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Ceramide accumulation accelerates nucleus pulposus cells degradation by p38MAPK activation

W.-J. WU[1], J. WANG[2], J. LIANG[2], Q. ZHOU[2], Y. LIANG[1]

[1]Department of Orthopedics, Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.
[2]Shanghai Institute of Traumatology and Orthopedics, Shanghai, China

Abstract. – OBJECTIVE: Ceramide is a lipid molecule that regulates life activities such as cell differentiation, proliferation, apoptosis, and aging. However, whether ceramide plays a role in the intervertebral disc degeneration (IDD) is not clear. The aim of this study is to explore the effect of ceramide during the nucleus pulposus (NP) cells degeneration.

PATIENTS AND METHODS: We used human NP cells and passaged them until the fourth generation to analyze the content of ceramide. Cell-permeable C6-ceramide was used to upregulate ceramide expression, and myriocin was used to inhibit the accumulation of ceramide. To understand the relation between p38MAPK and ceramide, SB203580 was used to inhibit the activation of p38MAPK. We tested the viability of NP cells by the detection of collagen II and p16 expression, the proliferation, and the apoptosis of NP cells.

RESULTS: Ceramide content was increased in NP cells from passage 1 (P1) to P4. The upregulation of ceramide accelerated the P1 NP cell degeneration by the reduction of collagen II production and proliferative cells population, increased p16 expression, and apoptotic cells population. However, the suppression of ceramide delayed the degeneration of P4 NP cells in the previous aspects. The accumulation of ceramide activated the phosphorylation of p38MAPK, and the inhibition of p38MAPK activation also alleviated the C6-ceramide-induced NP cell degeneration.

CONCLUSIONS: Ceramide accumulates during NP cell degradation, and the upregulated ceramide contributes to the NP cells degeneration by p38MAPK activation.

Key Words:
Ceramide, Nucleus pulposus cells, Apoptosis, Cell proliferation, p38MAPK.

Introduction

Chronic low back pain is a high-prevalence, difficult-to-treat orthopedic disease, which is one of the most serious diseases associated with chronic aging[1]. There are about 600 million people suffer low back pain worldwide, and 70%-85% of adults will experience different degrees of low back pain throughout their lives, which will greatly affect the patient's own quality of life, national medical expenses, and social productivity[2]. Most low back pain is tolerable and self-limiting. The acute phase lasts about 4 weeks, and the subacute phase lasts 4 to 12 weeks. However, some patients will develop into the chronic phase lasting up to 12 weeks. Clinically, large data based on histopathology and imaging indicate that most of the occurrence of low back pain begins with degenerative changes in the nucleus pulposus (NP) of the intervertebral disc[3]. The structure and biochemical composition of the NP changes during the progress of intervertebral disc degeneration (IDD). The decrease in proteoglycan (PG) and water content affects the carrying capacity of the intervertebral disc. In addition, the change in the composition of collagens and the decrease in its content has lost its original flexibility and scalability[4]. Therefore, exploring the mechanism of NP degeneration is one of the important directions of medical research on IDD.

Ceramide is a class of lipid molecules that has important regulatory effects on life activities such as cell differentiation, proliferation, apoptosis, and aging. When cells are stimulated by various external conditions such as ultraviolet radiation, heat shock, and chemotherapy drugs, endogenous ceramides begin to synthesize and may induce a series of physiological responses such as cell aging, growth inhibition, and apoptosis. Dadsena et al[5] reported ceramide triggers mitochondrial apoptosis by binding VDAC2. Boon et al[6] found ceramide promotes inflammation in type 2 diabetes. Jadhav et al[7] elucidated ceramide induces C2C12 myoblasts senescence which can be inhibited by Metformin. Gulbins et al[8] stated ceramide regulates neuronal stem cell proliferation in the hippo-

Corresponding Author: Yu Liang, MM; e-mail: Ly10334@rjh.com.cn

campus. Ceramide is also proved to have an ability in tumor suppressive action[9]. However, the effect of ceramide in the IDD still remains unknown.

In our study, we used the NP cells spontaneous degeneration model by repeated passage to explore the effect of ceramide in the progression of NP cell degradation. The inhibitor of ceramide named myriocin was applied to see whether the suppression of ceramide made sense to prevent the NP cell degeneration. What we found indicated that ceramide accumulated during the repeated passage of NP cells and the intervention of ceramide may be an effective way to alleviate NP cell degeneration.

Patients and Methods

NP Tissue Collection and NP Cells Isolation

We collected degenerative disc samples from patients who underwent NP discectomy due to disc herniation in Ruijin Hospital, Shanghai Jiaotong University School of Medicine. All of the written informed consents from the patients were signed before the surgeries. We divided the NP from the endplates and conserved them in cold cell culture medium for the following NP cell isolation. Before isolation, we washed the samples with phosphate-buffered saline (PBS) and then cut them into pieces as smaller as possible. The fragments were incubated with 0.15% type II collagenase for digestion at 37°C overnight. The next day, the solution was filtrated using a 100 μm pore strainer and resuspended with Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA). The cells were seeded in 6-well plates at 1×10^5 cells/well and passaged till the fourth generation. C6-ceramide and the p38MAPK inhibitor SB203580 used for NP cell culture were purchased from Sigma-Aldrich (St. Louis, MO, USA). This investigation has been approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine.

