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FULL LENGTH ARTICLE

Osteopontin inhibits osteoarthritis progression via the OPN/CD44/PI3K signal axis

Qing Liu [a,b,c], Hao Zeng [a], Yuhao Yuan [a], Zhiwei Wang [a], Ziyi Wu [a], Wei Luo [a,*]

[a] *Department of Orthopaedics, Xiangya Hospital, Central South University, 87th Xiangya Road, Changsha, Hunan 410008, PR China*
[b] *Department of Spine Surgery, The Second Xiangya Hospital, Central South University, 139th Renmin Middle Road, Changsha, Hunan 410011, PR China*
[c] *Molecular Oncology Laboratory, Department of Orthopaedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, IL 60637, USA*

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Abstract Chondrocyte degeneration and extracellular matrix component loss are the primary causes of osteoarthritis (OA). OA can be treated by inhibiting chondrocyte degeneration and increasing extracellular matrix component secretion. Osteopontin (OPN), a multifunctional protein, has gained immense attention with regard to its involvement in OA. This study aimed to explore the therapeutic value and mechanism of action of OPN in OA treatment. Results of the histomorphological analysis revealed a worn-off OA cartilage tissue surface, cartilage matrix layer deterioration, and calcium salt deposition. Compared to that in normal chondrocytes, in OA chondrocytes, the OPN, CD44, and PI3K protein and mRNA expression was upregulated. Further, siOPN, rhOPN, and rhOPN plus LS-C179404 interfered with OA chondrocytes. As verified in mice, OPN directly inhibited the expression level of PI3K in OA chondrocytes by binding with CD44. Morphological analysis of the knee joints demonstrated that OPN effectively inhibited OA progression via the OPN/CD44/PI3K signal axis. In conclusion, OPN activates intracellular PI3K signaling molecules by binding to CD44 on the cell surface to cause downstream cascading effects, thereby delaying chondrocyte degeneration and reducing cartilage matrix component loss; therefore, OPN is a potential therapeutic agent for OA.

* Corresponding author. Department of Orthopaedics, Xiangya Hospital, Central South University, Changsha, Hunan 410008, PR China.
E-mail addresses: 158112225@csu.edu.cn (Q. Liu), zjohn08@sina.com (H. Zeng), 504807763@qq.com (Y. Yuan), 44011294@qq.com (Z. Wang), wzy15021@163.com (Z. Wu), luowei0928@126.com (W. Luo).
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Introduction

Osteoarthritis (OA) is a chronic disabling degenerative joint disease; it is characterized by recurrent joint pain and progressive joint dysfunction. The morbidity and disability rates due to OA are high and the effects of drug treatment are limited; further, OA treatment is expensive, seriously threatens the physical and mental health of elderly patients, and imposes a burden on the society and the patient's family.[1,2] Thus, theoretical studies on OA are urgently needed to clarify its pathological mechanism, identify an effective therapeutic target, block the pathological process, and delay or terminate the disease course. In the initial stages of OA development, homeostasis of articular cartilage is disturbed due to the action of local or systemic factors. Articular cartilage exfoliation or fibrosis, subchondral osteosclerosis or cystic changes, periarticular hyperosteogeny, and cartilage matrix degradation are the core pathological changes observed during OA.[3] The pathological molecular mechanism of OA involves inflammatory mediator and cytokine overstimulation; this leads to disrupted cartilage synthesis and catabolism and subsequently chondrocyte degeneration and extracellular matrix component loss.[4–6] Chondrocytes, the only cell type in articular cartilage, primarily function in maintaining extracellular matrix balance. Therefore, chondrocyte proliferation–apoptosis balance directly affects the occurrence and development of OA.

Osteopontin (OPN), a member of the small integrin-binding ligand *N*-linked glycoprotein family, regulates various pathological and physiological processes, including wound healing, tumorigenesis, immune response, and inflammation, by interacting with its receptor integrins $\alpha9\beta1$, $\alpha4\beta1$, and αv ($\beta1$, $\beta3$, $\beta5$) and CD44 variants.[7–12] Chondrocytes, osteoblasts, osteocytes, and osteoclasts can secrete OPN, which is involved in the development, repair, and maintenance of metabolic homeostasis of normal articular cartilage.[12–14] OPN also regulates hyaluronic acid (HA), type II collagen, proteoglycan, and other cartilage matrix components, thereby functioning as a protective factor in OA development. Further studies on the mechanisms underlying OPN function in articular cartilage degeneration in OA will help reveal the pathogenesis of the disease.

OPN mediates intracellular signal transduction by binding receptors on the cell membrane. Integrin and cell surface adhesion glycoprotein are the main receptors of OPN.[15–17] CD44, a member of the adhesion receptor family, is involved in cell proliferation, differentiation, adhesion, and migration as well as intracellular signal transduction.[18–20] Its ligands, such as HA and OPN,[17] also play important roles in the pathological process of and cartilage metabolism in OA. OPN and CD44 are positively correlated with OA progression and tend to consistently affect some downstream products such as HA and matrix metalloproteinase-13 (MMP-13), which regulate cartilage metabolism.[14,21] Therefore, we hypothesized that OPN and CD44 cause cartilage degeneration in OA via a ligand–receptor reaction; however, which downstream signaling pathway regulates cartilage metabolism and affects OA progression remains unclear.

Recent studies have shown that the downstream signaling molecules regulated by the OPN–CD44 ligand–receptor reaction mainly include the phosphatidylinositol-3-kinase/protein kinase B (PI3K/Akt) signaling pathway, nuclear factor-κB signaling pathway, and mitogen-activated protein kinase signaling pathway.[22,23] The mechanism of the PI3K/Akt signaling pathway in chondrocyte proliferation and apoptosis has also been evaluated recently.[24,25] This pathway can effectively inhibit chondrocyte apoptosis via phosphorylation, and estradiol can promote chondrocyte proliferation via PI3K/Akt signaling pathway activation. These studies suggest the importance of the PI3K/Akt signaling pathway in the pathological process of chondrocyte degeneration in OA.

We hypothesized that OPN secreted by chondrocytes can inhibit the pathological process of OA by activating the intracellular PI3K/Akt/mTOR signaling pathway via a paracrine or autocrine mechanism combined with the membrane surface receptor CD44. Using *in vitro* and *in vivo* experiments, we investigated the role of the OPN/CD44/PI3K signal axis in the pathological process of cartilage degeneration in OA; we also clarified the role of OPN in OA with the aim of establishing an accurate therapeutic target for treating OA.

