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MINI-REVIEW

Frontier review of the roles of exosomes in osteoarthritis

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Abstract: Osteoarthritis (OA) is a common degenerative disease; however, its exact pathophysiology and early diagnosis are still a challenge. Growing attention to the exosomes may inspire innovations that would make the current management of OA more effective. The exosomes in synovial fluid are relatively stable, and they can be easily isolated by the relatively noninvasive procedure of liquid biopsy to provide diagnostic and monitoring value. Some miRNAs (miR-504, miR-146a, miR-26a, miR-200c, and miR-210) have been known to be secreted in exosomes of OA patients. On the other hand, intraarticular injection of platelet-rich plasma (PRP) is becoming a popular therapy for OA patients. PRP is also a source of exosomes and their numerous contents. It is evident from the literature that PRP-derived exosomes can induce chondrogenic gene expression in OA chondrocytes. Here, we review the latest findings on the roles of exosomes in OA with the emphasis on PRP-derived exosomes and their potential applications for treating OA.

Keywords: Exosomes; Osteoarthritis; Platelet-rich plasma

1. INTRODUCTION

Osteoarthritis (OA) is a common degenerative disease in the elderly which often causes disability and pain. Consequently, the societal burden and the health service demand are increasing. OA is characterized by cartilage degradation, osteophyte formation, synovial inflammation, subchondral bone sclerosis, and angiogenesis. The exact pathophysiology of OA is still unclear but is known to be associated with trauma, obesity, and metabolic diseases.[1] The diagnosis is made by X-ray and clinical history.

Nevertheless, the correlation between the images and the symptoms is often not consistent. Currently, the conservative treatment of OA is pain killer agents, physical therapy, and intraarticular agent injection; however, it can only achieve temporary symptom relief. Surgical intervention such as a total knee replacement is the final strategy, but the complications and further long-term rehabilitation are the matters of concern.[2] Therefore, the focus on molecular mechanisms of OA can help us find the novel approaches of diagnosis and treatment.

Exosomes are small extracellular vesicles structured by a double lipid bilayer membrane found in body fluids, including cerebrospinal fluid, blood, urine, and synovial fluid.[3] The size of exosomes is around 30 to 150 nanometers (nm) in diameter, much smaller than the cells.[4] Exosomes contain various molecules and function as signaling mediators between cells. The cargo of exosomes includes such molecules as microRNAs (miRNAs), messenger RNA (mRNAs), proteins, and DNA. It is generally known that the content of exosomes is influenced by the cell of origin.[5]

To date, there are multiple studies focused on the relationship between OA and exosomes.[6] Exosomes seem to play an essential role in OA-associated processes, which include the regulation of inflammatory responses, homeostasis, and cartilage modulation. There is a multitude of emerging clinical applications of exosomes in OA patients.[7] The exosomes derived from the mesenchymal stem cells and platelet-rich plasma were discovered to exert beneficial therapeutic effect on OA both *in vitro* and *in vivo*.[8,9] In this brief review, we describe the role of exosomes in the diagnosis of OA and their potency as biomarkers, as well as exosome-based therapeutics. The key features of this review are presented in Fig. 1.

2. THE ROLE OF EXOSOMES IN THE DIAGNOSIS OF OA

2.1. Synovial inflammation in OA

In OA patients, synovial inflammation is a characteristic of the early and late stages of the disease. The synovial membrane is a lining-like connective tissue in the joint capsule; it contains fibroblast-like synoviocytes (FLS) that secrete synovial fluid and hyaluronan that maintain intraarticular homeostasis and provide nutrients to the cartilage.[10] Synovitis and chronic inflammation may thicken the synovium, accompanied by extra macrophage accumulation. It results in decrease of the secretion of hyaluronan and movable range of the joint. The intraarticular microenvironment changes eventually affect the cartilage