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from NP cells with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's methods. RNA was reverse transcribed to complementary deoxyribose nucleic acid (cDNA) by Master Mix (Applied Biosystems, Foster City, CA, USA). RT-PCR was performed using SYBR Green Mix (Applied Biosystems, Foster City, CA, USA) to determine IL-1β, TNF-α, MMP-3, ADAMTS-5, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (as normalization) RNA levels. The primer sequences used for RT-PCR are listed in Table I based on the Primer Bank database. Relative mRNA expression levels were calculated by the $2^{-\Delta\Delta Ct}$ methods.

Western Blot (WB) Analysis

NP cells were lysed with radioimmunoprecipitation assay (RIPA) lysate (Beyotime, Shanghai, China), centrifuged at 4°C (12000 G for 5 min), the supernatant was removed, and the protein concentration was determined by bicinchoninic acid (BCA) method (Beyotime, Shanghai, China) to adjust samples with protein equally. The protein was subjected to vertical electrophoresis on a polyacrylamide gel, and then electrotransferred onto a nitrocellulose membrane. The membrane was blocked with 5% skimmed milk for 2 h and incubated with anti-p38 (ab170099, Abcam, Cambridge, MA, USA), anti-Phospho-p38 (p-p38, Cat#44-684G, Thermo Fisher Scientific, Waltham, MA, USA), anti-β-actin (ab179467, Abcam, Cambridge, MA, USA) antibodies at 4°C overnight. After washing, the membrane was incubated with the secondary antibody for 2 h at room temperature. The gray ratio of the bands of the protein was scanned by Scinn Corporation analysis software corresponding to the relative content of β-actin.

Immunofluorescence (IF) Staining

NP cells were fixed with 4% formaldehyde for 15 min at room temperature, washed with PBS, and

Table I. Primer sequences of the genes for RT-PCR.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
TNF-α	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
IL-1β	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
MMP-3	AGTCTTCCAATCCTACTGTTGCT	TCCCCGTCACCTCCAATCC
ADAMTS-5	GAACATCGACCAACTCTACTCCG	CAATGCCCACCGAACCATCT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

RT-PCR, quantitative reverse-transcription polymerase chain reaction.

then blocked with 5% bovine serum albumin (BSA) for 30 min. NP cells were following incubated with a primary antibody against p16 (ab51243, Abcam, Cambridge, MA, USA) or collagen II (ab34712, Abcam, Cambridge, MA, USA) overnight at 4°C and then incubated by Cy3-conjugated 488 antibody for 1 h in the dark. 4',6-diamidino-2-phenylindole (DAPI) was used to stain the cell nucleus. Finally, the fluorescence intensity of NP cells was visualized using an inverted microscope.

Flow Cytometry

The apoptotic ratio of NP cells was determined by flow cytometry using Annexin V-FITC/PI double-stained apoptosis detection kit (Keygen, Nanjing, China). NP cells were collected, washed with PBS, centrifuged, and labeled with Annexin V-FITC and propidium iodide (PI), and we used the FACS Calibur flow cytometer (BD Corporation, Franklin Lakes, NJ, USA) to detect the distribution of apoptotic rate. The proliferative ratio of NP cells was determined using the EdU assay kit (Keygen, Nanjing, China) according to the manufacturer's protocol.

Ceramide Quantitation

The ceramide content of NP cells was determined by liquid chromatography-mass spectrometry (LC-MS) method. Briefly, cells were harvested and then washed and diluted in 100 μL PBS. Sphingolipid extracts were prepared by Bligh-Dyer method and analyzed as previously described[10]. Quantification of ceramide was performed by the extracted ion chromatogram using a triple quadrupole mass spectrometer as reported[11].

Statistical Analysis

All data were displayed by the means values ± SD (standard deviation), and graphs were generated by GraphPad 5.0 (La Jolla, CA, USA) software. Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). A *p*-value below 0.05 was considered statistically significant.

Results

Ceramide Accumulated In Spontaneously Regressive NP Cells

To determine the content of ceramide in the NP cells of different degenerated degrees, we passaged NP cells from the first generation to the fourth to achieve the spontaneously regressive NP cells. As shown in Figure 1A and 1B, the expression of collagen II decreased significantly with the increased generation of NP cells, which meant NP cells in a higher generation were gradually weak in collagen II synthesis. Additionally, the p16 expression was raised as the NP cells going to a higher generation, indicating a senescent phenotype gradually appeared. Followingly, the result of ceramide content measurement suggested that the ceramide level was upregulated from the first generation to the fourth generation (Figure 1C). As with many other cellular aging processes[12-14], ceremide accumulated in aged NP cells. Apart from this, we also analyzed the proliferative and apoptotic ratio of NP cells by flow cytometry (Figure 1D, 1E). As expected, the proliferative NP cell population was decreased along with the cell continually passaged and the apoptotic cells increased obviously from passage 1 to passage 4, corresponding to a degenerated cell phenotype. This data indicated that the content of ceramide accumulated in the degenerated NP cells, which should be associated with the development of NP cell degeneration.