Materials and methods

Specimens and ethical statement

Normal knee joint cartilage tissue samples were obtained from patients with osteosarcoma undergoing hinge knee arthroplasty, and degenerative cartilage tissue samples (OA samples) was obtained from patients with OA (Kellgren–Lawrence Grade III-IV) undergoing surface knee arthroplasty. Patient characteristics have been summarized under Table 1. The study was conducted in accordance with

Table 1 Patients information.

Group	Average age (year)	Gender (F/M)	Diagnosis
OA	67.4	F:16 M:6	OA[a]
Normal	17.8	F:7 M:5	Osteosarcoma

[a] All patients with K-L grade III or IV.

ethical standards listed in the 1964 Declaration of Helsinki and approved by the Animal Research Center of Central South University and the Ethics Committee of Xiangya Hospital. Informed consent was obtained from the patients or their legal guardians.

Chondrocyte isolation, culture, and identification

The cartilage tissue samples obtained were immediately placed in phosphate-buffered saline containing antibiotics. Primary chondrocytes were isolated and cultured according to methods established in previous studies.[26] The isolated chondrocytes were identified using light microscopy, toluidine blue staining, and type II collagen immunohistochemical staining.

Cartilage tissue and chondrocyte staining

The obtained cartilage tissue samples were sequentially fixed with 4% paraformaldehyde, decalcified in 10% ethylenediaminetetraacetic acid, dehydrated using an alcohol gradient, and finally paraffin-embedded and prepared as sections cut from the paraffin block. Normal and OA cartilage tissue morphology was evaluated using hematoxylin & eosin (HE) staining, toluidine blue staining, saffron solid green staining, and alizarin red staining. The isolated chondrocytes, too, were stained with toluidine blue and alizarin red.

Cell proliferation capacity

The isolated chondrocytes were resuspended and equally inoculated into the wells of 96-well plates and then incubated at 37 °C for the purpose of detecting cell proliferation at 24, 48, 72, 96, and 120 h after inoculation. After changing the culture medium, 10 μL of CCK-8 reagent (7Sea Pharmatech, Shanghai, China) was added to each well and the plate was further incubated for 1 h. Absorbance was then recorded at 450 nm.

Immunohistochemical analysis

OPN and CD44 expression in the cartilage tissue was detected using immunohistochemical staining, as described in our previous report[27]; primary antibodies specific to OPN (ab69498; Abcam, Cambridge, UK) and CD44 (D261338; Sangon Biotech, Shanghai, China) were used. A horseradish peroxidase (HRP)-conjugated secondary antibody (AS064, ABclonal, Woburn, MA, USA) was used for binding the primary antibody, and 3,3′-diaminobenzidine tetrahydrochloride was used for signal detection.

Immunofluorescence staining

Immunofluorescence (IF) staining was performed to detect the specific expression of OPN, CD44, and PI3K in chondrocytes, as previously described.[27–29] Specific primary antibodies were used to detect OPN, CD44, and PI3K (D162051; Sangon Biotech, Shanghai, China), and phalloidin was simultaneously used to stain the cytoskeleton. Cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (Beyotime, Hangzhou, China), and a microscope (TCS SP8, Leica, Wetzlar, Germany) was used to capture images.

Immunoblotting analyses

Western blotting was performed to estimate protein expression, as described previously.[26–28,30,31] The same primary antibody used for IF staining was employed, and a HRP-conjugated secondary antibody (AS064, ABclonal) was used. β-actin was set as an internal control, and signals were visualized using an enhanced chemiluminescence substrate (NCM Biotech, Suzhou, China).

Cell transfection and intervention

An OPN gene silencing model of OA chondrocytes was constructed using a short interfering (si)-OPN plasmid (GenePharma, Shanghai, China). OA chondrocytes were transfected with Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. OPN upregulation was achieved using rhOPN (R&D Systems, Minnesota, MN, USA) at the recommended concentration of 1500 pg/mL. CD44-specific antagonists (LS-C179404, LifeSpan Bio, Seattle, WA, USA) were used to block ligand–receptor reactions between OPN and CD44.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cartilage tissue and cells as reported previously.[28,32,33] SYBR Green PCR Master Mix (Takara Bio, Shiga, Japan) was used to detect mRNA expression, and β-actin was set as an internal control. The sequences of the primers used have been shown in Table 2.

Animals and surgery

Thirty eight-week-old male mice were purchased from SJA Laboratory Animal Co., Ltd. (Hunan, China). An OA model was constructed by destabilizing the medial meniscus as described previously.[23,25] Intra-articular injection of drugs was started 2 weeks after the surgery, and the mice were divided into a normal control group, an OA control group, an OPN antibody group, an rhOPN group, and an rhOPN/LS-

Table 2 Primer sequences used in this study.

Primer	5′-3′ sequence
Actin	F:CACCCAGCACAATGAAGATCAAGAT R:CCAGTTTTTAAATCCTGAGTCAAGC
OPN	F:AGTTTCGCAGACCTGACATCC R:TTCCTGACTATCAATCACATCGG
CD44	F:TGACAACGCAGCAGAGTAATTC R:TTCCACCTGTGACATCATTCCT
PI3K	F:TACACTGTCCTGTGCTGGCTACT R:GAGATTCCCATGCCGTCGTA

C179404 group according to the treatment they received. Dosages of the drugs administered were strictly as per the instructions. After 6 weeks, the mice were sacrificed and knee joint samples were obtained. Paraffin sections were prepared and stained with saffron solid green to evaluate the effects of the drugs.

Statistical analysis

Data were analyzed using GraphPad Prism software (Version 8.0) and expressed as the means ± standard deviations (SDs). Student's t-test was used to assess between-group differences. $p < 0.05$ was considered statistically significant.

