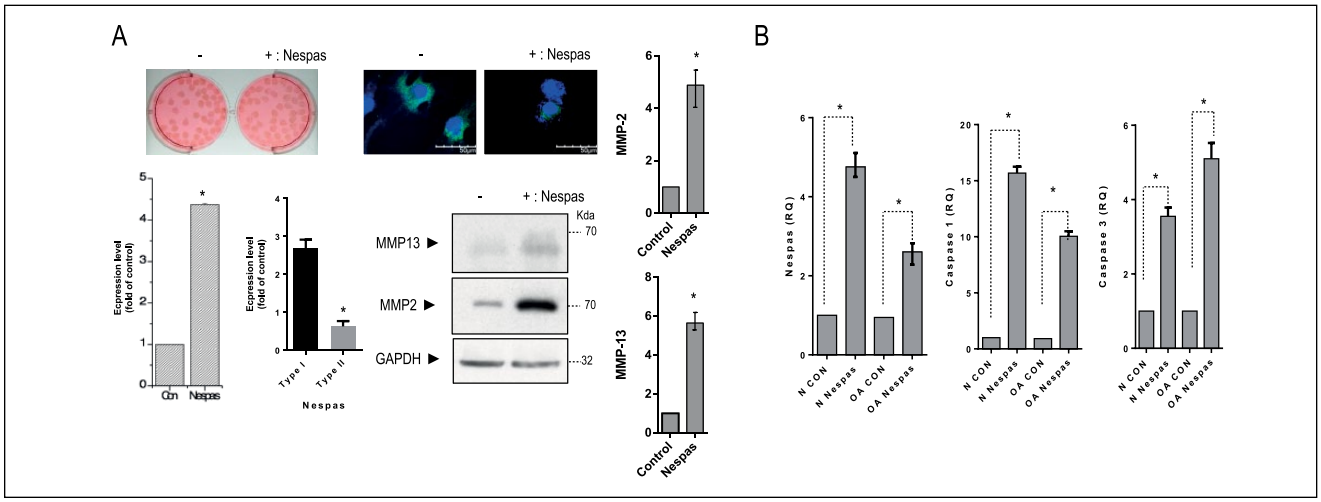


Figure 2. Nespas activates MMP-13 and stimulates apoptosis in human osteoarthritis (OA) chondrocytes. (**A**) Cells were cultured in alginate beads in the absence or presence of lentiviruses containing Nespas for 3 days and fluorescent images were captured (upper panel). The expression levels of Nespas and type I and type II collagen were measured by real-time polymerase chain reaction (RT-PCR), and protein levels of MMP-2 and -13 were analyzed by immunoblotting (lower panel). (**B**) The expression levels of Nespas, and caspase-1 and-3 were measured by RT-PCR using normal and OA chondrocytes in the absence or presence of Nespas. Results shown are representative of at least 3 independent experiments and shown as the mean, with error bars representing the 95% confidence interval (lower/upper limit); *, $P < 0.05$. Note: Color image is available on the online version of the article.

MMP upregulation and stimulation of chondrocyte apoptosis, were examined. Protein and RNA levels of MMP-2 and -13 of Nespas-expressing normal chondrocytes were examined (**Fig. 2A**, left panel). On Nespas introduction, the expression of MMP-2 and -13 was dramatically increased. In addition, the transcription levels of caspase-1 and -3 in Nespas-expressing normal and OA chondrocytes were significantly increased (**Fig. 2B**). We also observed that Nespas introduction disrupted mitochondrial dynamics and peroxisomal function as determined by staining with mitotracker and PMP70, respectively (**Fig. 3A**). In addition, mitochondrial membrane potential depolarization was increased on Nespas introduction (**Fig. 3B**).