Ceramide Overexpression Accelerated NP Cells Degeneration

To determine whether the upregulation of ceramide affects the phenotype of NP cells, we treated passage 1 NP cells with a cell-permeable analog of naturally occurring ceramides named C6-ceramide (ceramide)[15]. Under the stimulation of ceramide, the expression of collagen II decreased compared to the control with a dose-dependent. Besides, the p16 level significantly increased due to the ceramide treatment compared to the control (Figure 2A, 2B). The result of the LC-MS showed that ceramide was upregulated resulting from the ceramide analog treatment, especially in the concentration of 20 μM (Figure 2C). Ceramide also affected the viability of NP cells by increasing the apoptosis (Figure 2D) but decreasing the proliferation (Figure 2F) compared to the control. The overexpression of ceramide was proved to be related to the inflammatory response in many cells[16]. Therefore, we also tested the mRNA levels of some inflammatory factors during the NP cell degeneration, such as IL-1β, TNF-α, MMP-3, and ADAMTS-5, which were all increased after ceramide treatment (Figure 2E). The results indicated that the ceramide overexpression by an analog stimulation accelerated the NP cell degradation by the reduction of collagen II synthesis and cell

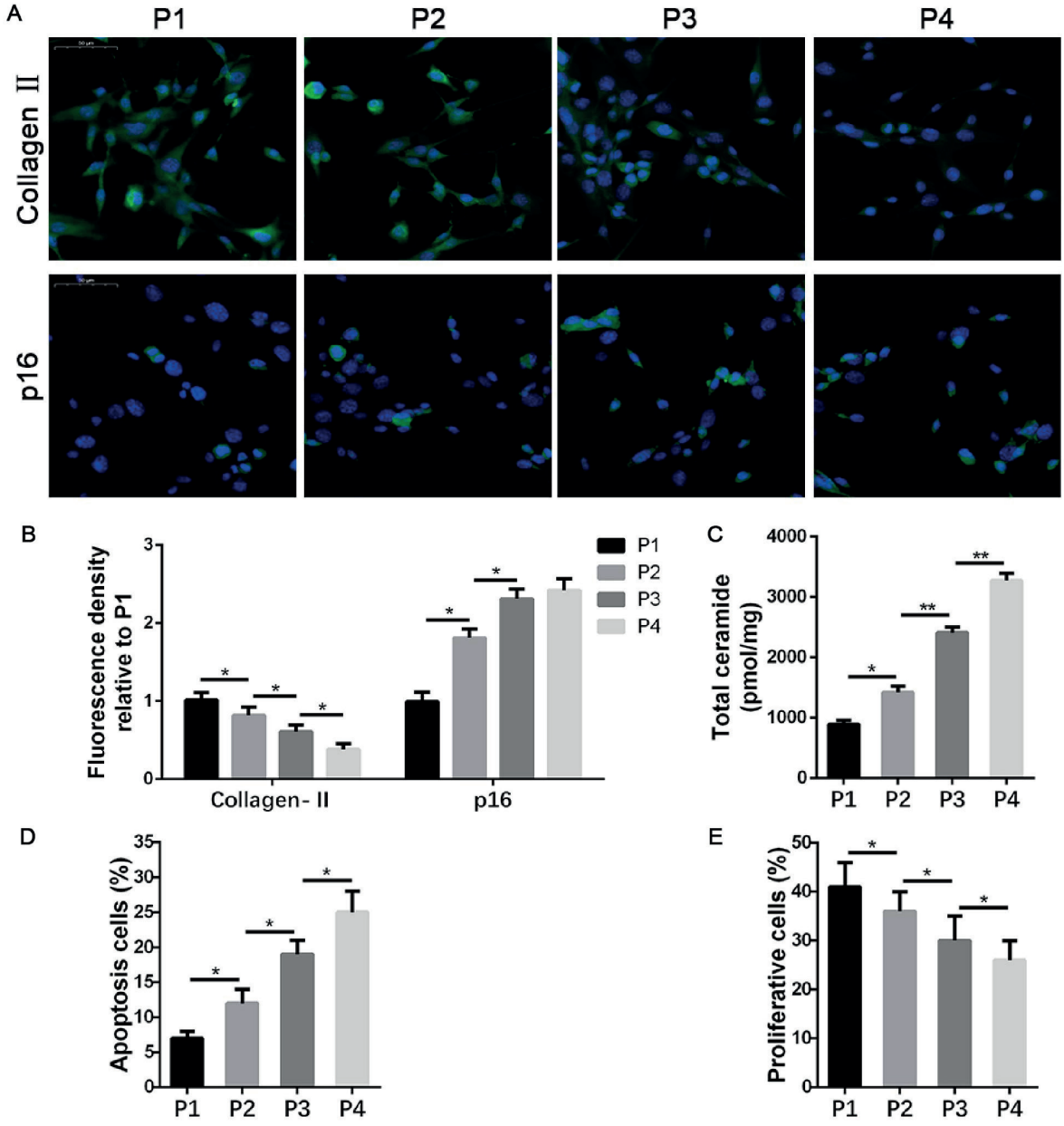

Figure 1. Ceramide accumulated in degenerated NP cells. NP cells from passage 1 (P1) cultured until the fusion rate up to 80% and passaged to P4. **A,** IF staining of collagen-II, and p16 (magnification: 400×). **B,** Quantification analysis. **C,** Total ceramide quantitation by LC-MS. The ratio of **D,** apoptotic or **E,** proliferative NP cells was determined by flow cytometry. Data are presented as mean ± SD of three independent experiments. (*p<0.05, **p<0.01).

viability, and the increase of senescent cells and the inflammatory response.

