Celastrol promotes chondrocyte autophagy by regulating mTOR expression

Siming Dai[1], Jiankun Fan[2], Yue Zhang[1,3], Zhenyong Hao[4], Huiming Yu[1], Zhiyi Zhang[1]

[1]Department of Rheumatology and Immunology, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150001, China;
[2]The Laboratory of Medical Genetics, Harbin Medical University, Harbin, Heilongjiang 150001, China;
[3]Shenzhen Futian Hospital for Rheumatic Diseases, Shenzhen, Guangdong 518000, China;
[4]Department of Orthopedic Surgery of Harbin Fifth Hospital, Harbin, Heilongjiang 150001, China.

Osteoarthritis (OA) is a debilitating disease with limited treatment options.[1] Autophagy serves as an important protective mechanism against the deterioration of cartilage and is negatively regulated by the mammalian target of rapamycin (mTOR).[2] In previous studies, celastrol could promote autophagy in various cell types and had the promising therapeutic potential for OA.[3] However, the effect and underlying mechanisms of celastrol on autophagy in OA remain unclear.

Our preliminary study using transcriptome sequencing and a network pharmacology analysis identified mTOR as a potential direct target of celastrol for protection against OA.[4] In this study, we aim to evaluate the effects of celastrol on mTOR activity in primary human osteoarthritic chondrocytes and to determine the mechanism by which it affects autophagy and endoplasmic reticulum (ER) stress.

Celastrol is a triterpenoid quinine methide isolated from the root of *Tripterygium wilfordii* Hook F. In laboratory studies and clinical practice, celastrol has a demonstrated favorable effect for slowing the progression of different forms of arthritis.[5] We first determined the optimal dose (200 nmol/L, for 24 hours) of celastrol for chondrocyte cultures by Cell Counting Kit-8 assay [Figure 1A]. Based on the previous bioinformatic analysis, we hypothesized that mTOR (a key regulator of autophagy) was a potential biological target of celastrol. Celastrol treatment reduced mTOR levels in primary OA chondrocytes, as measured by Western blotting [Figure 1B]. The expression of P62 and LC3 was used to assay autophagy. P62 is a marker of autophagy turnover, and its expression is inversely related to autophagic activity. LC3 indicates autophagosome formation, and its conversion from LC-I to LC3-II is generally regarded as marker of autophagy. The chondrocyte cells treated with celastrol had reduced P62 and increased LC3-II [Figure 1B], which indicated that celastrol could promote autophagy and contributed to the chondroprotective effect [Supplementary files, http://links.lww.com/CM9/A594].

Rapamycin is also an mTOR inhibitor. By comparing the pharmacological effects of celastrol and rapamycin on mTOR in OA chondrocytes, we found that celastrol treatment increased levels of phosphorylated pancreatic ER kinase (PERK), a marker of ER stress activation,[6] and LC3-II but decreased levels of P62, as well as caspase 12 (CASP12) and DNA damage-inducible transcript 3 (DDIT3), which indicated decreased proapoptotic effects [Figure 1C and 1D]. These results demonstrated that celastrol could promote chondrocyte autophagy and inhibit chondrocyte apoptosis, which could result in a therapeutic effect. Moreover, we observed a strong synergistic effect of combined treatment with celastrol and rapamycin, which suppressed mTOR but enhanced autophagy [Figure 1E], compared to treatment with celastrol or rapamycin alone ($P < 0.05$).

When celastrol was combined with MHY1485, an mTOR agonist, this reduced its inhibitory effect on mTOR signaling and did not result in activation of PERK. The combination of celastrol and MHY1485 also resulted in increased CASP12 and DDIT3, indicating a proapoptotic effect, as well as increased P62 and decreased LC3-II, indicating decreased autophagy [Figure 1G and 1H]. Thus, blocking autophagy with an mTOR agonist muted the pharmacological effects of celastrol on autophagy in

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001552

Correspondence to: Zhiyi Zhang, Department of Rheumatology and Immunology, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150086, China
E-Mail: zhangzhiyi2014@163.com

Chinese Medical Journal 2022;135(1)
Received: 29-01-2021; **Online:** 15-06-2021 **Edited by:** Lishao Guo

