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ROLE OF MESENCHYMAL STEM CELLS—DERIVED EXOSOMES IN OSTEOARTHRITIS TREATMENT

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ABSTRACT

Exosomes are nanovesicles that are involved in intercellular communication and are secreted by many types of cells. Exosomes secreted by stem cells can effectively transport bioactive proteins, messenger ribonucleic acids (mRNAs) and microribonucleic acids (miRNAs) organelles and play important roles in intercellular communication and the regulation of tissue regeneration. This transfer of bioactive molecules plays a main role in: tumor invasion and metastasis, immune and inflammation modulation, epithelial-mesenchymal transition and neurobiology. Mesenchymal Stem Cells (MSC) exosomes provide new perspectives for the development of an off-the-shelf and cell-free MSC therapy for the treatment of cartilage injuries and osteoarthritis. This report describes the progress in exosome studies and potential clinical use for osteoarthritis treatment.

Key words: exosomes; osteoarthritis; stem cell; treatment

INTRODUCTION

Osteoarthritis (OA, degenerative joint disease, osteoarthrosis) is the most common form of arthritis in dogs and cats. It is a chronic, progressive and irreversible joint disease, which ultimately results in degeneration of hyaline articular cartilage in association with alterations in the subchondral bone metabolism, periarticular osteophytosis, and a variable degree of synovial inflammation [6, 19, 30].

The treatment of osteoarthritis is most often conservative and multimodal. A majority of cases are managed with nonsteroidal antiinflammatory drugs or other analgesics combined with weight reduction, nutritional and exercise management. Osteoarthritis in dogs is associated with a variety of clinical signs such as stiffness, lameness, and gait alterations [10]. Exercise intolerance, muscle atrophy, joint swelling, capsular and extracapsular fibrosis, joint effusion, reduced range of motion, crepitus, and pain on joint manipulation are also present in many cases.

In recent years, OA therapy has been the subject of many studies in regenerative medicine. The use of MSCs for tis-

sue repair such as cartilage repair was first predicated on the hypothesis that these cells could differentiate into chondrocytes to replace the damaged tissue; it is now accepted that MSCs secrete factors to increased tissue repair. However, the studies demonstrated that MSCs secrete factors to promote proliferation and matrix synthesis of chondrocytes [42, 43]. The successful utilization of stem cells in OA treatment are presented by various studies based on animal models or clinical studies. However, the cell free therapy based on MSCs exosomes as a promising alternative for OA treatment has been based upon the fact that exosomes have been identified as the principal agent mediating the therapeutic efficacy of MSCs in several diseases such as myocardial ischemia/ reperfusion (I/R) injury, limb ischemia and pulmonary hypertension [12, 13, 15, 17, 20, 36,].

EXTRACELLULAR VESICLES

Extracellular vesicles (EVs) are released in the extracellular space by almost all cells. EVs are small, secreted bi-lipid membrane – enclosed particles and they are surrounded by a phospholipid bilayer and can be distinguished by their size and composition. Secreted membrane vesicles are classified as: microvesicles, ectosomes, membrane particles, exosome-like vesicles, apoptotic bodies, prostasomes, oncosomes, or exosomes, according to their biogenesis pathway, size, flotation density on a sucrose gradient, lipid composition, sedimentation force, and cargo content [8, 16, 35].

Generally, secreted membrane vesicles could be broadly divided into two classes: 1) vesicles formed by inward budding of endolysosomal vesicles and released through exocytosis; and 2) vesicles which are shed from the plasma membrane [16].

Exosomes and microvesicles are the two most representative vesicle types; exosomes, 40~100 nm in diameter and microvesicles 100 nm~1000 nm in diameter. They contain numerous proteins, lipids as well as messengers and micro RNAs responsible for intercellular communication. Cells often exchange substances and information through the release of these particles [21].

EXOSOMES

MSCs release a wide range of trophic factors to modulate the injured tissue environment and the regenerative processes including: cell migration, proliferation, differentiation, and matrix synthesis. It is the paracrine effects of MSCs and the secretion of trophic factors that mediate the tissue repair [22]. Wu and colleagues reported the trophic effects of MSCs on chondrocytes [38, 39]. Further, they described that human bone marrow MSCs can increase the proliferation and extracellular matrix synthesis of chondrocytes via the secretion of trophic factors. MSCs derives from different sources including bone marrow and adipose tissue and even synovial membranes may exert similar trophic effects.

