Role of macrophage polarization in osteoarthritis (Review)

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Abstract. Osteoarthritis (OA) is a disease involving the whole joint that seriously reduces the living standards of individuals. Traditional treatments include physical therapy, administration of anti-inflammatory and analgesic drugs and injection of glucocorticoids or hyaluronic acid into the joints. However, these methods have limited efficacy and it is difficult to reverse the progression of OA, therefore it is urgent to find new effective treatment methods. Immune microenvironment is significant in the occurrence and development of OA. Recent studies have shown that macrophages are important targets for the treatment of OA. Macrophages are polarized into M1 pro-inflammatory phenotype and M2 anti-inflammatory phenotype under stimulation of different factors, which release and regulate inflammatory response and cartilage growth. Accumulating studies have tried to alleviate OA by regulating macrophage homeostasis. The present study summarized the related studies, discuss the mechanism of various therapeutic reagents on OA, expound the molecular mechanism of drug effect on OA and attempted to provide clues for the treatment of OA.

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Abbreviations: ACLT, transection of the anterior cruciate ligament; ADAMTS, a disintegrin-like and metalloproteinase with thrombospondin motifs; ANG, angelicin; Arg-1, arginine-1; CCL, CC motif chemokine ligand; COX-2, cyclooxygenase-2; DMM, destabilization of the medial meniscus; ECM, extracellular matrix; EUP, Eucommia ulmoides polysaccharide; Evs, extracellular vesicles; GLP-1, glucagon like peptide-1; hASCs, human adipose stem cells; hUC-MSC, human umbilical cord mesenchymal stem cells; LPS, lipopolysaccharide; MFG-E8, milk fat globule epidermal growth factor 8; MIA, monosodium iodoacetate; MMP, metalloproteinase; MSCs, mesenchymal stem cells; OA, osteoarthritis; PAB, pseudolauric acid B; PTOA, posttraumatic osteoarthritis; ROS, reactive oxygen species; tBHQ, tert-butylhydroquinone; TRPV1, Transient receptor potential vanillin 1

Key words: macrophages, polarization, osteoarthritis, treatment, cartilage

Contents

1. Introduction

Osteoarthritis (OA) is a joint disease with a high prevalence and serious consequences. It leads to pain and decline in the quality of life of affected individuals (1,2). OA is characterized by synovitis, osteophyte formation and degeneration of joint-related tissues (such as articular cartilage), which may eventually result in changes in bone structure and disability of patients (3). Traditional treatments include physical therapy, anti-inflammatory and analgesic drugs and injecting glucocorticoid or hyaluronic acid into the joint (4-6). However, at present, most OA therapies have limited efficacy in symptom relief. There are still no drugs that can reverse the pathological process of OA (7). Although there is continuing research to reveal the mechanism of OA, more effective treatments are required. For the present review, the following terms were searched using the PubMed database (https://pubmed.ncbi.nlm.nih.gov/): 'osteoarthritis', 'macrophage polarization', 'treatment' and 'cartilage damage'; only papers published in English were assessed.

In OA, immune cells secrete inflammatory factors or mediators affecting cartilage structure through intercellular association to participate in the pathological process. Neutrophils promote OA by secreting proinflammatory mediators and producing enzymes that degrade cartilage. Dendritic cells activate T helper (Th)1 and Th17 cells, which produce pro-inflammatory cytokines, resulting in cartilage degradation, while Th2 cells secrete anti-inflammatory factors to protect cartilage. In addition, Th2 cells can activate B cells to secrete proinflammatory factors and antibodies to inhibit cartilage repair. Treg cells have beneficial effects on OA. They do not directly participate in cartilage repair,

but act on neutrophils to secrete anti-inflammatory factors. Natural killer cells are closely related to the differentiation of mesenchymal stem cells (MSCs) and osteoclasts. However, the largest number of immune cell types in OA synovium is macrophages (8). Macrophages, as an important part of the immune system, have long been considered to be an crucial participant in OA (9).

Macrophages, derived from hematopoietic stem cells, are immune cells that serve a key role in the immune response (10). Macrophages are activated by environmental factors such as TNF-α, IFN-γ and lipopolysaccharide (LPS) (8). When activated, they can absorb innate antigens or invading pathogens, express costimulatory molecules and secrete more inflammatory cytokines, which are essential for immune response and cartilage reconstruction (11). Macrophages are mainly polarized into M1 and M2 phenotypes (8). After being stimulated by IFN-γ, M0 macrophages are polarized into M1 macrophages, thereby expressing pro-inflammatory cytokines and participating in the process of pro-inflammation and degradation of extracellular matrix (ECM) (9). When M0 macrophages are stimulated by IL-4/IL-13, they polarized into M2 macrophages and express anti-inflammatory cytokines and thus exert anti-inflammatory and chondrogenic effects (12). Macrophage polarization participates in a number of pathological processes, including metabolic changes, virus infection, inflammatory environment and tumor immunity (8,9,11). Recent research has shown that it is beneficial to slow the progress of OA by regulating the changes of macrophage phenotype (12). This suggests that macrophage polarization is an emerging target to treat OA. The present study collected studies on the regulation of macrophage polarization and OA progress in order to elaborate its mechanism and provide clues for the subsequent development of treatment methods based on the regulation of macrophage phenotype.