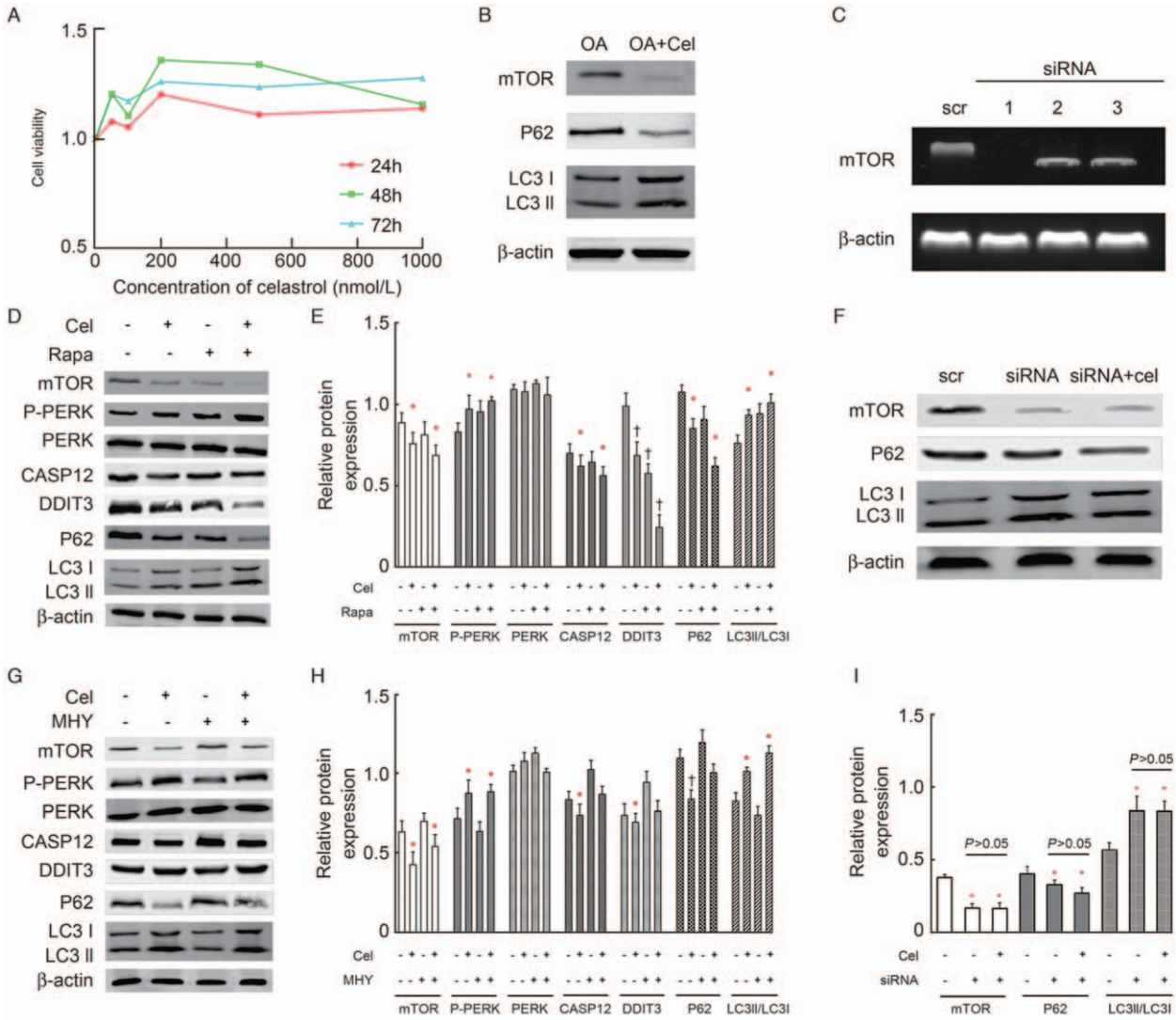

Figure 1: Celastrol promotes autophagy by inhibiting mTOR. (A) The optimal concentration of celastrol was determined by the CCK-8 assay. 200 nmol/L for 24 hours was determined to be the optimal dosage in this study (concerning the following period of time for drugs' treatment). (B) OA chondrocytes treated with 200 nmol/L celastrol for 24 h were used as experimental group. Protein levels of mTOR (the potential target of celastrol), P62, and LC3 were determined by Western blotting. (C) SqRT-PCR was performed to determine mTOR gene expression after silencing with siRNA. Transfection with an mTOR siRNA effectively knocked down mTOR gene expression. (D) OA chondrocytes in the celastrol and rapamycin experimental groups were treated with 200 nmol/L celastrol or 50 nmol/L rapamycin, respectively, for 24 h, and cells in the combined drug group were treated with both 200 nmol/L celastrol and 50 nmol/L rapamycin for 24 h. Protein levels were determined by Western blotting. A representative image is shown. (E) Densitometry was analyzed with ImageJ software and normalized to b-actin. Data were collected from three independent experiments and analyzed by a variance test, $^{*}P < 0.05$, $^{+}P < 0.01$. (F) OA chondrocytes were transfected with mTOR siRNA (final concentration of 50 nmol/L). After 48 h, cells were treated with 200 nmol/L celastrol for 24 h. Protein samples were collected, and protein levels were analyzed by Western blotting. (G) OA chondrocytes in the celastrol group were treated with 200 nmol/L celastrol for 24 h. Cells in the MHY1485 experimental group were treated with 10 μmol/L MHY1485 for 4 h. Cells in the celastrol and MHY1485 group were first treated with 10 μmol/L MHY1485 for 4 h, and this was followed by 200 nmol/L celastrol for 24 h. Protein levels were determined by Western blotting, and a representative image is shown. (H) Densitometry was analyzed by ImageJ software and normalized to β-actin. Data was collected from three independent experiments and analyzed using a variance test, $^{*}P < 0.05$, $^{+}P < 0.01$. (I) Densitometry was analyzed by ImageJ software and normalized to β-actin. Data were collected from three independent experiments and analyzed by a variance test, $^{*}P < 0.05$. Cel: Celastrol; CASP12: Caspase 12; CCK-8: Cell Counting Kit-8; DDIT3: DNA damage-inducible transcript 3; OA: Osteoarthritis; Rapa: Rapamycin; scr: Scramble; SiRNA: Small interfering RNA; SqRT-PCR: Semi-quantitative reverse transcription and polymerase chain reaction.

chondrocytes; this indicates that mTOR is an intracellular target of celastrol and that there may be antagonistic effects of celastrol and MHY1485.