Ceramide Suppression Alleviated NP Cells Degeneration

To determine whether the suppression of ceramide protects the NP cells naturally degeneration, we used myriocin to suppress ceramide levels by de novo synthetic pathway. We passaged NP cells from the first generation to the fourth generation with or without myriocin (1 mM or 2 mM). Under the treatment of myriocin, we found the collagen II expression was mainly maintained compared to the cells without myriocin treatment, and 2 mM was effective to alleviate the degenerative progress of NP cells. The marker of senescent cell p16 was inhibited in a dose-dependent compared to the control with no myriocin intervention (Figure

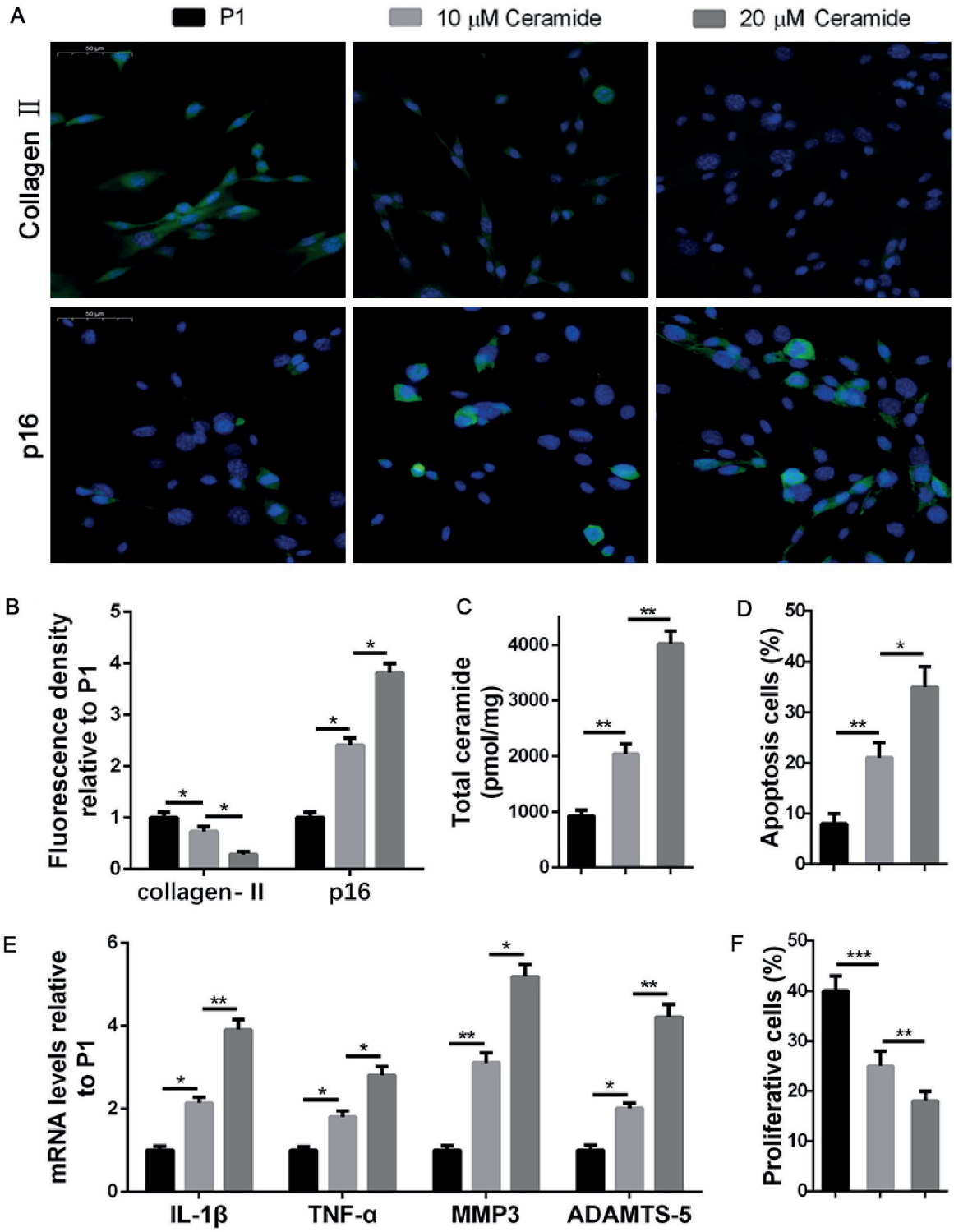

Figure 2. Ceramide overexpression promoted NP cells degeneration. NP cells in P1 were treated with C6-ceramide (10 μM or 20 μM) for 24 h and changed the medium. **A,** IF staining of collagen-II, and p16 (magnification: 400×). **B,** Quantification analysis. **C,** Total ceramide quantitation by LC-MS. **D,** Ratio of apoptotic NP cells was determined by flow cytometry. **E,** mRNA expression levels were assayed by RT-PCR. **F,** Ratio of proliferative NP cells was determined by flow cytometry. Data are presented as mean ± S.D. of three independent experiments. (*p<0.05, **p<0.01, ***p<0.001).