2. Development of OA

OA may be caused by factors such as heredity and mechanical load (13-15). OA can influence the entire joint, including cartilage, synovial tissue, subchondral bone, joint capsule, ligaments and periarticular muscles (16). However, cartilage degeneration may be the most important pathological process of OA and accumulating researches focus on preventing its degeneration (3). The inflammatory environment generated by cartilage injury will cause chondrocyte apoptosis and hypertrophy, ECM decomposition, eventually leading to cartilage injury and aggravating OA (17). Cartilage covers the end of bone and is a type of hyaline cartilage without nerves and blood vessels (18). Typically, it consists of ECM and chondrocytes (19). The ECM consists of matrix, collagen type 1A (col1A) and collagen type 2A1 (col12A1) (20). Proteoglycan is the main component of matrix and participates in chondrocyte synthesis and degradation (12). The cascade effect is amplified by the production of MMP or a disintegrin-like and metalloproteinase with thrombospondin motifs (ADAMTS) by chondrocytes, synovium fibroblasts and macrophages when mechanical loading on cartilage causes damage (8). The ECM degradation enzyme ADAMTS5 is a proteoglycan enzyme of the ADAMTS family (21), whose main function is to clear large proteoglycans such as aggrecan, leading to the destruction of cartilage and OA (22). MMPs, especially collagenase MMP1 and MMP13, are believed to cut collagen type II, the main structural component of cartilage, leading to irreversible loss of ECM structure and function (23). Therefore, the regulation of cartilage homeostasis is particularly important for the development of OA.

Existing studies have shown that the imbalance of ECM composition synthesis and degradation is an important marker of exacerbating OA (8,12,23,24). However, the mechanism of imbalance between cartilage damage and repair remains to be elucidated. Trauma can lead to bone and joint friction or increased enzyme activity, followed by the formation of 'wear' particles that are then eaten by resident macrophages (8). However, once the immune system is unable to eliminate these 'wear' particles completely, they act as mediators of inflammation, inducing chondrocytes to release degrading enzymes (22). Synovial macrophages phagocytose molecules produced by collagen or proteoglycan breakdown and release pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 (23). These cytokines can act on chondrocytes to further release degradation enzymes and prevent the generation of type II collagen, thus further increasing cartilage degradation (25). In conclusion, the mechanism of cartilage destruction aggravating OA may be: i) The increased production of degrading enzymes leads to decreased synthesis or increased decomposition of collagen fibers or ii) the apoptosis and senescence of chondrocytes. The morphologic change of articular cartilage goes through several processes: At first, fibrotic areas and cracks appear on the surface of the cartilage, leading to cartilaginous softening and cartilage thickness reduction (24). When the articular cartilage is completely destroyed, the underlying subchondral bone plate is completely exposed (25). The longer OA lasts, the more pronounced these changes become.

There are now a variety of induction methods to damage joints in animals and build models to simulate OA (26-28). One type of model is to simulate OA by surgical instability of joint structure and joint injury. These include anterior cruciate ligament (ACLT) transection or medial meniscus (DMM) instability, which may result in changes in the mechanical load of the joint (26). In addition, a closed non-invasive injury model has been established, which can simulate OA injury after mechanical injury regardless of whether there is ACL fracture or not (27). Another model triggers joint damage by injecting drugs into the joint that disrupt cartilage homeostasis. Intra-articular injection of monosodium iodoacetate (MIA) inhibits glycolysis of chondrocytes, which leads to chondrocyte death and induces inflammation, thus simulating OA (26). Occasionally collagenase is injected into the joint, which will destroy the stability of the joint and cause inflammation, leading to OA-like joint injury (26).

