TRPM7 Mediates Chondrocyte Senescence in Osteoarthritis by Inducing PDK4-Dependent MAM Formation and cGAS-STING Activation

Cheng Sun, Hao Liu, Renpeng Zhou, Wei Hu

Department of Clinical Pharmacology, The Second Affiliated Hospital of Anhui Medical University, Hefei, 230601, China

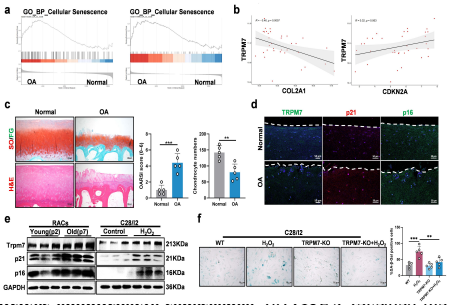
huwei@ahmu.edu.cn

Disclosure: None

Introduction: Chondrocyte senescence is a key factor in the progression of osteoarthritis (OA), but the molecular mechanisms driving chondrocyte senescence remain largely unknown. TRPM7, a channel kinase that regulates Ca^{2+} signaling and cellular stress responses, has been implicated in cartilage degeneration (Zhou R, et al., ***RB***, 2022) . Furthermore, activation of the cGAS-STING pathways promotes cellular senescence. We hypothesized that TRPM7 promotes OA progression through a PDK4-cGAS-STING axis. This study aimed to define TRPM7's role in chondrocyte senescence and cartilage degeneration and to elucidate the underlying signaling mechanisms.

Materials & Methods: Human OA and healthy cartilage were assessed histologically and molecularly. Chondrocyte senescence was analyzed by SA-β-gal staining and expression of p21/p16. TRPM7, PDK4, and cGAS–STING activity were evaluated via immunoblotting and immunofluorescence. Functional studies involved knocking out TRPM7 in vitro and in vivo, knocking out PDK4 in vitro, or inhibiting STING.

Results: TRPM7 is upregulated in OA cartilage and drives chondrocyte senescence. To evaluate the role of TRPM7 and chondrocyte senescence in OA progression, we performed gene set enrichment analysis (GSEA) on transcriptome data from cartilage tissues of OA patients and healthy controls (GSE114007, GSE117999). Gene set enrichment analysis (GSEA) revealed that cellular senescence pathways were significantly enriched in OA cartilage compared with healthy controls **(Fig. 1a)**. Transcriptome analysis further showed that TRPM7 expression correlated positively with CDKN2A and negatively with COL2A1 **(Fig. 1b)**, suggesting a potential link between TRPM7 and chondrocyte senescence. Histological and molecular analyses of human OA cartilage confirmed severe matrix loss and structural damage, accompanied by increased TRPM7, p21, and p16 expression **(Fig. 1c–d)**. Additionally, TRPM7, p16, and p21 expression were significantly increased in the chondrocyte replicative senescence and H_2O_2-induced chondrocyte senescence **(Fig. 1e)**. Knockout of TRPM7 reverses H_2O_2-induced chondrocyte senescence **(Fig. 1f)**. Together, TRPM7 is a key mediator of chondrocyte senescence and OA progression.



drives chondrocyte senescence. (a) GSEA revealed the biological process of cellular senescence in OA and normal cartilage. (b) Correlation analysis of TRPM7 expression with CDKN2A and COL2A1. (c) Representative SO/FG and H&E staining image of cartilage tissue, OA severity was quantified based on chondrocyte density, cartilage thickness, and Osteoarthritis Research Society International (OARSI) scoring; (d) IF analysis of TRPM7, p21 and p16 expression in cartilage tissue. (e) Western blot analysis of TRPM7, p21and p16 in primary rat articular chondrocytes (RACs) and C28/I2 cells. (f) SA-β-gal staining in TRPM7-KO C28/I2 treated with H_2O_2.

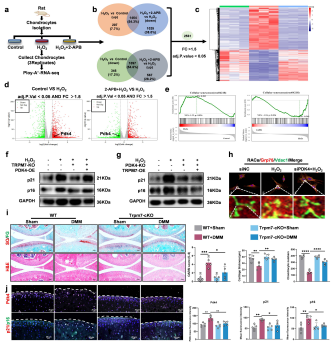


Fig. 2. PDK4 is involved in TRPM7-mediated senescence of articular chondrocytes. (a) Schematic of RNA-seq experimental design and sample preparation. (b–c) Venn diagram and heatmap showing TRPM7-regulated gene sets. (d) Volcano plot highlighting PDK4 expression across control, H_2O_2, and H_2O_2+2-APB groups. (e) GSEA showing enrichment of cellular senescence pathways. (f–g) Western blot of p21 and p16 in C28/I2 cells. (h) IF analysis of co-localization of MAMs components (Grp75 and Vdac1) in RACs treated with siPDK4 and H_2O_2. (i) Representative SO/FG and H&E staining of cartilage. (j) IF analysis of PDK4, p21, and p16 in cartilage.