Results

Serious wear of OA cartilage and loss of matrix components

The tissue samples revealed a significant difference between OA and normal cartilage. The OA cartilage surface was severely worn off and cartilage defects were visible locally (Fig. 1A). The HE staining results revealed that the OA cartilage surface was rough and had undergone a furrow-like change. There were many flocculent cartilage matrices, and the entire cartilage layer was uneven in thickness and layered. Toluidine blue staining revealed loss of extracellular matrix components such as HA and

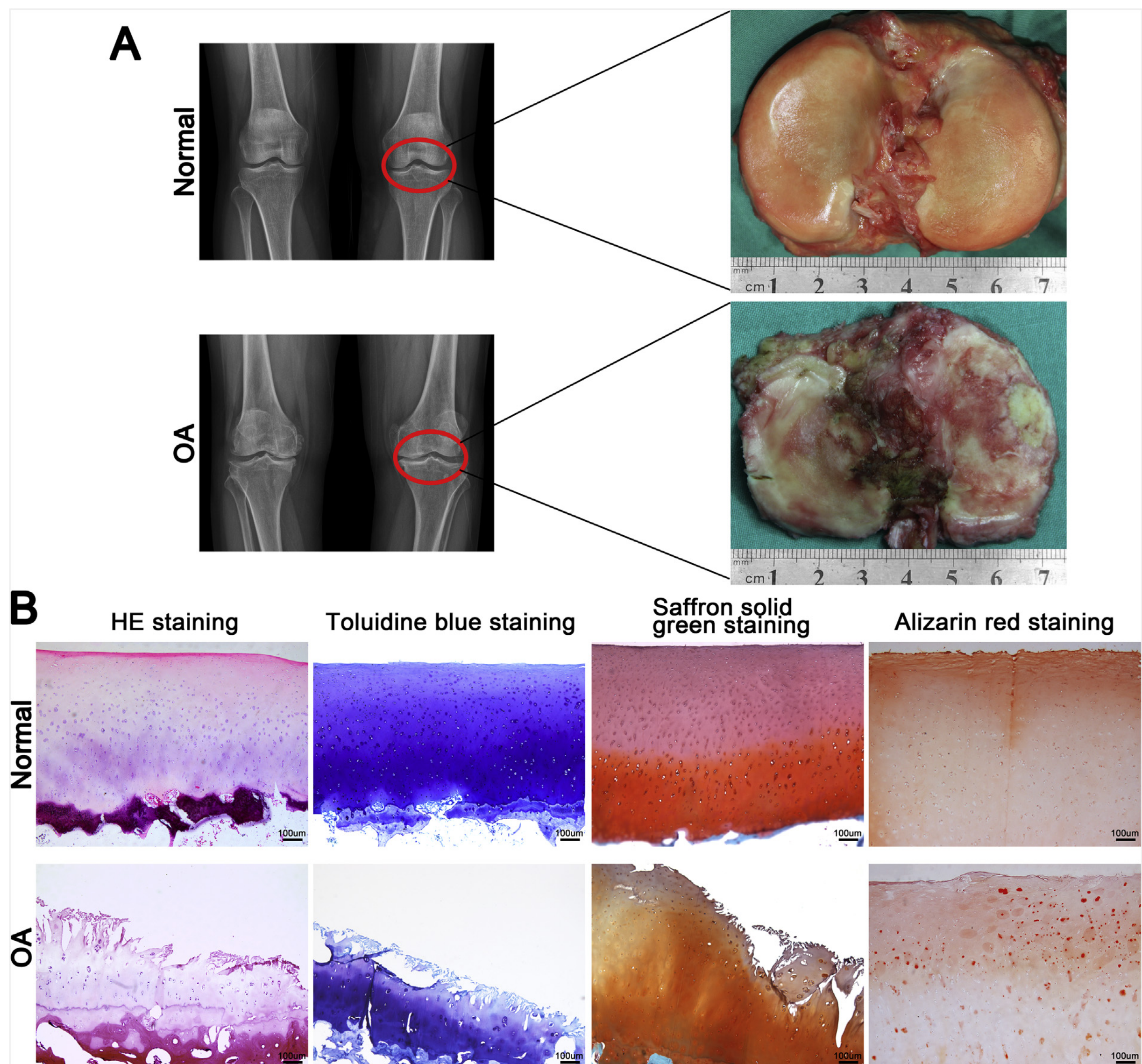

Figure 1 OA cartilage wears more seriously than normal cartilage and matrix components were largely lost. (**A**) The surface of normal articular cartilage appears smooth and flat; the cartilage layer is thick, and the color is white. The surface of OA cartilage appears rough with obvious cracks. The texture of cartilage is slightly hard, and part of articular cartilage layer completely disappears. (**B**) Compared to that for normal cartilage tissue surface, for the OA cartilage surface, HE staining revealed a fluff-like change, toluidine blue staining and saffron solid green staining revealed extracellular matrix component loss, and alizarin red staining showed a considerable calcium salt deposition.

glycosaminoglycan, and saffron and green staining revealed a decrease of lacunae in OA cartilage; scattered calcium deposition was also observed in OA cartilage (Fig. 1B). Although OA chondrocytes isolated *in vitro* are not significantly different from normal chondrocytes in terms of morphology and proliferation ability (Fig. 2A, B), the glycosaminoglycan content in OA chondrocytes was higher than that in normal chondrocytes (Fig. 2A, C) and was more likely to cause calcium deposition (Fig. 2A, D).

OPN, CD44, and PI3K were upregulated in OA cartilage tissue and chondrocytes

Immunohistochemical analysis was performed to detect the expression of OPN and CD44 proteins in cartilage tissue. Compared to that in normal cartilage, both OPN and CD44 were highly expressed in OA cartilage (Fig. 3). This could have been so because damaged cartilage tissue starts to repair itself, resulting in the upregulation of OPN expression and subsequent signaling cascade responses. Furthermore, the distribution and expression of OPN, CD44, and PI3K were detected in chondrocytes using IF staining. OPN was primarily distributed in the cytoplasm and nucleus of chondrocytes (Fig. 4A), and the expression level of OPN was significantly higher in OA chondrocytes than in normal chondrocytes ($P < 0.001$). Likewise, CD44 was primarily distributed in the cell membrane and mediated internal and external signal transmission in cells (Fig. 4B); the expression of CD44 was significantly upregulated in OA chondrocytes compared to that in normal chondrocytes ($P < 0.01$). PI3K, an important signaling molecule, mainly transmits signals on the cell membrane and causes downstream cascading effects. PI3K was largely expressed in the cytoplasm (Fig. 4C), and its expression was significantly upregulated in OA chondrocytes than in normal