Overexpression of Nespas Suppressed miRs Targeting ACSL6

To identify the signaling network modulated by Nespas during OA pathogenesis, we analyzed changes in mRNA and miRNA expression on Nespas introduction. Among genes tested, expression levels of genes involved in peroxisomal activities and genes in biochemical and enzymatic pathways involved in

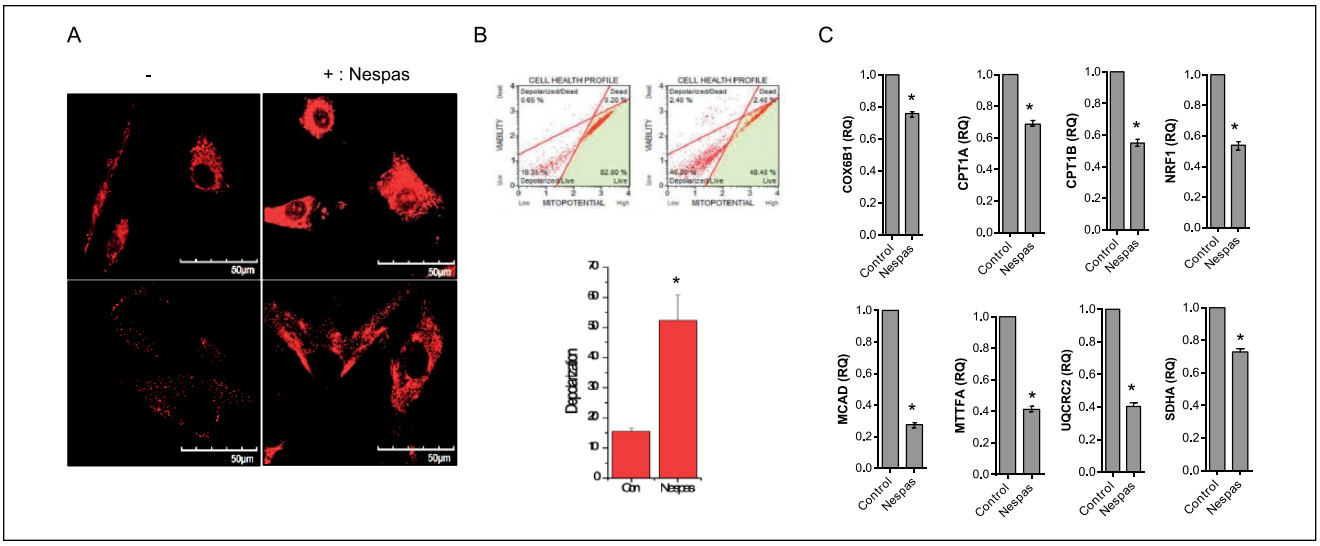


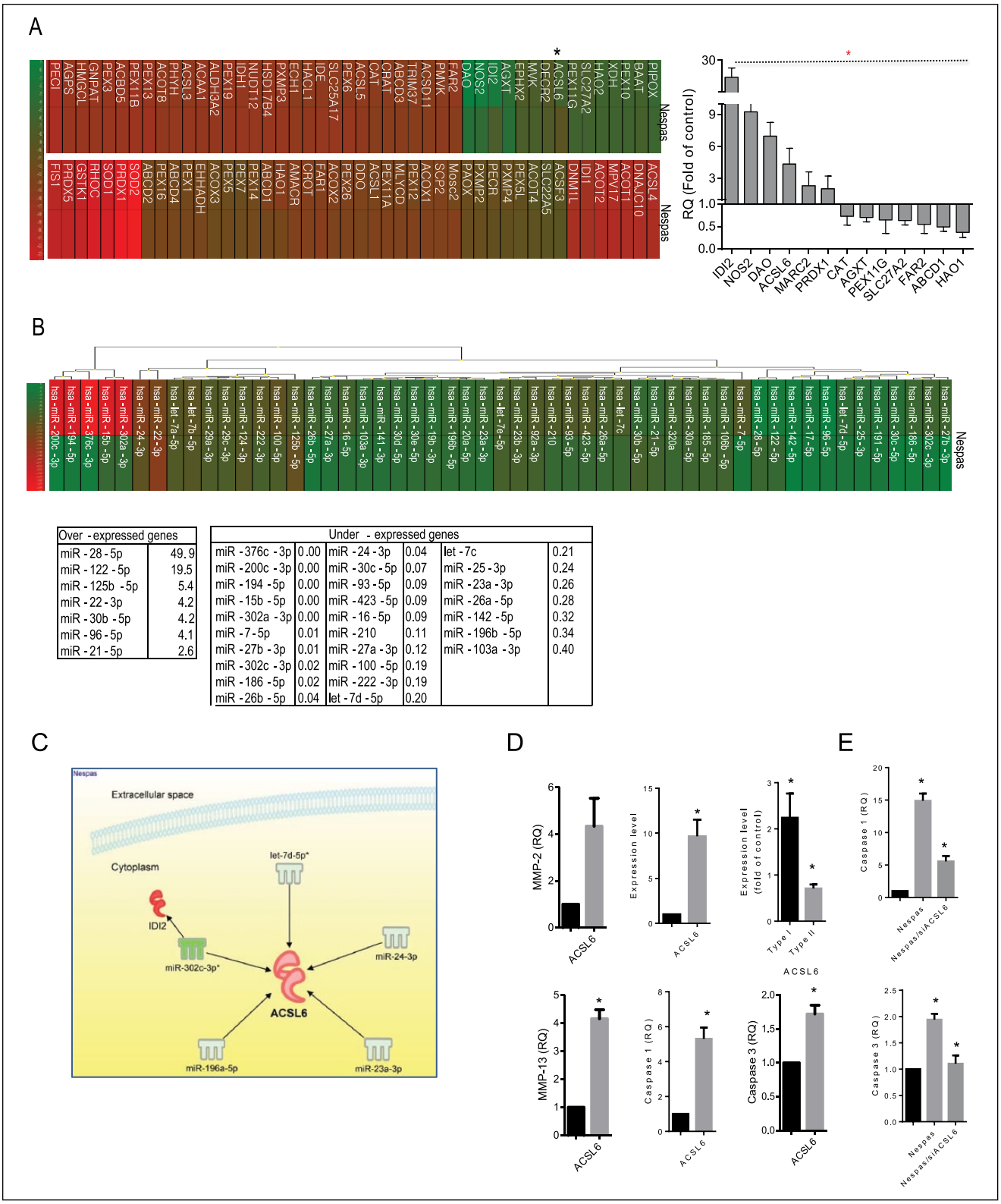
Figure 3. Nespas disrupts mitochondria and peroxisome function. (**A**) Normal chondrocytes were stained with Mitotracker and PMP70 in the absence or presence of lentiviruses containing Nespas. (**B**) Cell death and mitopotential were analyzed in the absence or presence of lentiviruses containing Nespas. (**C**) Expression levels of mitochondrial genes were analyzed by real-time polymerase chain reaction. Results shown are representative of at least 3 independent experiments and presented as the mean signal, with error bars representing the 95% confidence interval (lower/upper limit); *, $P < 0.05$.