Finally, we transfected OA chondrocytes with a small interfering RNA (siRNA) silencing the mTOR gene. Chondrocytes treated with celastrol for 24 h after transfection served as the experimental group. As depicted in Figure 1F and 1I, both celastrol treatment and siRNA silencing of mTOR significantly promoted autophagy. However, there was no appreciable difference in the outcomes of the two treatment groups, which indicates that mTOR is the key target of celastrol in OA chondrocytes.

In conclusion, celastrol can promote autophagy by reducing mTOR levels, thereby protecting primary human OA chondrocytes. Combination treatment with celastrol and rapamycin can produce an even stronger effect. Combining celastrol with the mTOR agonist MHY1485

reduced its effects on OA chondrocytes, and celastrol also had no effect on chondrocytes treated with siRNA targeting mTOR. In future work, an OA mouse model will be used to further determine the therapeutic target and potential mechanism of celastrol in OA.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81771749 and No. 81771748).

Conflicts of interest

None.

References

1. Ye ZZ, Zhang ZY, Li ZG, Huang CB, Zhang Y. Toward wiping out osteoarthritis in China: research highlights. Chin Med J 2020;133:883–885. doi: 10.1097/cm9.0000000000000746.
2. Zhang Y, Vasheghani F, Li YH, Blati M, Simeone K, Fahmi H, *et al*. Cartilage-specific deletion of mTOR upregulates autophagy and protects mice from osteoarthritis. Ann Rheum Dis 2015;74:1432–1440. doi: 10.1136/annrheumdis-2013-204599.
3. Feng K, Chen H, Xu C. Chondro-protective effects of celastrol on osteoarthritis through autophagy activation and NF-κB signaling pathway inhibition. Inflamm Res 2020;69:385–400. doi: 10.1007/s00011-020-01327-z.
4. Dai SM, Wang H, Wang M, Zhang Y, Zhang ZY, Lin ZG. Comparative transcriptomics and network pharmacology analysis to identify the potential mechanism of celastrol against osteoarthritis. Clin Rheumatol 2021. doi: 10.1007/s10067-021-05726-3. Published ahead of print.
5. Cascão R, Fonseca JE, Moita LF. Celastrol: A spectrum of treatment opportunities in chronic diseases. Front Med (Lausanne) 2017;4:69. doi: 10.3389/fmed.2017.00069.
6. Li YH, Tardif G, Hum D, Kapoor M, Fahmi H, Pelletier JP, *et al*. The unfolded protein response genes in human osteoarthritic chondrocytes: PERK emerges as a potential therapeutic target. Arthritis Res Ther 2016;18:172. doi: 10.1186/s13075-016-1070-6.

How to cite this article: Dai S, Fan J, Zhang Y, Hao Z, Yu H, Zhang Z. Celastrol promotes chondrocyte autophagy by regulating mTOR expression. Chin Med J 2022;135:92–94. doi: 10.1097/CM9.0000000000001552