3A, 3B). As expected, the ceramide content was decreased in passage 4 resulting from the stimulation of myriocin (Figure 3C). The result of flow cytometry showed that myriocin meanwhile prevented NP cells apoptosis and protected their proliferation (Figure 3D, 3F). Then, we detected

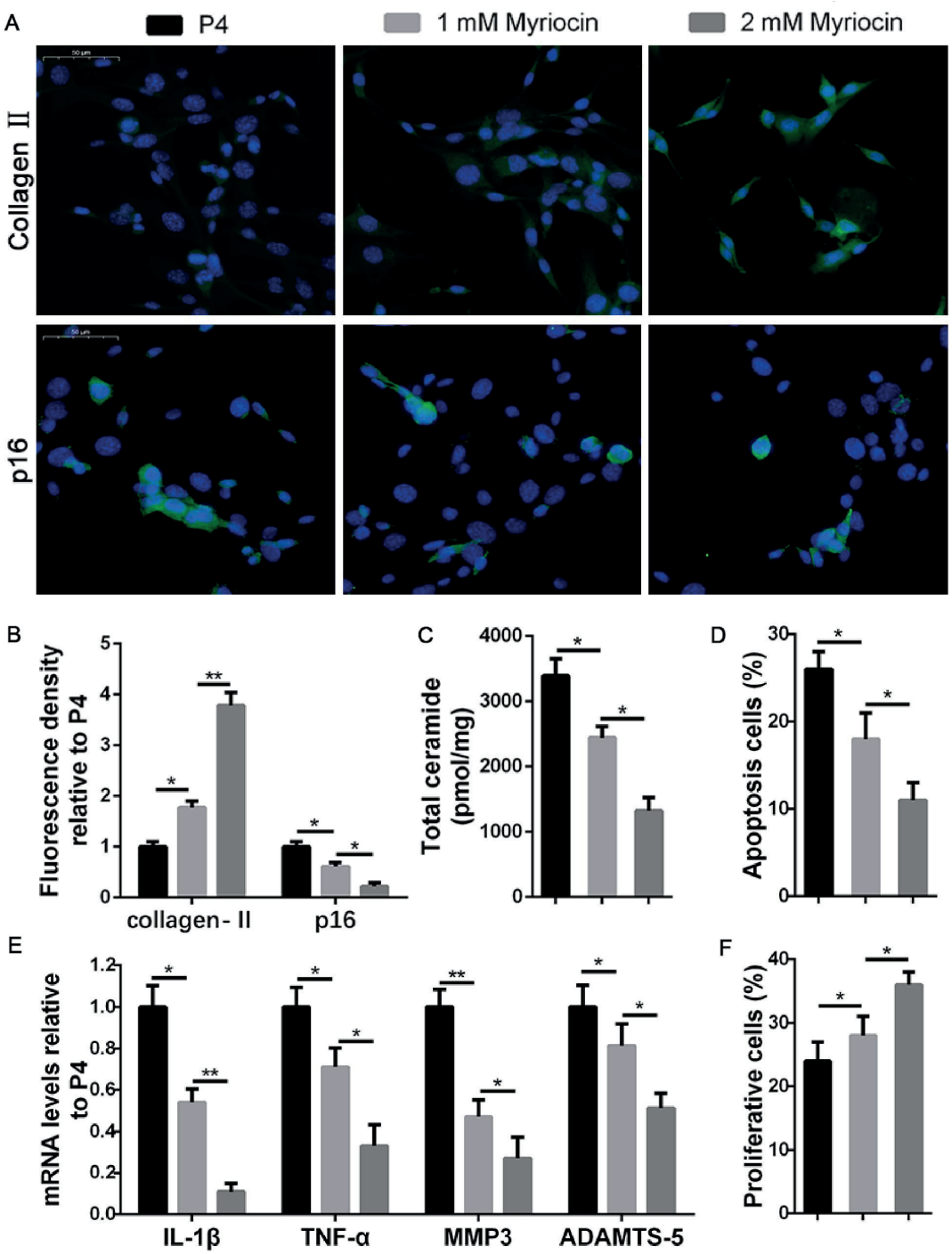

Figure 3. Ceramide inhibition alleviated NP cells degeneration. NP cells were treated with or without myriocin (10 mM or 2 mM) from P1 to P4. **A,** IF staining of collagen-II, and p16 (magnification: 400×). **B,** Quantification analysis. **C,** Total ceramide quantitation by LC-MS. **D,** Ratio of apoptotic NP cells was determined by flow cytometry. **E,** mRNA expression levels were assayed by RT-PCR. **F,** Ratio of proliferative NP cells was determined by flow cytometry. Data are presented as mean ± SD of three independent experiments. (*p<0.05, **p<0.01).