PDK4 is involved in TRPM7-mediated senescence of OA articular chondrocytes. To explore TRPM7-mediated chondrocyte senescence, RNA-seq was performed under control, H_2O_2, and H_2O_2+2-APB conditions **(Fig. 2a).** Analysis identified 2,561 DEGs (FC>1.5, FDR<0.05), with PDK4 notably upregulated by H_2O_2 and reversed by 2-APB **(Fig. 2b–d)**, suggesting PDK4 as a downstream effector of TRPM7. GSEA confirmed enrichment of senescence-related pathways, reversed by 2-APB **(Fig. 2e)**. Functional assays showed that knockout of TRPM7 blocked H_2O_2-induced p21/p16 upregulation, while PDK4 overexpression could not rescue the effect, whereas TRPM7 overexpression failed to induce senescence in PDK4-KO cells, confirming TRPM7 acts upstream of PDK4 **(Fig. 2f-g)**. This finding was further validated in the cartilage tissue of OA model mice with TRPM7 knockout **(Fig. 2i-j)**. Additionally, H_2O_2-induced enhanced GRP75-VDAC1 co-localization, while PDK4 silencing attenuated these interactions, demonstrating PDK4's role in MAMs assembly **(Fig. 2h)**.

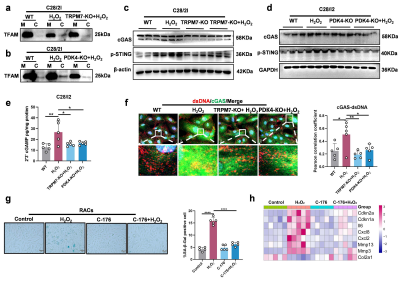


Fig. 3. cGAS-STING pathway mediates TRPM7-induced, PDK4-dependent mtDNA release and promotes chondrocyte senescence. (a-b) Western blot analysis of TFAM in TRPM7-KO and PDK4-KO C28/I2 cells treated with H_2O_2. (c-d) Western blot analysis of cGAS and p-STING protein levels in TRPM7-KO and PDK4-KO C28/I2 cells treated with H_2O_2. (e) Enzyme-linked immunosorbent assay (ELISA) measuring 2'3'-cGAMP activity in H_2O_2 induced TRPM7-KO and PDK4-KO C28/I2 cells. (f) IF staining showing co-localization of DNA and cGAS after TRPM7 or PDK4 knockout. (g) SA-β-gal staining in RACs treated with C-176 and H_2O_2. (h) Heatmap showing the levels of aging-related genes in H_2O_2 induced-RACs treated with C-176 by qPCR analysis, normalized with GAPDH.

cGAS-STING pathway mediates TRPM7-induced, PDK4-dependent mtDNA release and promotes chondrocyte senescence. Subcellular fractionation showed H_2O_2-induced TFAM translocation from mitochondria to cytoplasm, which was blocked by TRPM7 or PDK4 knockdown **(Fig. 3a–b)**. TRPM7 or PDK4 inhibition also reduced cGAS and p-STING expression **(Fig. 3c–d)** and lowered intracellular cGAMP levels **(Fig. 3e)**. IF staining confirmed decreased mtDNA–cGAS co-localization upon TRPM7 or PDK4 knockout **(Fig. 3f)**, indicating suppression of cGAS-STING activation. Functionally, STING inhibition with C-176 reduced H_2O_2-induced β-gal-positive cells, downregulated aging-related genes (Cdkn2a, Cdkn1a, Cxcl8, Cxcl2, Il-6, Mmp13, Mmp3), and delayed Col2a1 degradation **(Fig. 3g–h)**. These results demonstrate that TRPM7 and PDK4 drive chondrocyte senescence via the cGAS-STING pathway.

Conclusions: In conclusion, we demonstrated, for the first time, that TRPM7 was the key regulatory target in chondrocyte senescence and OA progression. Meanwhile, we elucidated a novel mechanism regulating chondrocyte senescence, in which TRPM7 upregulates PDK4, thereby activating the cGAS-STING pathway and exacerbating chondrocyte senescence and OA progression. Targeting TRPM7 and its downstream effectors may offer novel theoretical insights and therapeutic strategies for OA prevention and treatment.

Significance: This study identifies the TRPM7–PDK4–cGAS-STING axis as a central mechanism of OA pathogenesis and highlights TRPM7 and its downstream effectors as promising therapeutic targets for preventing chondrocyte senescence and cartilage degeneration.

References: 1. Zhou, Renpeng et al. Redox biology vol. 55 (2022): 102411.