3. Macrophages and OA

OA is characterized by the infiltration and invasion of macrophages, the release of a great quantity of pro-inflammatory and pro-catabolic mediators in the articular cavity and the increase of synovial vascular distribution. These mediators are recognized by synovial immune cells and activate the immune cells to release more cytokines, resulting in chronic and repeated inflammation. In short, these fragments released into the

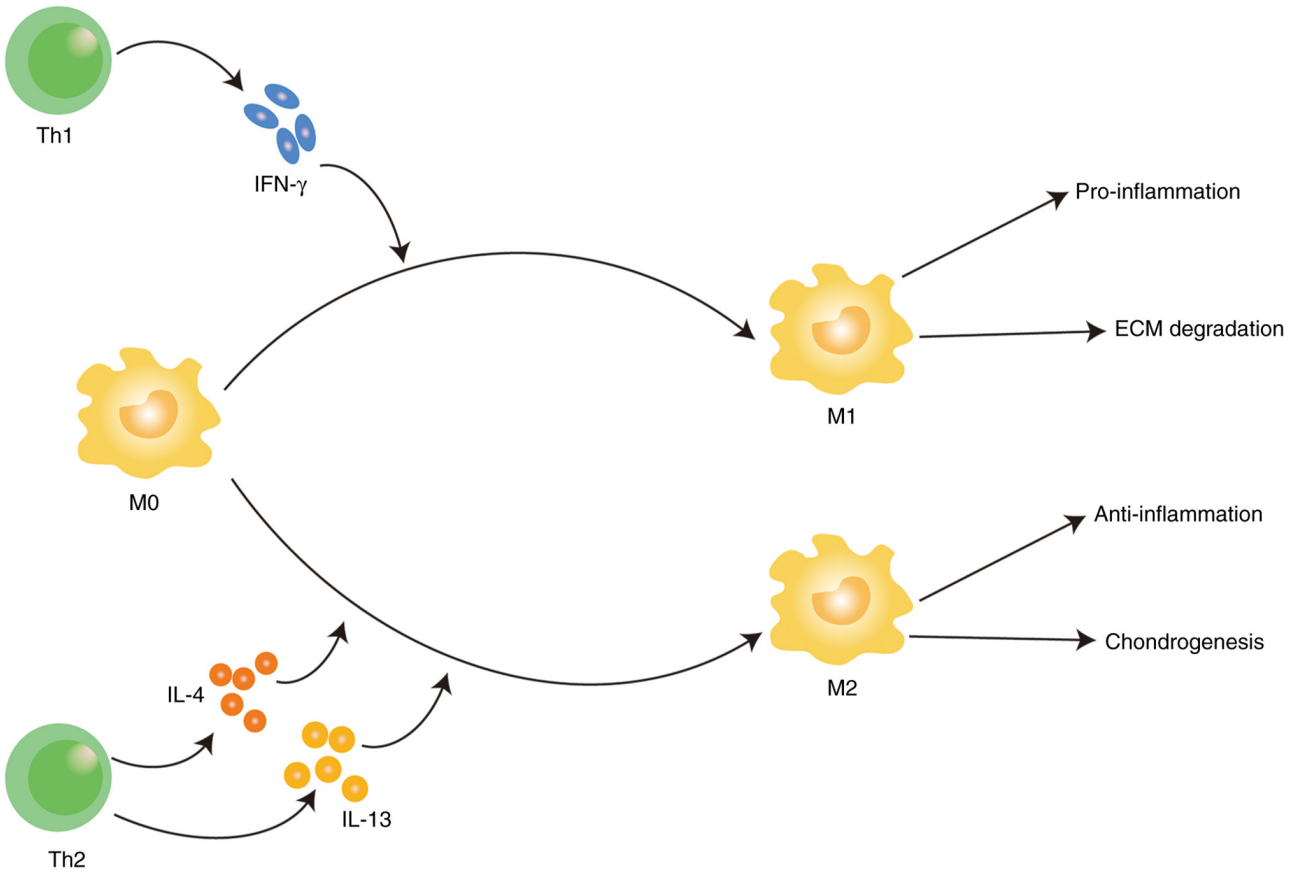

Figure 1. Macrophage polarization regulates OA. OA, OA, osteoarthritis; Th, T helper cell; ECM, extracellular matrix.

synovial cavity stimulate macrophages to produce and release inflammatory mediators (such as cytokines, chemokines, lipid mediators and death-associated molecular patterns itself) into the synovial fluid (29). In turn, these mediators activate chondrocytes that produce metalloproteinases, leading to a vicious cycle between cartilage and synovium (30). The pathological process is generally described as mechanical stress directly damages cartilage or activates chondrocytes, producing abnormal levels of degradation enzymes and reactive oxygen species (ROS), leading to cartilage destruction. Subsequently, microcrystals, osteochondral fragments and ECM degradation products are released from the articular cavity. These molecules and products trigger inflammatory macrophages to secrete chemokines, cytokines, MMP, ROS and lipid mediators. They can directly degrade ECM components or imbalance of chondrocyte homeostasis, leading to imbalance of cartilage matrix degradation and synthesis, thereby aggravating OA.