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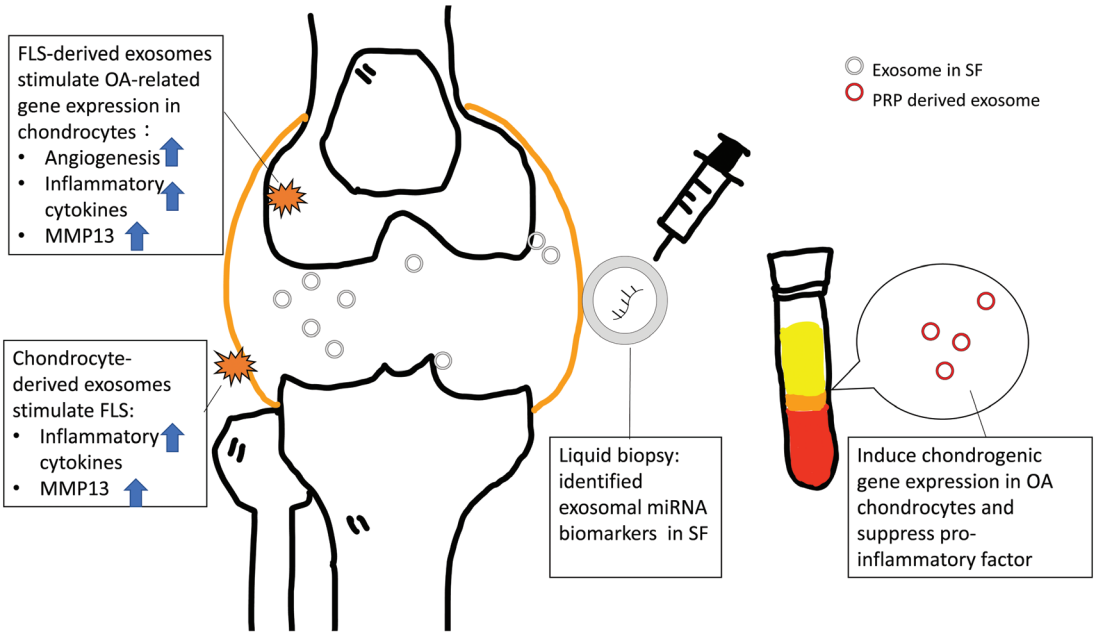

Fig. 1. Exosome involved pathogenesis in OA knee. FLS-derived exosomes stimulate OA-related gene expression in chondrocytes. Meanwhile, chondrocyte-derived exosomes stimulated FLS to produce inflammatory cytokines. These secreted exosomes in SF could be potential biomarkers through liquid biopsy. PRP, which is a widely used therapy in OA patients. Exosomes derived from PRP have a chondroprotective and anti-inflammatory effect. FLS = fibroblast-like synoviocytes; OA = osteoarthritis; PRP = platelet-rich plasma; SF = synovial fluid.

integrity. Various research has been dedicated to the involvement of exosomes in this disease progression.

2.2. Exosomes in synovial fluid

FLS are capable of releasing exosomes that are secreted into the synovial fluid and taken up by chondrocytes. A recent study reported that IL-1β-stimulated FLS could increase exosome secretion.[11] It was found that FLS-derived exosomes stimulate OA-related gene expression in chondrocytes, which results in angiogenesis and production of inflammatory cytokines and MMP13 metallopeptidase. Furthermore, the chondrocytes treated with inflammatory cytokines produce exosomes that stimulate FLS to produce inflammatory cytokines and MMP13.[12] Thus, exosomes are believed to participate in the communication between FLS and chondrocytes. Chondrocytes are a crucial cell type for the production of extracellular matrix in the cartilage, which is crucial for the maintenance of joint function. In the process of OA development, microenvironment changes result in cartilage catabolic change. Recent research revealed that both FLS and chondrocytes can secrete more exosomes in the inflammatory stage. These exosomes are released into the synovial fluid and then stimulate the macrophages to release more inflammatory cytokines and metalloproteinases.[13] The treatment of chondrocytes with FLS-derived exosomes was shown to induce epigenetic changes. FLS-derived exosomes were reported to upregulate *MMP13* and *ADAMTS5* and downregulate *ACAN* and *COL2A1* gene expression in particular chondrocytes.[12]