peroxisomal function were altered (**Fig. 4A**). The mRNA transcripts of *ID2, NOS2, DAO, ACSL6, Mosc2*, and *PPDX1* were significantly upregulated whereas transcripts of *CAT, AGXT, PEX11G, SLC27A2, FAR2, ABCD1*, and *HAO1* were significantly downregulated. Among the miRNAs tested, significantly upregulated and downregulated miRs were shown in **Figure 4B**. Furthermore, interactome analysis using miRNAs and genes modulated by Nespas was analyzed by a web-based software application, ingenuity pathway analysis (IPA) (**Fig. 4B**). We analyzed which miRNAs whose expressions were altered by Nespas could target the gene whose expression was altered by Nespas using IPA. According to IPA, Acyl-CoA synthetase 6 (ACSL6), which was upregulated on Nespas introduction, could be an OA modulator because miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p were suppressed by Nespas introduction (**Fig. 4C**). To determine whether ACSL6 was involved in OA pathogenesis, we introduced ACSL6 into normal chondrocytes (**Fig. 4D**). Overexpression of ACSL6 in normal chondrocytes as confirmed by RT-PCR significantly upregulated transcription levels of type I collagen, caspase-1, and caspase-3 (**Fig. 4D**) and this caspase-1 and -3 upregulation by Nespas introduction was suppressed by ACSL6 knockdown (**Fig. 4E**). Moreover, these upregulations were suppressed by the co-introduction of miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p with Nespas (**Fig. 4F**). These data suggest an inter-network among Nespas:miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p:ACSL6 during the regulation of caspase-1 and -3 during OA pathogenesis. To confirm this, we evaluated the expression levels of ACSL6 and miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p in OA patients compared to normal patients. Transcription of ACSL6 was significantly increased in OA chondrocytes whereas miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p were significantly suppressed in OA chondrocytes compared to normal chondrocytes (**Fig. 4G**).

Discussion

Osteoarthritis is a degenerative joint disease characterized by cartilage degeneration, subchondral bone sclerosis, and osteophyte formation.[31] Recently, there has been increasing interest in lncRNAs because of their diverse biological functions.[32,33] Recently, several lncRNAs, such as H19,[28] MEG3,[34] PCGEM1,[35] and GAS5,[36] were found to function in cartilage degradation and are considered promising therapeutic targets for OA. However, the identification and regulatory mechanisms of lncRNAs as they relate to OA pathogenesis remains largely unexplored, and the applications of lncRNAs as therapeutic targets to prevent and/or treat cartilage degradation are very limited. In the current study, we suggested a new possible target and insight into the regulatory mechanism of cartilage homeostasis.

Nespas is known to be mainly express in the progress zone, mesenchyme and ectoderm of the limb, and the neural tube,[31] and Nespas imprinting was reported to be involved in Albright's hereditary osteodystrophy[37] and pseudohypoparathyroidism type 1a.[38] In this study, we found that Nespas was involved in OA pathogenesis. We compared Nespas expression in severely damaged regions of TKR cartilage compared to healthy intact regions of TKR cartilage. Since



Over - expressed genes	
miR - 28 - 5p	49.9
miR - 122 - 5p	19.5
miR - 125b - 5p	5.4
miR - 22 - 3p	4.2
miR - 30b - 5p	4.2
miR - 96 - 5p	4.1
miR - 21 - 5p	2.6

Under - expressed genes					
miR - 376c - 3p	0.00	miR - 24 - 3p	0.04	let - 7c	0.21
miR - 200c - 3p	0.00	miR - 30c - 5p	0.07	miR - 25 - 3p	0.24
miR - 194 - 5p	0.00	miR - 93 - 5p	0.09	miR - 23a - 3p	0.26
miR - 15b - 5p	0.00	miR - 423 - 5p	0.09	miR - 26a - 5p	0.28
miR - 302a - 3p	0.00	miR - 16 - 5p	0.09	miR - 142 - 5p	0.32
miR - 7 - 5p	0.01	miR - 210	0.11	miR - 196b - 5p	0.34
miR - 27b - 3p	0.01	miR - 27a - 3p	0.12	miR - 103a - 3p	0.40
miR - 302c - 3p	0.02	miR - 100 - 5p	0.19		
miR - 186 - 5p	0.02	miR - 222 - 3p	0.19		
miR - 26b - 5p	0.04	let - 7d - 5p	0.20		

(Figure 4. continued)