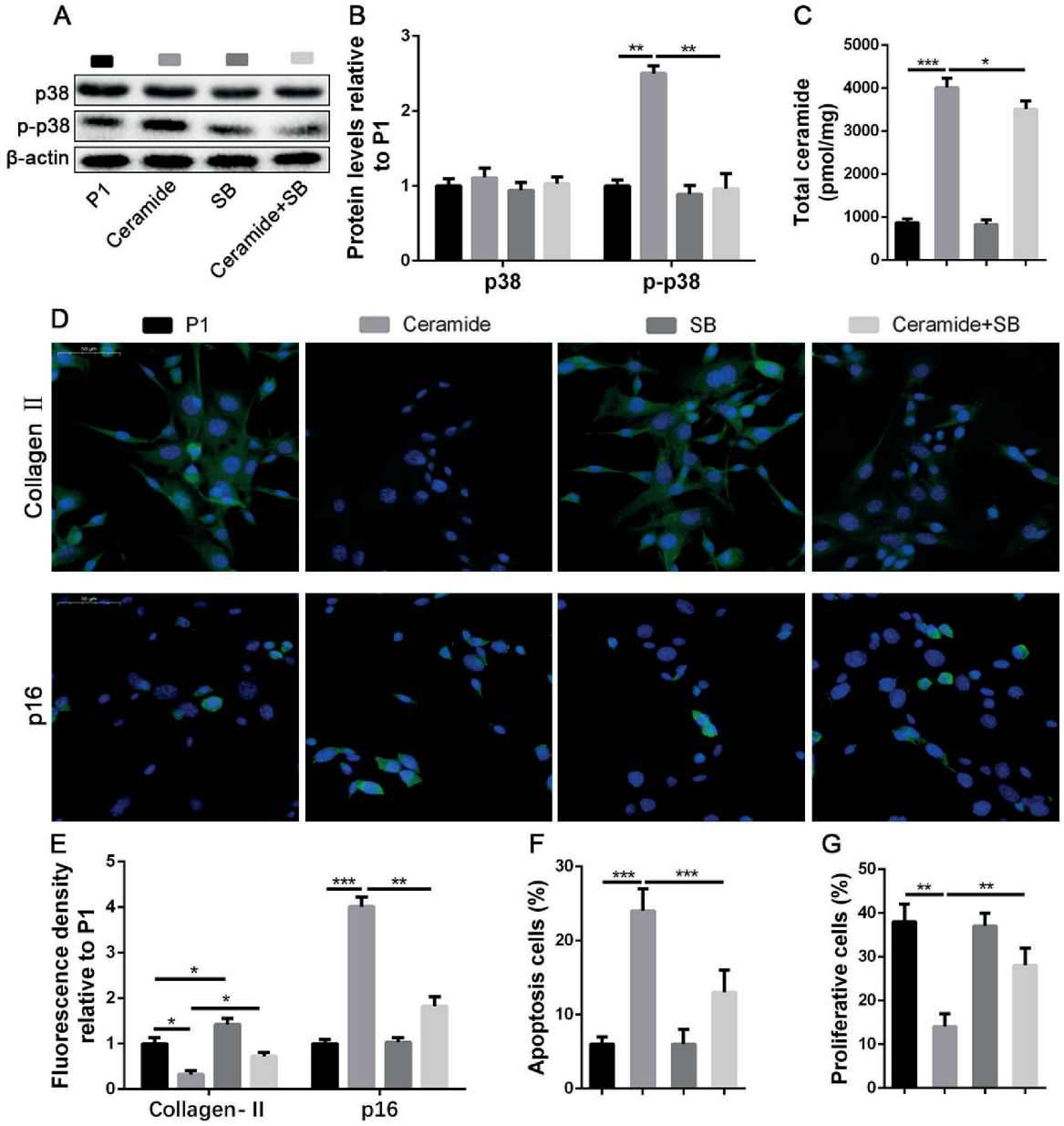

Figure 4. Suppress p38MAPK activity rejected ceramide-induced NP cell degeneration. P1 NP cells were treated with C6-ceramide (20 μM) or SB (50 nM) for 24 h and then changed the medium. Besides, P1 NP cells were pre-treated with C6-ceramide (20 μM) for 24 h and then treated with SB (50 nM) for another 24 h. **A,** Protein expression of p38 and p-p38 was determined by WB and **B,** quantification analysis. **C,** Total ceramide quantitation by LC-MS. **D,** IF staining of collagen-II, and p16 (magnification: 400×). **E,** Quantification analysis. The ratio of **F,** apoptotic or **G,** proliferative NP cells was determined by flow cytometry. Data are presented as mean ± SD of three independent experiments. (*p<0.05, **p<0.01, ***p<0.001).

the mRNA levels about the inflammatory factors and found the suppression of ceramide by myriocin decreased the IL-1β, TNF-α, MMP-3, and ADAMTS-5 expression (Figure 3E). In this part, we considered that the suppression of ceramide alleviated NP cell degeneration by the protected collagen II content and cell viability, and reduced senescent cells and the inflammatory response.

p38 Suppression Rejected Ceramide Induced NP Cells Degeneration

Like ceramide, the p38MAPK signaling pathway can be activated during extracellular stimulation such as ultraviolet irradiation, heat shock, high osmotic pressure, and inflammatory factors, and regulates various physiological processes, such as cell differentiation, cell cycle, and inflam-

matory response. Therefore, we wondered whether ceramide affected NP cells metabolism crosslinking with p38 activity. We used SB203580 (SB) to suppress p38MAPK activation. As shown in Figure 4A and 4B, the expression of total p38 was no significantly different among these groups. However, the 20 μM ceramide treatments markedly upregulated the p-p38 expression, which was suppressed by SB again. So, the overexpression of ceramide accompanied by the activation of p38. In addition, SB decreased the accumulation of ceramide even a small drop (Figure 4C). The result of IF showed that SB could partly reverse the ceramide-induced collagen II downregulation and p16 upregulation (Figure 4D, 4E). Finally, apoptosis and proliferation were also measured and the result suggested that SB treatment protected the NP cell proliferation and prevented the apoptosis progress compared to the ceramide treated group (Figure 4F, 4G). We found the suppression of p38MAPK phosphorylation could alleviate the upregulation of ceramide-induced NP cell degeneration, indicating that ceramide might accelerate the NP cell degeneration by p38MAPK activation.