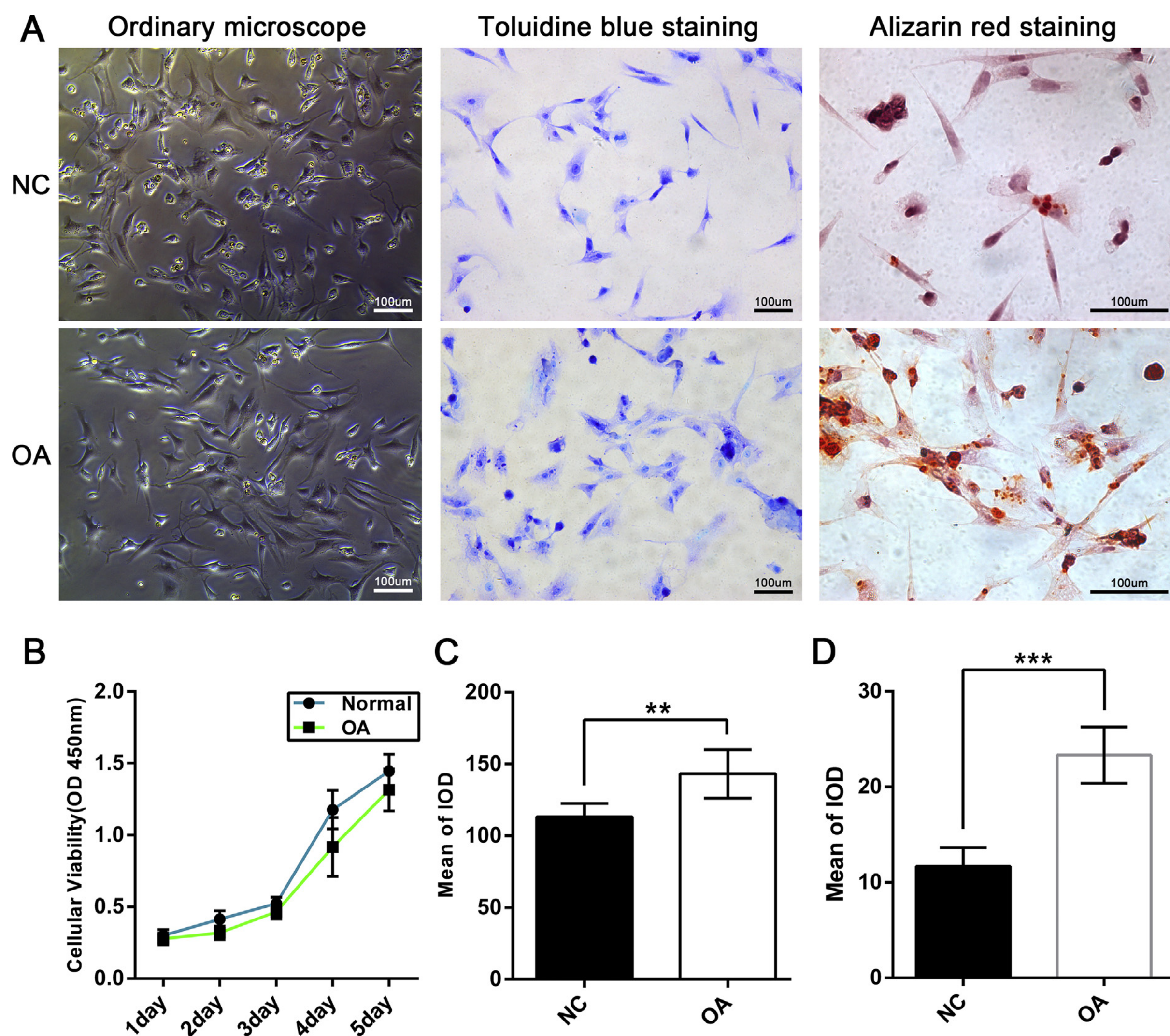

Figure 2 The cell viability of OA chondrocytes was similar to normal chondrocytes but with matrix mineralization. (**A**, **B**) There was no significant difference between the normal and OA chondrocytes in terms of morphology and cell viability. (**A**, **C**) Toluidine blue staining showed that the glycosaminoglycan content was higher in OA chondrocytes than in normal chondrocytes ($P < 0.01$). (**A**, **D**) Alizarin red staining showed that OA chondrocytes were more prone to calcium deposition than normal chondrocytes ($P < 0.001$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

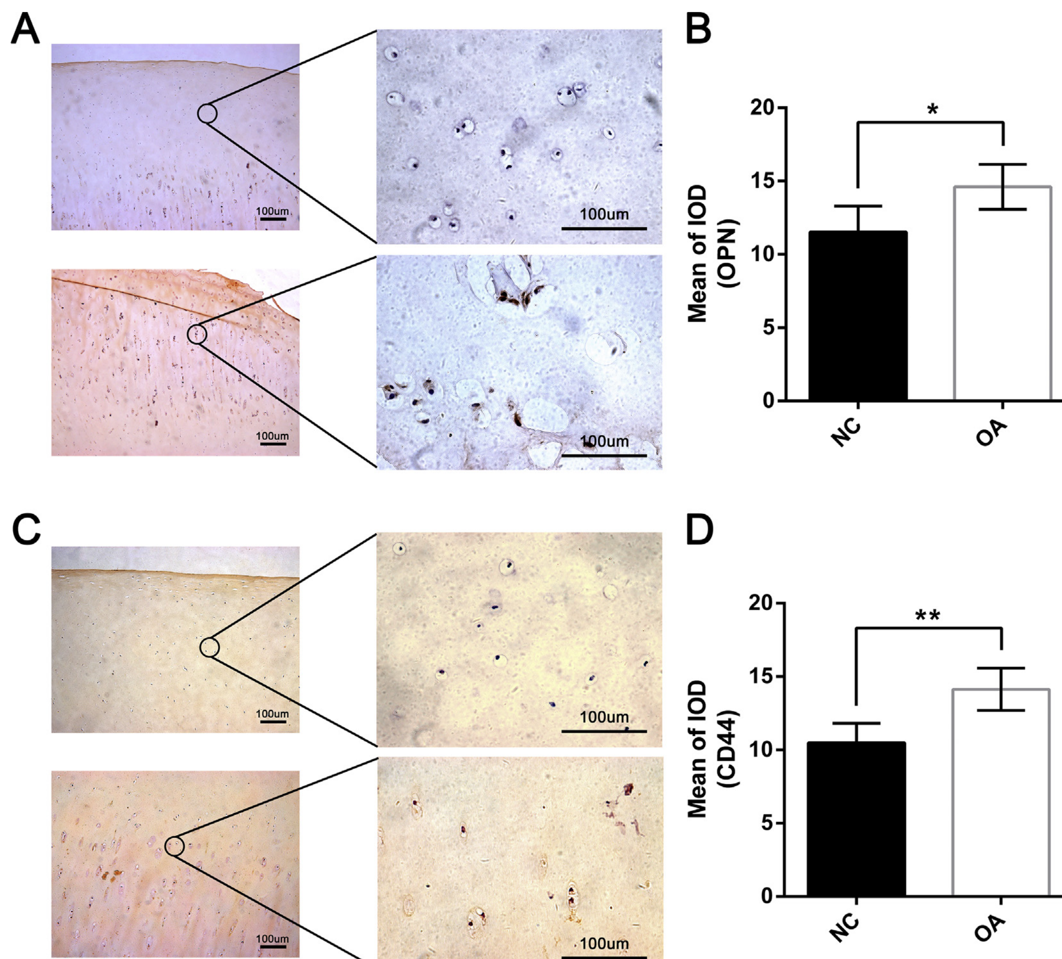

Figure 3 Upregulated expression of OPN and CD44 in OA cartilage. (**A**, **B**) Expression of OPN was significantly higher in OA cartilage than in normal cartilage ($P < 0.05$). (**C**, **D**) Expression of CD44 was significantly higher in OA cartilage than in normal cartilage ($P < 0.01$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

chondrocytes ($P < 0.001$). More importantly, the mRNA and protein expression levels of OPN, CD44, and PI3K were higher in OA chondrocytes than in normal chondrocytes (Fig. 5A–C).