Macrophages can regulate the local microenvironment under physiological and pathological conditions and respond quickly to various stimuli (31). After stimulation, macrophages are mainly activated into two phenotypes: M1 and M2. Macrophages is polarized to M1 type by IFN-γ released by Th1 cell and has high expression of proinflammatory cytokines, including TNF-α, IL-1β, IL-6, IL-12, IL-23 and cyclooxygenase-2 (COX-2), therefore, have strong pro-inflammatory and pro-tumor functions (32). M2 macrophages are polarized by the STAT pathway activated by Th2 cytokines such as IL4/IL-13 through interleukin receptor, which up regulates arginine-1(Arg-1), CC motif chemokine ligand (CCL) 17 and CCL2, so it has anti-inflammatory effect (33) (Fig. 1). Th1 cells release IFN-γ to polarize M0 macrophages into M1 macrophages, resulting in pro-inflammation and ECM degradation; Th2 cells release IL-4 and IL-13 polarizes M0 macrophages into M2 macrophages, resulting in anti-inflammation and chondrogenesis.

Therefore, macrophages are not only essential immune cells, but also attractive therapeutic targets. In-depth understanding of the molecular characteristics related to the dynamic changes of macrophage polarization and their pathways is crucial to elucidate the molecular basis of disease progression and design of new macrophage therapy strategies (34). Identifying precise targets that control the transition between given steady states is a huge challenge.

4. Macrophages are emerging targets for OA treatment

OA is an immune disease caused by dysfunction of immune microenvironment. Reconstructing the balance of immune microenvironment by regulating the polarization of macrophages is an effective measure to treat OA. The factors affecting OA development include chemical components and biomolecules. Increasing studies have shed light on regulating inflammation by reversing macrophage polarization and progress has been made. The following is a review of the regulation of macrophages on OA, providing clues for a deeper understanding of the regulation mechanism of macrophages in OA (Table I).

Table I. OA treatment strategies based on macrophage.

Type	Name	OA sample; cells	OA sample; animal
Chemical compound			
	Liraglutide	Mouse primary chondrocytes, RAW 264.7 cells	MIA-induced OA mouse model
	Tert-butylhydroquinone	Mouse chondrocytes and synovial macrophages	DMM-induced OA mouse model
	Angelicin	Mouse bone marrow macrophage	DMM-induced OA mouse model
	Fargesin	Mouse primary chondrocytes, RAW 264.7 cells	OA mouse model induced by injection of collagenase
	Pseudolaric acid B	RAW 264.7 cells, bone marrow derived macrophages (BMDM)	DMM-induced OA mouse model
	Eucommia ulmoides Polysaccharides	RAW 264.7 cells	Anterior cruciate ligament transection rabbit model
Cell			
	Human adipose stem cells	Primary human adipose stem cells, RAW264.7 cells	MIA-induced OA mouse model
	Human umbilical cord mesenchymal stem cells	3rd-5th generation human umbilical cord mesenchymal stem cells, CP-R092 cells, mouse synovial macrophages	Anterior cruciate ligament transection mouse model
	Human umbilical cord mesenchymal stem cells	Primary human umbilical cord mesenchymal stem cells, mouse derived macrophages and rat derived chondrocytes	Anterior cruciate ligament transection mouse model
	Transient receptor potential vanillin 1	RAW 264.7 cells	MIA-induced OA rat model
Protein			
	Milk fat globule epidermal growth factor 8 8	Mouse primary chondrocytes, RAW 264.7 cells	MIA-induced OA mouse model
	E3 ubiquitin ligase itch protein	Mouse bone marrow macrophage	MIA-induced OA mouse model

OA, osteoarthritis; MIA, monosodium iodoacetate; DMM, destabilization of the medial meniscus.

Compounds

Liraglutide. Glucagon like peptide-1 (GLP-1) is secreted by intestinal L cells and is a gastrointestinal hormone that processes food intake. Insulin has a series of extrapancreatic functions associated with anti-inflammatory properties (35). The GLP-1 analogue liraglutide is a human GLP-1 applied in the patients with diabetes (36). New data suggest that liraglutide inhibits ROS generation, pro-inflammatory cytokine secretion (37).

In vitro, when liraglutide is added to LPS-stimulated RAW264.7 cells, M1 macrophage phenotype reverts to M2 phenotype (38). Liraglutide significantly reduced the expression of MMP and ADAMTS and made chondrocytes anti-catabolic. From a cellular perspective, the decrease of proinflammatory factors in macrophages and chondrocytes is dose-dependent (38). *In vivo* results show that in the MIA-induced model, liraglutide injection significantly reduced the secretion of IL-6, nitric oxide and prostaglandin E2 and improved the synovitis severity score in mice (38).