Discussion

Ceramide belongs to a class of highly hydrophobic families and is the central molecule of the sphingomyelin signaling pathway[17]. Ceramide acts as a second messenger effector and participates in the activation of multiple protein kinases and protein phosphatases, and it regulates signal processes such as apoptosis, proliferation, differentiation, and growth arrest playing an important physiological role in cell homeostasis[18]. NP degeneration of the intervertebral disc is a multi-factor complex disease, and it is currently difficult to achieve the delay or even reverse the process of degeneration. Since now, the relation between ceramide and IDD is not clear. In our study, we noticed that the content of ceramide accumulated within the degeneration of NP cells from P1 to P4 generations[19]. Therefore, we held the viewpoint that ceramide played a role in the development of IDD.

We treated NP cells in the P1 generation with exogenous ceramide to made it overexpressed. C6-ceramide can rapidly induce human umbilic vein endothelial cells senescence[20]. Chan et al[21] found ceramide promoted inflammatory processes and apoptosis in the epithelium of the lung. Ceramide also triggers oxidative stress and nitric oxide synthase on human endothelial cells[22]. Therefore, ceramide accelerates different cell types of degradation with different manners. In our study, the cell-permeable analog efficaciously increased the ceramide content and made the phenotype of P1 NP cells similar to the P4, indicating the accumulation of ceramide promotes the degenerated degree of NP cells. On the contrary, the suppression of ceramide accumulation by myriocin could prevent NP cell degeneration containing the aspects of collagen II synthesis of, inflammation response, apoptosis, senescence, and proliferation. Myriocin is proved to suppress inflammatory and fungal by inhibiting ceramide de novo synthesis in chronic lung inflammation with compromised immunity[23]. Kurek et al[24] stated myriocin destroyed ceramide *de novo* synthesis mediating lipid metabolism in the liver of streptozotocin treated rats.

The mechanism underlines ceramide in the intervention of cell metabolism is complicated, that ceramides can activate c-Jun N-terminal kinase (JNK)[25], nuclear transcription factor κB (NF-κB)[26], toll-like receptor (TLR)[27] signaling pathway, and p38MAPK pathway[28]. p38MAPK signaling pathway regulates various physiological processes such as cell differentiation and apoptosis, cell cycle, and inflammatory response. We suggested that ceramide contributed to NP cell degeneration by the activation of p38MAPK. We found the p-p38 level was upregulated by the treatment of C6-ceramide. Additionally, the suppression of p38MAPK activation partly reversed the pro-degenerated function of ceramide accumulation. This result indicates that ceramide promotes the activation of p38MAPK contributing to the NP cell degeneration. However, the detailed mechanism of p38MAPK crosslinking with ceramide and the relation between other signaling pathways with ceramide need further exploration.

Conclusions

In summary we elucidated that ceramide accumulated during the development of NP cell degeneration. The overexpressed ceramide accelerates NP cells degeneration in decreasing proliferation, advancing the apoptosis, promoting senescence, and suppressing inflammation of NP cells. Besides, the suppression of ceramide can alleviate NP cells natural degeneration, and these effects underlining ceramide may be associated with the activation of p38MAPK. We believe cer-