In vitro verification of the OPN/CD44/PI3K signal axis

siRNA2 was found to have the highest silencing efficiency, reaching up to 80% (Fig. 5D, E). Thus, in subsequent analyses, we used siRNA2 to silence the OPN gene. The results showed that the mRNA and protein expression of CD44 in OA chondrocytes was significantly upregulated, whereas that of PI3K was downregulated after siOPN intervention. After rhOPN intervention, the mRNA and protein expression of CD44 and PI3K in chondrocytes was upregulated. When rhOPN and LS-C179404 were simultaneously used to treat chondrocytes, the protein levels of CD44 and PI3K were decreased, whereas the CD44 mRNA level was upregulated (Fig. 5F–H). Thus, downregulation of OPN expression provides feedback to upregulate CD44 expression, and a direct relationship exists between CD44 and PI3K; further, LS-C179404 can directly inhibit PI3K expression.

In vivo verification of the OPN/CD44/PI3K signal axis in mice

Destabilization of the medial meniscus method was used to construct a mouse model of knee OA (Fig. 6A). The results of staining the paraffin section with saffron solid green were consistent with those of the *in vitro* staining. Analysis of the sagittal position revealed an obvious joint space in the normal knee joint; additionally, the cartilage surface was complete and the cartilage layer was thick (Fig. 6B). In the OA control group, although a joint space was present, the cartilage layer was obviously thinner (Fig. 6C). Compared to that in the OA control group, in the rhOPN group the cartilage layer was significantly thicker and the degree of OA was significantly improved (Fig. 6D). However, the degree of OA was severe and the joint space was narrowed or even disappeared, with the cartilage layer completely peeled off, in the OPN antibody and rhOPN plus LS-C179404 intervention groups (Fig. 6E, F). Further

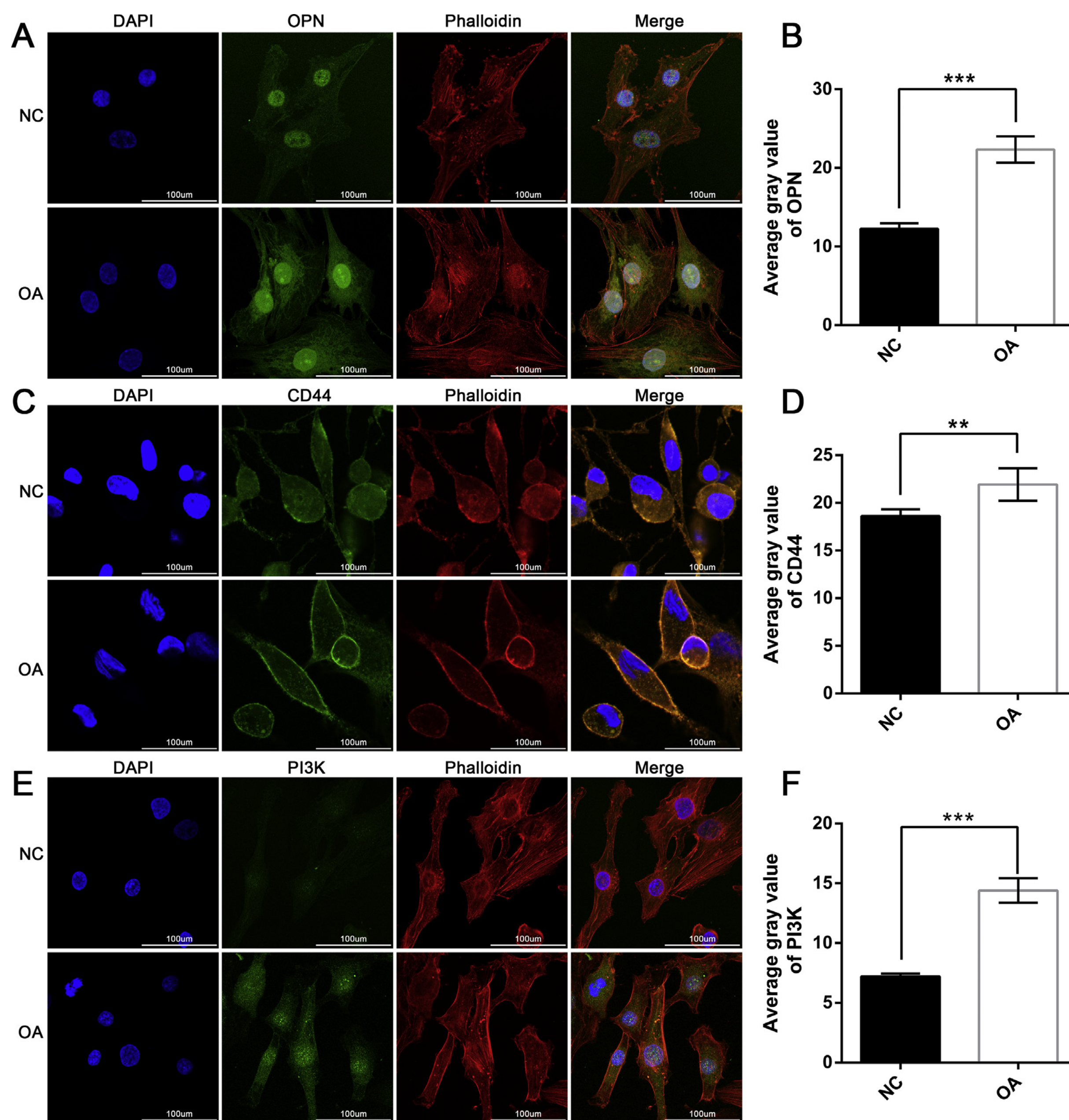

Figure 4 OPN, CD44 and PI3K were upregulated in OA chondrocytes. (**A**, **B**) OPN was primarily distributed in the cytoplasm, and OPN expression was higher in OA chondrocytes than in normal chondrocytes ($P < 0.001$). (**C**, **D**) CD44 was mainly distributed in the cell membrane and scattered in the cytoplasm ($P < 0.01$). Compared to that in normal chondrocytes, CD44 was highly expressed in OA chondrocytes. (**E**, **F**) CD44 was mainly distributed in the cytoplasm and nucleus. Compared to that in normal chondrocytes, CD44 was highly expressed in OA chondrocytes ($P < 0.001$). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

observation of the femoral and patellofemoral joints revealed that OA occurrence is related to the whole knee joint and not just the tibiofemoral joint (Fig. 6). Thus, the cartilage surface integrity was impaired, and cartilage layer thinning and joint space narrowing were the main pathological changes in OA.