Liraglutide has been applied in clinical trials. A randomized controlled trial applied liraglutide in patients with knee arthritis, but it did not reduce knee pain compared with placebo (39). More clinical experiments are needed to verify the anti-inflammatory effect of liraglutide. In conclusion, liraglutide may improve OA by changing macrophage polarization and reducing cartilage degradation.

Tert-butylhydroquinone. Tert-butylhydroquinone (tBHQ) is a compound with strong antioxidant activity (40). There is increasing evidence that tBHQ may be used to treat diseases related to oxidative stress (41).

In vitro, tBHQ significantly reduces the expression of phosphorylated (p-)STAT1 and p-STAT3 induced by LPS and effectively inhibits the M1 polarization of synovial macrophages. From a signaling pathway perspective, tBHQ inhibited the production of ROS through MAPK and NF-κB signaling pathways, which can alleviate OA (42). In addition, IL-1β can induce chondrocyte apoptosis, promote inflammation and inhibit differentiation. tBHQ treatment prevents this effect of IL-1β (42). In DMM-induced mouse model, tBHQ significantly reduces the expression levels of IL-6, TNF-α and IL-1β and inhibits the development of OA in mice (42). In brief, tBHQ may be used for treating OA.

Angelicin. In China, *Angelica sinensis* is a commonly used herbal medicine to treat a variety of ailments. It is reported that *Angelica sinensis* extract has antioxidant and anti-OA effects (43,44). Angelicin (ANG) is an active ingredient isolated from *Angelica sinensis*, which has the functions of anticoagulation, analgesia and hemostasis. Therefore, ANG is considered to have a strong immunomodulatory effect (45).

ANG significantly inhibits the transformation of macrophages from M0 to M1 and the repolarization from M2 to M1 is also significantly inhibited (46). From the perspective of signaling pathway, ANG regulated macrophage polarization by activating the STAT pathway to reduce secretion of pro-inflammatory factors. In *in vivo* experiments, ANG effectively inhibits LPS-induced M1 polarization (46). The shape of cartilage in the ANG treatment group was similar to that of healthy cartilage, with smooth cartilage surface and complete cartilage matrix. Although experimental data indicate that ANG is effective in alleviating OA, more studies are needed to expand the applicability of this study. Overall, these results suggest that ANG has certain curative effects on OA.

Fargesin. Fargesin is a type of lignan with pharmacological activity and is extracted from *Flos magnoliae* (FM), which has long been used in the treatment of emphysema, sinusitis and headaches. It is reported that FM extracts have significant anti-allergic and anti-inflammatory effects (47). Recent studies have found that fargesin is critical for inflammatory response and glucose metabolism (48).

In terms of signaling pathways, fargesin promotes the polarization of macrophages into M2 through p38/ERK, P65/NF-κB pathways. In addition, anti-inflammatory factors in synovium are increased after fargesin treatment (49). Following fargesin treatment, the markers of cartilage catabolism (MMP13, collagen type X and Runt-related transcription factor 2) decrease and the markers of cartilage formation (col2a1 and Sox9) increase. This is through the mechanism of paracrine macrophages. *In vivo*, fargesin treatment alleviates synovitis and cartilage damage in CIOA-induced mice (49). Therefore, in-depth study of the signaling pathways regulating macrophage polarization and reprogramming or preventing macrophages from para-secreting cytokines to chondrocytes may be one direction for alleviation of OA.

Pseudolauric acid B. Pseudolaric acid B (PAB) is extracted from the dried cortex of the roots of *Pseudolarix kaempferi* Gord. It has a number of properties, such as antifungal, anti-angiogenesis and anti-inflammation (50). PAB may inhibit angiogenesis by promoting proteasome-mediated degradation (51).

In vitro experiments show that PAB inhibits the degradation of IκBα by inhibiting the phosphorylation of P65. At the same time, PAB inhibits NF-κB signaling by stabilizing peroxisome proliferator activated receptor γ to prevent M1 polarization and angiogenesis, thereby further reducing synovitis and OA progression (51). *In vivo*, mice treated with PAB show significantly reduced cartilage destruction and improved synovitis (52). These results expand the potential clinical application of PAB and strengthen the possibility that early targeting of synovitis or inhibiting angiogenesis may prevent OA (52).