amide could be a potential target for the treatment strategy of IDD.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Sampara P, Banala RR, Vemuri SK, Av GR, Gpv S. Understanding the molecular biology of intervertebral disc degeneration and potential gene therapy strategies for regeneration: a review. Gene Ther 2018; 25: 67-82.
2) Kennon JC, Awad ME, Chutkan N, DeVine J, Fulzele S. Current insights on use of growth factors as therapy for Intervertebral Disc Degeneration. Biomol Concepts 2018; 9: 43-52.
3) Chou R. In the clinic. Low back pain. Ann Intern Med 2014; 160: C1-C6.
4) Patil P, Niedernhofer LJ, Robbins PD, Lee J, Sowa G, Vo N. Cellular senescence in intervertebral disc aging and degeneration. Curr Mol Biol Rep 2018; 4: 180-190.
5) Dadsena S, Bockelmann S, Mina J, Hassan DG, Korneev S, Razzera G, Jahn H, Niekamp P, Muller D, Schneider M, Tafesse FG, Marrink SJ, Melo MN, Holthuis J. Ceramides bind VDAC2 to trigger mitochondrial apoptosis. Nat Commun 2019; 10: 1832.
6) Boon J, Hoy AJ, Stark R, Brown RD, Meex RC, Henstridge DC, Schenk S, Meikle PJ, Horowitz JF, Kingwell BA, Bruce CR, Watt MJ. Ceramides contained in LDL are elevated in type 2 diabetes and promote inflammation and skeletal muscle insulin resistance. Diabetes 2013; 62: 401-410.
7) Jadhav KS, Dungan CM, Williamson DL. Metformin limits ceramide-induced senescence in C2C12 myoblasts. Mech Ageing Dev 2013; 134: 548-559.
8) Gulbins A, Grassme H, Hoehn R, Wilker B, Soddemann M, Kohnen M, Edwards MJ, Kornhuber J, Gulbins E. Regulation of neuronal stem cell proliferation in the hippocampus by endothelial ceramide. Cell Physiol Biochem 2016; 39: 790-801.
9) Galadari S, Rahman A, Pallichankandy S, Thayyullathil F. Tumor suppressive functions of ceramide: evidence and mechanisms. Apoptosis 2015; 20: 689-711.
10) Merrill AJ, Sullards MC, Allegood JC, Kelly S, Wang E. Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. Methods 2005; 36: 207-224.
11) Munoz-Olaya JM, Matabosch X, Bedia C, Egido-Gabas M, Casas J, Llebaria A, Delgado A, Fabrias G. Synthesis and biological activity of a novel inhibitor of dihydroceramide desaturase. ChemMedChem 2008; 3: 946-953.
12) Rivas DA, Morris EP, Haran PH, Pasha EP, Morais MS, Dolnikowski GG, Phillips EM, Fielding RA. Increased ceramide content and NFkappaB signaling may contribute to the attenuation of anabolic signaling after resistance exercise in aged males. J Appl Physiol (1985) 2012; 113: 1727-1736.
13) Zhao L, Spassieva SD, Jucius TJ, Shultz LD, Shick HE, Macklin WB, Hannun YA, Obeid LM, Ackerman SL. A deficiency of ceramide biosynthesis causes cerebellar purkinje cell neurodegeneration and lipofuscin accumulation. PLoS Genet 2011; 7: e1002063.
14) Cutler RG, Kelly J, Storie K, Pedersen WA, Tammara A, Hatanpaa K, Troncoso JC, Mattson MP. Involvement of oxidative stress-induced abnormalities in ceramide and cholesterol metabolism in brain aging and Alzheimer's disease. Proc Natl Acad Sci U S A 2004; 101: 2070-2075.
15) Kester M, Bassler J, Fox TE, Carter CJ, Davidson JA, Parette MR. Preclinical development of a C6-ceramide NanoLiposome, a novel sphingolipid therapeutic. Biol Chem 2015; 396: 737-747.
16) Rivera IG, Ordonez M, Presa N, Gomez-Larrauri A, Simon J, Trueba M, Gomez-Munoz A. Sphingomyelinase D/ceramide 1-phosphate in cell survival and inflammation. Toxins (Basel) 2015; 7: 1457-1466.
17) Chakraborty M, Jiang XC. Sphingomyelin and its role in cellular signaling. Adv Exp Med Biol 2013; 991: 1-14.
18) Cheng Q, Li X, Wang Y, Dong M, Zhan FH, Liu J. The ceramide pathway is involved in the survival, apoptosis and exosome functions of human multiple myeloma cells in vitro. Acta Pharmacol Sin 2018; 39: 561-568.
19) Chen Q, Shi F, Yang X, Liu L, Song Y. [Comparative study on biological characteristics between different generations of rabbit nucleus pulposus cells]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2018; 32: 660-667.
20) Gao YF, Chen X, Su XQ, Zhai ZH. [Rapid induction of senescence-like changes in human umbilic vein endothelial cells(HUVECs) by C6 ceramide]. Shi Yan Sheng Wu Xue Bao 2001; 34: 333-335.
21) Chan C, Goldkorn T. Ceramide path in human lung cell death. Am J Respir Cell Mol Biol 2000; 22: 460-468.
22) Li H, Junk P, Huwiler A, Burkhardt C, Wallerath T, Pfeilschifter J, Forstermann U. Dual effect of ceramide on human endothelial cells: induction of oxidative stress and transcriptional upregulation of endothelial nitric oxide synthase. Circulation 2002; 106: 2250-2256.
23) Caretti A, Torelli R, Perdoni F, Falleni M, Tosi D, Zulueta A, Casas J, Sanguinetti M, Ghidoni R, Borghi E, Signorelli P. Inhibition of ceramide de novo synthesis by myriocin produces the double effect of reducing pathological inflammation and exerting antifungal activity against A. fumigatus

airways infection. Biochim Biophys Acta 2016; 1860: 1089-1097.

24) Kurek K, Wiesiolek-Kurek P, Piotrowska DM, Lukaszuk B, Chabowski A, Zendzianendzian-Piotrowska M. Inhibition of ceramide de novo synthesis with myriocin affects lipid metabolism in the liver of rats with streptozotocin-induced type 1 diabetes. Biomed Res Int 2014; 2014: 980815.

25) Tirodkar TS, Lu P, Bai A, Scheffel MJ, Gencer S, Garrett-Mayer E, Bielawska A, Ogretmen B, Voelkel-Johnson C. Expression of ceramide synthase 6 transcriptionally activates acid ceramidase in a c-Jun N-terminal kinase (JNK)-dependent manner. J Biol Chem 2015; 290: 13157-13167.

26) Gorgani-Firuzjaee S, Ahmadi S, Meshkani R. Palmitate induces SHIP2 expression via the ceramide-mediated activation of NF-kappaB, and JNK in skeletal muscle cells. Biochem Biophys Res Commun 2014; 450: 494-499.

27) Kiefer K, Casas J, Garcia-Lopez R, Vicente R. Ceramide imbalance and impaired TLR4-mediated autophagy in BMDM of an ORMDL3-overexpressing mouse model. Int J Mol Sci 2019; 20:

28) Liao L, Zhou Q, Song Y, Wu W, Yu H, Wang S, Chen Y, Ye M, Lu L. Ceramide mediates Ox-LDL-induced human vascular smooth muscle cell calcification via p38 mitogen-activated protein kinase signaling. PLoS One 2013; 8: e82379..