Discussion

The present study revealed some important findings: (1) The OA cartilage tissue surface was severely worn off and cartilage matrix component loss was large; further, significant calcium salt deposition was observed. However, excessive cartilage matrix component secretion was noted in OA cartilage cells; (2) OPN, CD44, and PI3K expression in OA chondrocytes was upregulated, and OPN expression directly upregulated CD44 and PI3K expression; (3) OPN could upregulate the expression of downstream PI3K by reacting with CD44 to form receptor ligands, ultimately regulating extracellular matrix component secretion and chondrocyte life cycle; and (4) *In vitro* animal experiments demonstrated the important role of the OPN/CD44/PI3K signal axis in OA and confirmed the protective effect of OPN

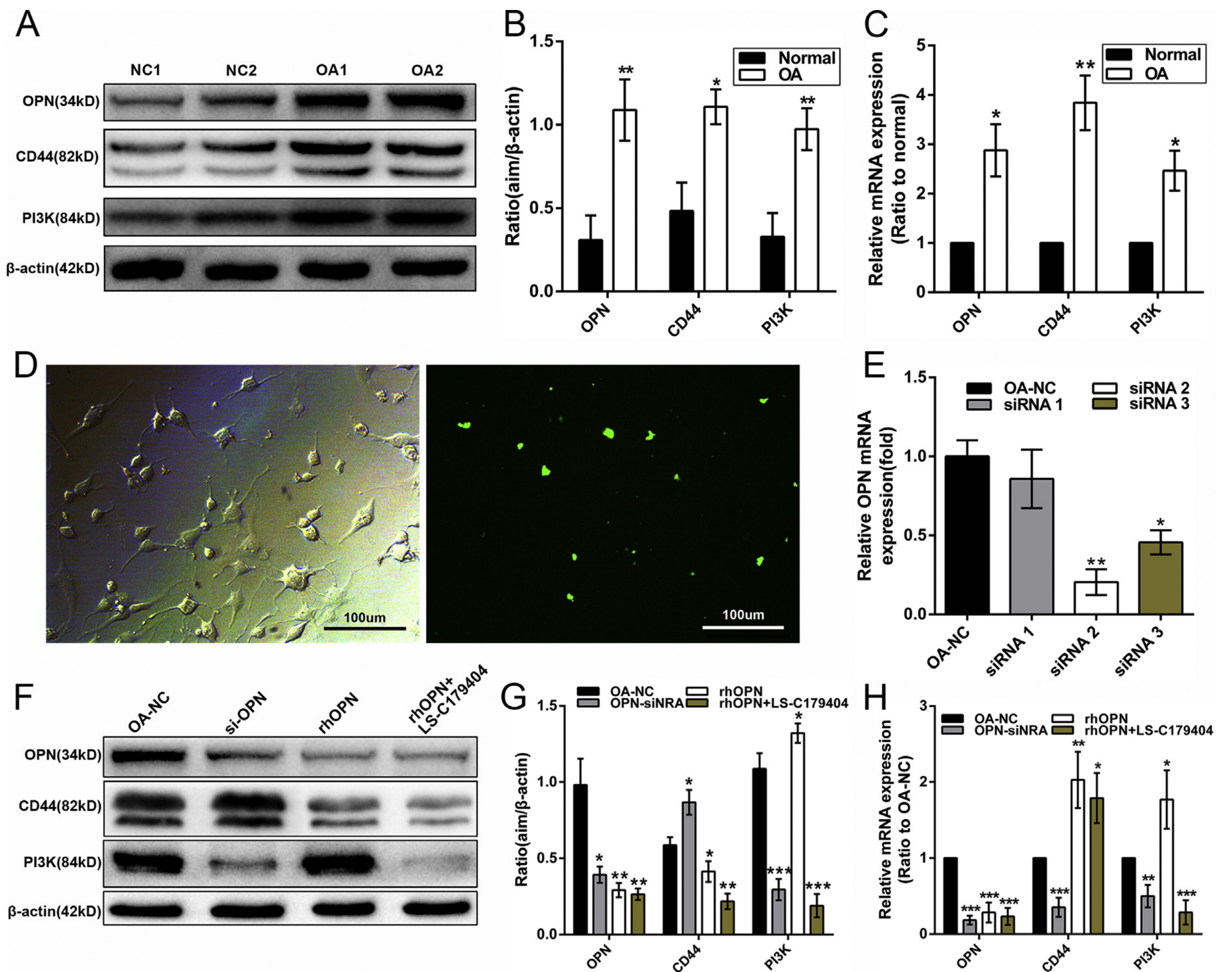

Figure 5 OPN inhibits the progress of OA through the OPN/CD44/PI3K signal axis *in vitro* experiments. (**A**, **B**) Western blot analysis revealed the expression of OPN, CD44, and PI3K in chondrocytes. Quantitative analysis showed that the expression levels were upregulated in OA chondrocytes ($P < 0.05$). (**C**) RT-PCR analysis showed that the mRNA levels of OPN, CD44, and PI3K in chondrocytes were upregulated ($P < 0.05$). (**D**, **E**) Light micrographs and fluorescence images of si-OPN transfection. Silencing efficiency showed that siRNA2 had the highest silencing efficiency ($P < 0.01$). (**F**—**H**) si-OPN, rhOPN, and rhOPN plus LS-C179404 were used to treat the cells, and the protein and mRNA expression levels of OPN, CD44, and PI3K were then detected. Quantitative analysis confirmed direct correlation between the expression of these molecules ($P < 0.05$). *$P < 0.05$, **$P < 0.01$.

and its specific mechanism in OA treatment. The mechanism of OA was predicted according to our results and those of previous studies (Fig. 7). Through *in vitro* and *in vivo* experiments, OPN was found to be capable of partially activating intracellular PI3K by binding CD44 on the surface of chondrocytes, resulting in increased PI3K expression and downstream Akt/mTOR activation, subsequently resulting in extracellular matrix synthesis and inhibiting chondrocyte apoptosis. Additionally, OPN protects against OA via the OPN/CD44/PI3K signal axis by delaying OA progression. These observations provide theoretical support and a research basis for precision medicine in terms of OA treatment.