2.3. Liquid biopsy and biomarkers

FLS and chondrocyte-derived exosomes in synovial fluid play a potential role in OA pathogenesis, including promoting inflammation and degeneration of articular cartilage. The content of exosomes is varied at different stages of the disease. The characteristic can bring us information to diagnose OA. Currently, the severity of OA is classified by the Kellgren-Lawrence system, which includes five grades depending on bony features evaluation through plain film imaging.[14] The strategies of treatment of OA, such as intra-articular hyaluronic acid injection or surgical intervention, are adjusted according to this guideline. However, some discordance exists between imaging assessment and clinical picture; therefore, additional biomarkers such as exosomes can be of benefit. The content of exosomes is relatively stable, and they can be easily isolated by relatively noninvasive procedure of liquid biopsy to provide diagnostic and monitoring value.[15] Kolhe and colleagues reported that some miRNAs (miR-504, miR-146a, miR-26a, and miR-210) were secreted in exosomes of OA patients. Moreover, female OA patients had specific estrogen-responsive miRNAs that targeted the Toll-like receptor signaling pathway.[16] It may explain the high prevalence of females among OA patients. Domenis and colleagues found that exosomes in synovial fluid could stimulate proinflammatory factor release, and the disease progression could be monitored accordingly.[13] Further studies are needed to monitor the response of synovial fluid exosome biomarkers after treatment in OA patients. On the other hand, Murata and colleagues identified that exosomal miRNA biomarkers derived from plasma and synovial fluid are different between rheumatoid arthritis and OA.[17] Withrow and colleagues identified that exosomes are present in the synovial fluid of OA patients at different concentrations and quantities than in healthy people. The profiling of cargo of these exosomes using PCR array showed that miR-200c level was increased in OA patients.[12] Further studies can be focused on the identification of exosomal biomarkers that can be used for tracking the progression of OA.

3. PRP-DERIVED EXOSOMES

Platelet-rich plasma (PRP) is an autologous derivative of whole blood containing supraphysiological concentration of platelets and numerous growth factors that can enhance bone regeneration, cartilage, and tissue repair. PRP intraarticular injection

is becoming a popular therapy for musculoskeletal diseases, although the results are still variable. PRP injections into OA patients have been shown to influence the entire joint environment.[18] Some meta-analyses of the efficacy of PRP injections compared with placebo or other therapeutic means for the treatment of knee OA[19] revealed significant pain reduction[20] and functional improvement.[21]

Besides the growth factors, PRP is also a source of exosomes and their numerous content.[22,23] Many of these exosomes are released from the platelets.[24] The properties of platelet-derived exosomes are dependent on the microenvironment in which they reside.[25] Iyer and colleagues found that PRP-derived exosomes can accelerate muscle injury recovery in a rat model.[26] Tao et al. found that exosomes derived from human PRP prevent apoptosis induced by glucocorticoid-associated endoplasmic reticulum stress in rat osteonecrosis of the femoral head via the Akt/Bad/Bcl-2 signaling pathway.[27] Alexander and colleagues categorized two kinds of autologous blood products and found that their derived exosomes are sufficient to induce chondrogenic gene expression in OA chondrocytes.[9] In meanwhile, the exosomes could suppress proinflammatory factor release accordingly.

In an animal study, Liu and colleagues found that PRP-derived exosomes had a similar therapeutic effect on OA when compared with activated PRP. These exosomes contained growth factors and activated the Wnt/β-catenin signaling pathway.[28] Further studies are needed to qualify and quantify the PRP-derived exosomes, which may lead to their application in the clinical practice.

In conclusion, OA is a whole joint disease that affects the cartilage, subchondral bone, and synovium. Exosomes, the nano-sized vesicles responsible for communication between cells, are involved in OA pathophysiology. Synovial fluid-derived exosomes are implicated in the curial mechanisms of disease development and reflect disease severity, therefore, can potentially be utilized as biomarkers to monitor the disease progression. Further studies are needed to identify the content of exosomes that can be used as more accurate and reliable biomarkers. Second, PRP-derived exosomes may exert therapeutic effects in OA patients. Precise characterization of PRP-derived exosomes and connection to the clinical outcome is essential in future studies. In conclusion, exosomes can be of enormous value to OA patients in the future.

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