Eucommia ulmoides polysaccharide (EUP). EU is a traditional herbal medicine that has been used in China for thousands of years (53). In recent years, a polysaccharide extracted from EU, as a drug component, has been studied for its immunomodulatory effect (53). It is reported that EUP has anti-inflammatory and anti-hypertensive effects. In terms of osteogenesis, it inhibits osteoclast and promotes osteogenesis (54-56).

EUP inhibits the expression of cytokines IL-6, IL-1β and IL-18, which inhibit chondrocyte apoptosis. In the extracellular matrix, cytokines activate MMPs to cut collagen fibers, so as to alleviate the progress of OA. In addition, the expression of osteogenesis and cartilage regeneration related genes such as BMP-6, TGF-β and Arg-1 increase under the treatment of EUP (57). At the same time, EUP treatment significantly decreases the expression of M1 macrophage markers (F4/80 and inducible nitric oxide synthase), while a M2 macrophage marker (CD206) increases (57). Micro-CT of living rabbit model showed that cartilage regeneration and subchondral bone reconstruction were evident following EUP treatment.

A retrospective study combined meloxicam with EUP in patients with OA showed significant improvement in pain, stiffness and dysfunction (58). Therefore, it can be considered that EUP alleviates the progress of OA and has the potential to treat OA.

Cells

Human adipose stem cells (hASCs). hASCs are differentiated from mesenchymal stem cells (MSCs), which can secrete growth factors, chemokines and cytokines or other substances to improve the surrounding microenvironment and promote the growth of surrounding cells (59). hASCs secrete extracellular vesicles (EVs) to convey information between cells. EVs as carriers carry protein and other signal molecules, serving a role in targeting cells, such as chondrocytes and monocytes (60). EVs can improve OA by protecting chondrocytes from apoptosis and stimulating macrophages to polarize into the anti-inflammatory phenotype. Therefore, hASCs have been applied in regulating cell growth, regulating inflammatory response and repairing tissue defects (60,61).

In vitro, LPS was added to RAW264.7 cells to induce inflammation. The expression of proinflammatory cytokines (COX-2, IL-1, IL-6 and TNF-α) decreased significantly following hASCs-EVs treatment. In addition, hASCs-EVs promoted the transformation of macrophage to M2 subtype (62). In a mouse OA model, the number of M1 macrophages in OA synovium decrease and the expression of proinflammatory IL-1β in synovium and cartilage is also inhibited following intra-articular injection of EVs (62). In addition, hASCs-EVs treatment can regulate immune reactivity and effectively prevent proteoglycan degradation. It also reduces cartilage damage and slowed the progression of OA (62). In the DMM model, hASCs-EVs reduces the number of MMP-13 positive cells and reduces cartilage damage (62). Although this previous article shows that hASCs-EVs are helpful in reducing cartilage damage and OA, it is necessary to optimize the injection dose and study the mechanism in the future. Chen *et al* (63) studied the injection of ADSCs into the joint to treat knee OA and it effectively reduced pain scores and improved functional scores. In conclusion, hASCs-EVs should be considered as a potential treatment for OA.