OPN is a multifunctional extracellular matrix protein and mainly binds members of the integrin receptor family on the cell surface; it plays an important role in mediating cell adhesion and recruitment, cytokine expression, intracellular signal transduction, tumor occurrence and metastasis, tissue mineralization and reconstruction, cellular immune regulation, angiogenesis, etc.[4,12,14,34,35] Chondrocytes, osteoclasts, and osteoblasts can reportedly secrete OPN, and the role of OPN in bone and cartilage tissue has become a research hotspot. Ann[11] reported that OPN can promote calcium pyrophosphate dihydrate deposition in cartilage tissue and aggravate OA progression. Yumoto et al[10] reported that OPN deficiency can protect the knee joints of mice from anti-type II collagen antibody-induced OA. Based on these results, high OPN expression in cartilage tissue can aggravate OA progression. However, OPN appears to play a dual role in OA development. Matsui et al[36] showed that in two models of knee joint OA due to joint destabilization and age-related effects, after knocking out the *OPN* gene, there was a sharp loss in articular cartilage proteoglycan and cartilage matrix network structure remodeling. Attur[37] confirmed that OPN inhibits the release of IL-1, NO, and PGE2 in chondrocytes to inhibit OA progression. OPN was previously found to be related to the progression of knee OA. In the knee cartilage and synovial fluid, OPN expression increases with joint injury aggravation. OPN can also upregulate

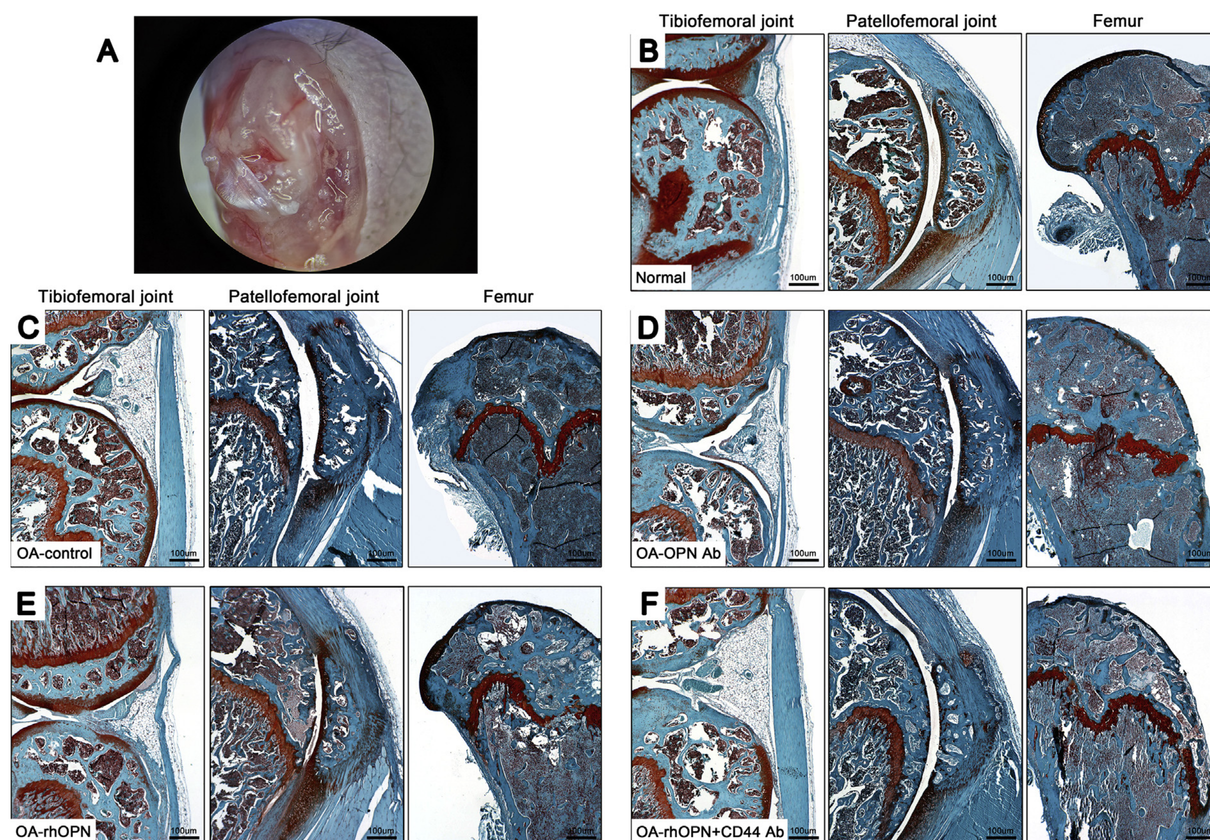

Figure 6 OPN inhibits the progress of OA through the OPN/CD44/PI3K signal axis in mice. (**A**) Construction of a mice knee OA model via destabilization of the medial meniscus. (**B**) Morphology of normal knee joint showed that the cartilage surface was smooth and integrated and that the stroma layer was thick. (**C**) In the OA control group, the cartilage layer was abraded, weak, or even completely absent, and osteosclerosis was obvious. (**D**) In the OPN antibody intervention group, the cartilage was severely abraded, patellofemoral joint space was narrowed, and osteosclerosis was obvious. (**E**) In the rhOPN group, the cartilage surface was uniform, the cartilage matrix layer was thick, and the joint space was slightly narrower, which was obviously superior to that observed in the OA control group. (**F**) In the rhOPN plus LS-C179404 intervention group, severe cartilage wear off, nearly complete absence of the cartilage layer, and obvious hardening of the subchondral bone were observed, which was obviously inferior to that observed in the OA group.

proteoglycan and type II collagen in human knee OA chondrocytes. Thus, OPN protects against OA in the knee joint.[12,14,35,38] In this study, we confirmed that at the protein and mRNA levels, OPN expression was significantly higher in OA chondrocytes than in normal chondrocytes; we also determined the mechanism underlying OPN action by studying the intracellular signaling pathway.