The exosomes secreted by stem cells can effectively transport bioactive proteins, messenger ribonucleic acids (mRNAs) and microribonucleic acids (miRNAs) organelles and play important roles in intercellular communication and the regulation of tissue regeneration. Presently, exosomes are the most clearly defined class of secreted membrane vesicles reported to date [16]. They are formed by the invagination of endolysosomal vesicles to form multi-vesicular bodies [7, 16, 40]. It has been reported that exosomes collected from mesenchymal stem cells showed a protective effect against ischemia/reperfusion injury, because of their immunosuppressive and anti-inflammatory effects [2, 13, 17, 23, 41].

Exosomes were first discovered as a vehicle for discarding unwanted transferrin by maturating sheep reticulocytes [24]. Recent studies have demonstrated, that exosomes contain mRNA [31] miRNA [27] and consequently it can be transferred into recipient cells to modulate protein synthesis. All these studies suggest that exosomes extentions may mediate intercellular communication through protein–protein interactions and exchange of proteins and genetic materials [14].

The presence of exosomes in: various physiological fluid/human blood [3], human urine [3, 26], bronchial lavage fluids [1] and the large diversity in exosome – secreting cell types/B cells [28], dendritic cells [45] mast cells [29], T cells [25], platelets [11], Schwann cells [9], tumour cells [37], mesenchymal stem cells [13], human embryonic kidney cells [32], various cancer cell lines [4] and sperm [33] indicate that the secretion of exosomes is a general cellular function.

MSCS EXOSOMES IN OA TREATMENT

Recent animal model-based studies suggest that MSCs exosomes have significant potential as a novel alternative to whole cell therapies in OA management. According to the results of the latest animal-based study, the MSCs exosomes derived from human embryonic stem cells mediate cartilage repair by enhancing proliferation, attenuating apoptosis and modulating immune reactivity in a rat model with osteochondral defect on bilateral trochlear grooves. The MSCs exosomes increase M2 macrophage infiltration with a concomitant decrease in M1 macrophages and inflammatory cytokines and have effects on chondrocyte migration, proliferation and matrix synthesis [42]. In the previous study, Zhang et al. compared the therapeutic effects of human embryonic MSC-derived exosomes and phosphate buffered saline (PBS) in a rat model with osteochondral defects on bilateral trochlear grooves also.

Generally, exosome-treated defects showed enhanced gross appearance and improved histological scores, complete restoration of cartilage and subchondral bone, extracellular matrix deposition than the contralateral PBS-treated defects [43].

Biomedical research on human embryos, including the acquisition and manipulation of embryonic stem cells is prohibited in the Slovak Republic.

Z h u et al. [44] compared the exosomes secreted by induced pluripotent stem cell derived MSCs (iMSCs-Exos) and synovial membrane derived MSCs (SMMSC-Exos) in a mouse collagenase-induced OA model. In this study it was demonstrated that while iMSC-Exos and SMMSC-Exos both stimulated chondrocyte migration and proliferation, iMSC-Exos had a greater effect than SMMSC-Exos. These authors found that the injection of iMSC-Exos significantly attenuated OA in a mouse model of collagenase-induced OA. Histological analysis demonstrated that the repaired cartilage in the iMSC-Exos group presented typical hyaline features similar to normal cartilage. The immunohistochemistry analysis indicated that the expression of collagen II, a specific marker of hyaline cartilage, was similar in the iMSC-Exos and normal control groups [44].

In other mouse collagenase-induced OA models, the exosomes were isolated from the conditioned medium of bone marrow-derived murine (BM-MSCs). BM-MSC-derived exosomes exerted anti-apoptotic effects on OA-like chondrocytes, immunosuppressive function and inhibited macrophage differentiation. BM-MSC-derived exosomes are potent to protect cartilage and bone from degradation in the collagenase induced OA murine model [5].

In other *in vitro* animal studies, exosomes derived from human induced pluripotent stem cells [18] and human synovial mesenchymal stem cells [34] demonstrated anti-inflammatory properties, prevented cartilage degradation and protective effects of exosomes.

CONCLUSIONS

The general approach to osteoarthritis treatment includes pharmacological and support therapies, rehabilitation and physiotherapy. Pain management involves the use of non- steroidal antiinflammatory drugs, corticosteroids, anticonvulsants and other analgetics. In many cases, this therapy is insufficient. The treatment of osteoarthritis is an interesting area for regenerative medicine. Exosomes derived from different sources of stem cells are the subject of many studies concerning cartilage repair or osteoarthritis treatment. Nowadays, the efficacy of MSCs exosomes in osteoarthritis treatment are demonstrated only *in vivo* and *in vitro* animal based studies. The next step in research is a clinical study that would demonstrate MSCs exosome's safety, efficacy and properties in osteoarthritis treatment.

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