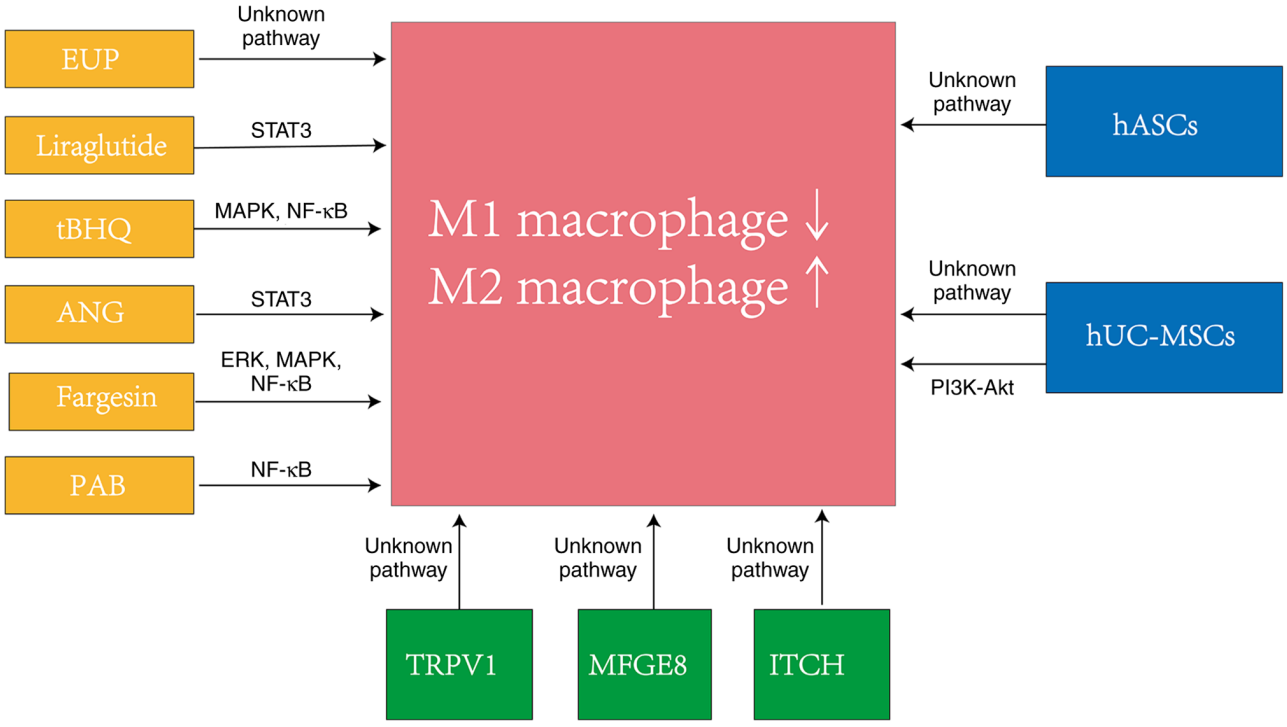

Figure 2. Substances and pathways that regulate the polarization of macrophages. EUP tBHQ, tert-butylhydroquinone; ANG, angelicin; PAB, pseudolauric acid B; hASCs, human adipose stem cells; hUC-MSC, human umbilical cord mesenchymal stem cells; TRPV1, Transient receptor potential vanillin 1; MFG-E8, milk fat globule epidermal growth factor 8.

Human umbilical cord mesenchymal stem cells (hUC-MSCs). There are two studies that reveal the effect of EVs secreted by hUC-MSCs on OA. The first study shows that hUC-MSC-EVs regulated OA *in vitro* mainly through the following: i) Releasing EVs to inhibit the infiltration of synovial M1 macrophages; ii) reducing the expression of CD14 and IL-1β, and iii) increasing the expression of CD206 and IL-10. *In vivo* experimental data show that in a rat model, hUC-MSC-EVs treatment can reverse the cartilage rupture and subchondral bone thickening (64).

The second study shows that hUC-MSC-EVs may transmit target proteins and microRNA through PI3K/Akt signaling pathway, so as to achieve the repolarization of M2 macrophages. In addition, the paracrine secretion of polarized M2 macrophages induced by hUC-MSC-EVs decreases the expression of MMP and apoptosis of chondrocytes. An *in vivo* experimental study reported that hUC-MSC-EVs can improve the progression of OA caused by ACLT (65).

Although hUC-MSCs have been proved to be effective in alleviating OA, there are still a number of problems with sEVs. The pain caused by repeated injections and the optimal dosage of injections are all difficult issues (64). However, hUC-MSC and their derivatives (such as EVs) have made remarkable achievements in promoting OA repair. These two studies further elucidated the molecular mechanism of hUC-MSC-EVs in the remission of OA and greatly improved the possibility of clinical application.

Proteins

Transient receptor potential vanilloid 1 (TRPV1). TRPV1 is highly expressed on primary afferent neurons and participates in the conduction of pain (66). Activation of TRPV1 is closely associated with inflammation. However, TRPV1 exhibits both pro-inflammatory and anti-inflammatory effects, suggesting that the mechanism by which TRPV1 regulates inflammation may be complex (67).

In vitro experimental studies show that at the level of signaling pathways, TRPV1 inhibits the polarization of M1 macrophages in synovium through Ca^{2+}/CaMKII/Nrf2 axis, thereby reversing the progress of OA (68). A rat OA model showed that activation of TRPV1 could reduce knee swelling, improve synovitis score and reduce M1 macrophage level, so as to reduce cartilage destruction and osteophyte formation (68).

In a study in which the target disease was knee OA, the pain of patients was significantly reduced after the application of TRPV1 antagonist (69). In conclusion, this previous paper clarifies that TRPV1 is an attractive research direction and reveals a new mechanism of local capsaicin in the treatment of OA.

Milk fat globule epidermal growth factor 8 (MFG-E8). MFG-E8, also known as lactomyxin, is a peripheral secreted glycoprotein that can phagocytize apoptotic cells (70). In addition to being used to clear apoptotic cells, accumulating evidence shows that MFG-E8 serves a critical role in inhibiting inflammation, repairing injury, arterial remodeling and improving prognosis (70).