CD44, a single-chain transmembrane glycoprotein with multiple cytokine-binding sites, plays a key role in regulating intracellular and extracellular signaling.[19,20,39] Zhang reported that the CD44 expression level was positively correlated with knee OA severity,[20,40] and Cai et al[41] found that HA hydrogels bind chondrocytes via CD44, thereby inhibiting oligonucleic glycylic acid release and regulating OA progression. In addition, studies have shown that intra-articular injection of HA inhibits synovial neovascularization and fibrosis and maintains articular cartilage integrity via a CD44-dependent mechanism.[42] These results confirm that OPN and CD44 have an intrinsic relationship with OA; however, the specific mechanism remains unclear. In this study, OPN-siRNA and rhOPN were used to treat OA chondrocytes and the correlation between OPN and CD44 was verified at the protein and mRNA levels. The results showed that the expression level of CD44 was upregulated by OPN; further, the mechanism of OPN and CD44 in knee OA cartilage degeneration involved a ligand—receptor reaction. However, it remains unclear which downstream signal pathway regulates cartilage metabolism and affects OA progression.

PI3K is an intracellular phosphatidylinositol kinase involved in cell apoptosis, proliferation, and differentiation as well as glucose transport. Increased PI3K activity is closely related to cell proliferation and apoptosis; as a classical intracellular pathway, the PI3K/Akt/mTOR signaling pathway plays an important role in cell apoptosis and tumor development.[43—47] Numerous studies have shown that the PI3K/Akt/mTOR signaling pathway plays an important role in OA. Chen[24] reported that PI3K/Akt/mTOR signaling can selectively inhibit MMP-13 production to prevent cartilage degradation, thereby protecting against knee OA. Based on previous reports, Huang[48] reported that estradiol (E2) promoted chondrocyte proliferation in an OA model of rats via the PI3K/Akt/mTOR signaling pathway and that blocking this pathway can eliminate the proliferation

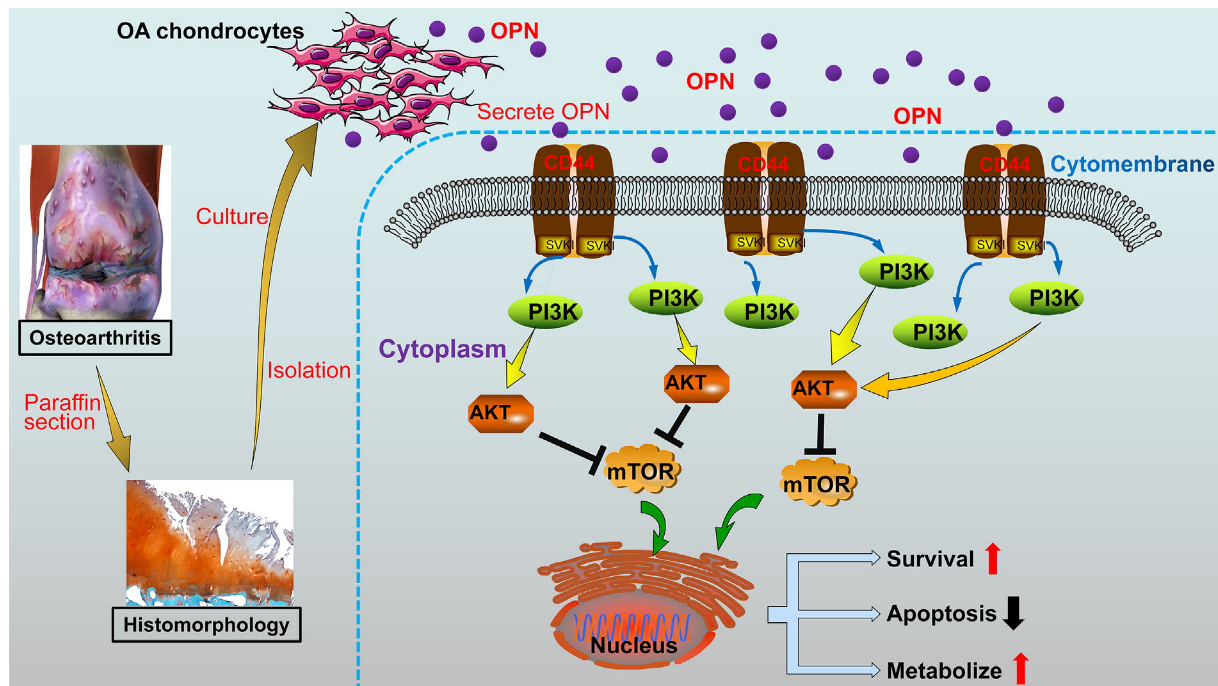

Figure 7 Mechanism of OPN inhibiting OA progression through OPN/CD44/PI3K signal axis.

of OA chondrocytes. In the present study, we found that PI3K expression was higher in OA chondrocytes than in normal chondrocytes. Further, OPN—siRNA, rhOPN, and LS-C179404 were used to treat OA chondrocytes. The expression level of PI3K in OA chondrocytes was positively correlated with the mRNA and protein expression level of CD44, and the mRNA and protein expression level of CD44 and PI3K were directly related in OA chondrocytes. The present study also revealed the mechanism underlying the role of the OPN/CD44/PI3K signal axis in OA. Because this experiment was based on previous studies, the PI3K/Akt/mTOR signaling pathway in chondrocytes was not reverified, and this is a limitation of the present study. Therefore, in future studies, we will determine the exact effect of OPN and CD44 on the PI3K/Akt/mTOR signaling pathway in OA to provide theoretical support and a research basis for precision medicine in terms of OA treatment.

Conclusions

In conclusion, we verified that OPN is highly expressed in OA chondrocytes and can promote extracellular matrix component synthesis and inhibit chondrocyte apoptosis by activating the PI3K/Akt/mTOR signaling pathway via its interaction with CD44, thereby regulating the progression of knee OA.

Ethical review committee statement

This study has been approved by the Animal Research Center of Central South University and the Ethics Committee of Xiangya Hospital.

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Conflict of interests

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

CRediT authorship contribution statement

Qing Liu: Conceptualization, Formal analysis, Writing - original draft. **Hao Zeng:** Conceptualization, Formal analysis. **Yuhao Yuan:** Formal analysis, Writing - review & editing. **Zhiwei Wang:** Formal analysis, Writing - review & editing. **Ziyi Wu:** Writing - review & editing. **Wei Luo:** Conceptualization, Formal analysis, Writing - original draft, Writing - review & editing.

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Abbreviations

OA Osteoarthritis
OPN Osteopontin

HA Hyaluronic acid
PI3K Phosphatidylinositol-3-kinase
HE Hematoxylin & eosin
IF Immunofluorescence
MMP-9 Matrix metallopeptidase 9

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