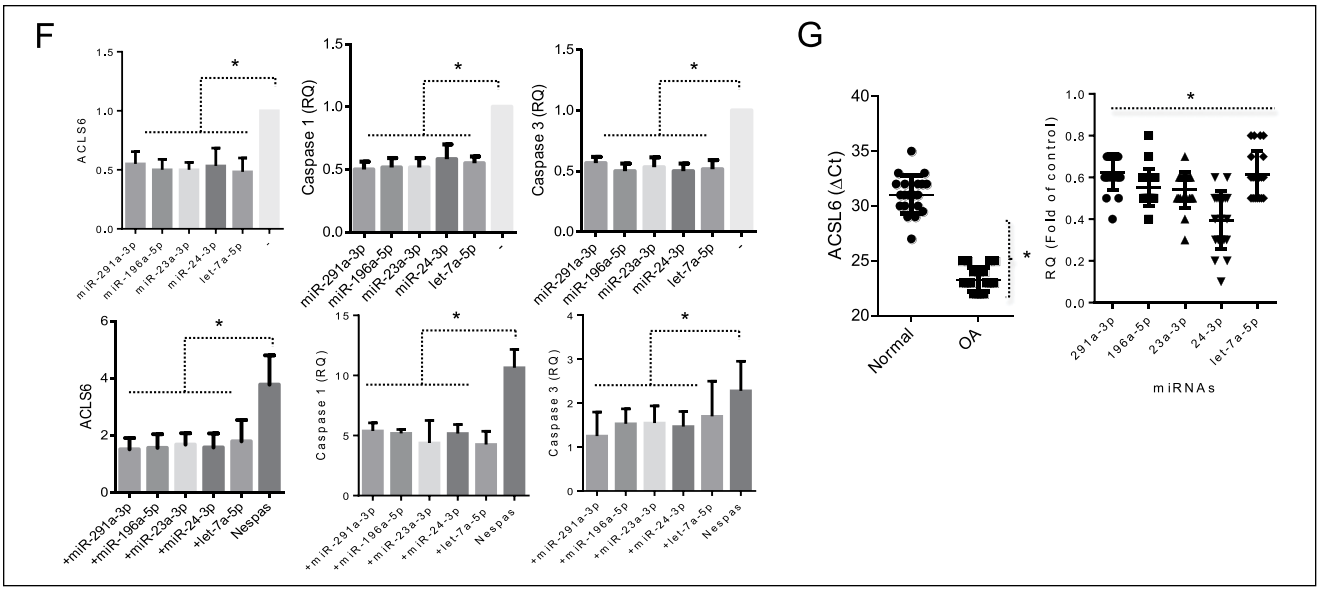


Figure 4. Nespas disrupts mitochondria and peroxisome function. (**A**) The expression levels of peroxisome-related genes were measured by real-time polymerase chain reaction (RT-PCR) in the absence or presence of lentiviruses expressing Nespas and gene expression is expressed as a heat-map and bar graph. "Red" color of heat-map represents significant increase in expression levels. (**B**) miRNA profiles were analyzed in the absence or presence of lentiviruses containing Nespas and are expressed as a heat-map and table. (**C**) The interactome between the profile of peroxisome-related genes and miRNAs in the absence or presence of lentiviruses containing Nespas as analyzed by IPA. (**D**) Normal chondrocytes were infected with lentiviruses containing ACSL6 for 3 days and expression levels of ACSL6, MMP-2 and -13, type-I and -II, and caspase-1 and -3 were analyzed by RT-PCR. (**E**) Normal chondrocytes were infected with lentiviruses containing ACSL6 and Nespas and caspase-1 and -3 mRNA levels were analyzed by RT-PCR. (**F**) Normal chondrocytes were treated with miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p in the absence (upper panel) or presence (lower panel) of lentiviruses containing Nespas for 3 days and expression levels of ACSL6, caspase-1 and -3 were analyzed by RT-PCR and represented as fold of control. (**G**) Expression levels of ACSL6, miR-291a-3p, -196a-5p, -23a-3p, -24-3p, and let-7a-5p were analyzed in human normal and OA chondrocytes. Results shown are representative of at least 3 independent experiments and shown as the mean, with error bars representing the 95% confidence interval (lower/upper limit); *, $P < 0.05$. Note: Color image is available on the online version of the article.

the age and sex of tissue donors may influence lincRNA expression levels, we used 20 cartilage samples from female OA patients in their 70s. We found that Nespas expression was increased in the OA chondrocytes isolated from damaged cartilage regions as compared to chondrocytes isolated from intact cartilage regions. Nespas expression in the healthy intact region of OA cartilage was similar to the expression in normal cartilage, indicating that age may not affect Nespas expression.