At the signaling pathway level, MFG-E8 targets chondrocyte growth and macrophage repolarization through the NF-κB pathway to prevent OA (71). In addition, lack of MFG-E8 leads to dysregulation of articular cartilage synthesis and decomposition homeostasis. Exogenous administration of MFG-E8 can promote the transformation of macrophages into M2 and reduce the release of inflammatory cytokines

during the development of OA (72). In a DMM-mediated mouse model, MFG-E8 can prevent OA from developing from cartilage injury to osteophyte formation (72).

In conclusion, these results broaden the field of clinical application of MFG-E8. Targeting MFG-E8 through intra-articular supplements represents a method to delay the development of OA.

E3 ubiquitin ligase ITCH protein. Ubiquitin E1, E2 and E3 ligases can mediate ubiquitination. This modification occurs at the level of protein translation. Proteins usually degrade and lose their biological functions after ubiquitination. ITCH is a monomeric protein that serves a key role in different cellular environments, including DNA damage, immune response, T cell differentiation and cell death (73).

In vitro data show that ITCH-deficient macrophageswill promote the pro-inflammatory polarization of macrophages and aggravate the progress of post-traumatic OA (PTOA). *In vivo* studies show that in the mouse OA model, the decrease of ITCH protein level is related to the severity of PTOA, indicating that the decrease of ITCH protein level may aggravate human OA (74).

In conclusion, ubiquitination and degradation of ITCH protein may be involved in PTOA process and studying the mechanism of its degradation may be an exciting direction for preventing the progression of OA (Fig. 2).

Fig. 2 shows the relevant pathways of substances involved in macrophage polarization regulation in different studies. A previous study reported that the pathway underlying how hUC-MSCs regulate macrophage polarization is unknown (62), whereas another previous study reported that hUC-MSCs act through the PI3K/Akt pathway (64).

5. Conclusion

OA is a chronic disease that causes physical disability. By 2030, the number of OA patients in the United States is expected to increase to ~67 million (75). Traditional treatments include physical therapy, anti-inflammatory and analgesic drugs and injections of glucocorticoids or hyaluronic acid into the joints. However, these therapies are ineffective and have side effects. For example, non-steroidal anti-inflammatory drugs are associated with cardiovascular risk and gastrointestinal bleeding problems (76,77). The optimal dose and time of intra-articular hormone injection remain controversial (6). Gene editing, including RNAi and CRISPR/Cas9 is an emerging therapy for OA. RNAi is regulated by small interfering RNA (siRNA) at the level of gene transcription (78). In OA, a number of animal studies confirm that RNAi can reduce cartilage degradation and alleviate OA (79-81). However, there are currently no clinical trials using siRNA for OA treatment. CRISPR/Ccas9 has great potential in the treatment of OA. One study summarizes the potential targets of CRISPR/Cas9 in the treatment of OA (82). However, CRISPR/Cas9 has ethical and effective targeting range problems. Notably, gene editing can be easily completed using CRISPR/Cas9, which raises concerns regarding the application of this technology to embryos (83). Single guide RNA (sgRNA) can only bind to a specific region near the PAM sequence on DNA (82). The PAM sequence for Cas9 is 5'-NGG-3', where 'N' can be any nucleotide base, but the third base must be G. This results in a significant reduction in the potential target locations for DNA editing (83).

However, these OA treatment methods can only improve the symptoms of OA but cannot reverse its process. Immune microenvironment serves an important role in OA (18). Osteoarthritis is characterized by the invasion and activation of macrophages and lymphocytes. Macrophages will polarize into M1 macrophages or M2 macrophages following stimulation. Regulating the phenotype of macrophages is important in the treatment of OA and may be the most promising treatment.

The novelty of the present study is from the perspective of immunology; it collated the latest studies of the enriched signal pathways and target molecules of macrophage polarization in OA, so as to provide insights into the treatment of OA. The pathways involved are ERK, PI3K/Akt, MAPK, STAT and NF-κB pathways, different substances including compounds, cells and proteins can alleviate OA by regulating the balance of M1 and M2 macrophages polarization. This suggests that maintaining the homeostasis of M1 macrophages and M2 macrophages is important for the treatment of OA. In addition, in the *in vivo* models of these studies, cartilage destruction has been improved, which again shows that cartilage homeostasis is very important for the development of OA.

Unfortunately, most of these studies are only in the preclinical stage and further experiments are needed to verify the efficacy of their clinical use. It may bring more benefits to the future research on macrophage regulation.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

BSC, HXH and ZMC contributed to the study design, participated in the review process and prepared the first draft. YYS, CC and GFB contributed to collecting the relevant literature and important information, generating figures and modified the manuscript. BSC, ZMC, CSW and GHX conceived the review and approved the final version of the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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