Further functional studies are critical to confirm the role of Nespas and to explore potential targets of Nespas in OA. To do so, we cultured established human chondrocytes isolated from cartilage of OA patients in alginate bead culture. Overexpression of Nespas in alginate bead chondrocyte cultures induced typical OA characteristics, that is, downregulation of type II collagen, upregulation of type I collagen and caspase, and activation of MMP-13.

Furthermore, Nespas induction into alginate bead chondrocyte cultures dysregulated cellular organelles such as mitochondria and peroxisomes. It is well accepted that peroxisomes and mitochondria have a strong interrelation as they show complementary activities, share proteins, and communicate with each other. Metabolically, these organelles cooperate to degrade fatty acids.[39] Very long- and branched-chain fatty acids are first shortened in peroxisomes, then are transferred to the mitochondria for further breakdown[40] through intracellular activation by long-chain acyl-CoA synthetases (ACSL).[41] Here, we found that the expression of several miRNAs such as miR-24-3p, -23a-3p, 196a-5p, and -302c-3p, and let-7d-5p were significantly reduced by Nespas overexpression. Additionally, these miRs potentially targeted Acyl-CoA synthetase 6 (ACSL6), suggesting that dysregulation of the mitochondria and peroxisomes on Nespas overexpression may be due to the upregulation of miRs targeting ACSL6.

Acyl-CoA synthetase (ACSL) catalyzes the formation of fatty acyl-CoAs from ATP, CoA, and long-chain fatty acids, and these acyl-CoAs then enter degradative pathways (i.e., β-oxidation or fatty acid retroconversion) or synthetic pathways (i.e., glycerolipid synthesis or

cholesterol ester formation).[42] Among ACS, ACSL6 encodes a key enzyme that activates polyunsaturated long chain fatty acids with arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid as preferred substrates.[42] Overexpression of ACSL6 impairs β-oxidation and increases phosphatidylcholine levels in human skeletal muscle.[43] Genetic inhibition of ACSL6 is associated with increased cellular respiration and mitochondria biogenesis in rat skeletal muscle through activation of the AMPK/PGC-1α pathway.[43] Here, we also found an increased level of ACSL6 in human OA chondrocytes and ACSL6 overexpression in human chondrocytes induced typical OA characteristics, such as downregulation of type II collagen and upregulation of type I collagen and caspase-3. Moreover, the co-introduction of Nespas and ACSL6 decreased the levels of caspase-1 and -3, and co-introduction with miR-24-3p, -23a-3p, 196a-5p, or -302c-3p, or let-7d-5p, which seem to potentially target ACSL6 as determined by IPA with Nespas, decreased the expression level of caspase-1 and -3.

Collectively, we showed here that the highly upregulated Nespas in OA chondrocytes was identified as a novel lncRNA and is associated with the molecular pathogenesis of OA. Elevated Nespas suppressed the level of ACSL6-targeting miRs and upregulation of ACSL6 stimulated OA pathological processes.

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Authors' Note

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. E-J Jin had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. SP, ML, CC, and E-JJ were involved in study conception and design. SP, ML, and CC were involved in data acquisition. E-JJ participated in data analysis and interpretation.

Ethical Approval

Ethical approval for this study was obtained from the Institutional Review Board of Wonkwang University (WKUH201605-HRBR-041).

Informed Consent

Written and verbal informed consent was obtained from all subjects before the study.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Supplemental Material

Supplementary material for this article are available online.

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