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Extracellular vesicles in cartilage homeostasis and osteoarthritis

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Abstract

Purpose of review—Extracellular vesicles (EV) carry bioactive molecules that can be transferred between cells and tissues. This purpose of this review is to describe how EV regulate functions of cells in cartilage and other joint tissues. The potential application of EV in the treatment of osteoarthritis (OA) and as biomarkers will also be discussed.

Recent findings—EV are found in synovial fluid, in articular cartilage and in the supernatants of synoviocytes and chondrocytes. EV in cartilage have been proposed to be involved in cross talk between cells in joint tissues and to affect extracellular matrix turnover and inflammation. EV from arthritic joints can promote abnormal gene expression and changes in cartilage extracellular matrix, including abnormal mineralization. Promising results were obtained in the therapeutic application of mesenchymal stem cell derived EV for cartilage repair and experimental OA.

Summary—EV have emerged as vehicles for the exchange of bioactive signalling molecules within cartilage and between joint tissues to promote joint homeostasis and arthritis pathogenesis. As the molecular content of EV can be customized, they offer utility in therapeutic applications.

Keywords

extracellular vesicles; exosomes; cell-cell communication; chondrocytes; synoviocytes; osteoarthritis

INTRODUCTION

Osteoarthritis (OA) represents the most common musculoskeletal disorder [1]. It is a complex and multifaceted disease, characterized by the degradation of articular cartilage, subchondral bone remodeling, joint inflammation and changes in meniscus and ligaments [2]. Various risk factors for OA have been identified and these include aging, joint injury, excessive chronic

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Conflicts of interest

There are no conflicts of interest.

mechanical stress, genetic factors, and metabolic disorders [3]. Although several pathogenesis pathways have been characterized [4], current knowledge is incomplete and has not led to effective approaches for prevention or treatment. These limitations can be overcome by advances in the understanding of molecular mechanisms that are involved in the maintenance of joint tissues which involves communication of cells within the different joint tissues. Cells are able to communicate with neighboring or distant cells through cytokines and hormones. In addition, extracellular vesicles (EV) that are released from cells have attracted attention as novel a mechanism of cell-cell communication. EV transfer bioactive molecules to recipient cells to modulate their activity [5]. In this review, we focus on the role of EV in cell-cell communication within and among joint tissues during homeostasis and OA pathogenesis and address the potential therapeutic application of EV.

EXTRACELLULAR VESICLES

Extracellular vesicles (EV) had previously been regarded as inert cellular debris, that was generated as a consequence of cell damage or the result of dynamic plasma membrane turnover. However, recently cell-cell communication via EV has become the center of attention in research of diseases and tissue repair. EV contain bioactive molecules, including proteins, mRNAs, microRNAs, lipids, and DNA [6]. EV have been classified depending on size and biogenesis pathway. Exosomes are small EV (30–150 nm in diameter) that are generated in multivesicular endosome (MVE)/multivesicular bodies (MVB) and are released when these compartments fuse with the plasma membrane. Microvesicles/microparticles (MVs) (50–1000 nm in diameter) are released by budding from the surface of the plasma membrane [7]. Exosome biogenesis is a very tightly regulated process governed by multiple signaling molecules, and begins with receptor activation that is unique to each cell type [8]. Detailed and conclusive characterization of the various types of EV has not yet been accomplished. The International Society for Extracellular Vesicles provided a minimal set of biochemical, biophysical and functional standards [9]. Size alone can not distinguish exosomes from MVs [10]. Furthermore, some proteins previously used as exosome markers, such as MHC class II or class I molecules, heat-shock proteins, flotillins, or actin, are present in all types of EV, and thus cannot be considered as either exosome or even EV-specific markers [11]. New specific markers of medium and large size EV (e.g., actinins), of endosome-derived exosomes (co-expressing three tetraspanins CD9/CD63/CD81 and including TSG101 and syntenin-1), and of non-endosomal EV (some ITGs) have been proposed [7, 11]. There remains a continuing need to better understand the molecular mechanisms of the biogenesis and release of EV and to discover better markers for the various types. The released EV have surface receptors/ligands of their cell source and have potential to interact with specific target cells [6]. EV directly stimulate target cells by receptor-mediated interactions or transfer of the enclosed bioactive molecules [8].

EV FROM CARTILAGE AND CHONDROCYTES

EV have long been known to be present in the pericellular matrix of articular cartilage and growth plate cartilage [12–17]. Various terms have been used to describe them, including matrix vesicles (MaV), articular cartilage-derived extracellular vesicles (ACEV) or apoptotic bodies but there is no conclusive distinction (Table 1). Originally, MaV were described in

growth plate as derived from budding or disintegrating cells that are associated with hydroxyapatite deposition. Alkaline phosphatase activity is abundant in MaV and is used as a marker for their identification [13]. MaV also contain pyrophosphate-generating nucleoside triphosphate pyrophosphohydrolase (NTPPH) activities. Matrix vesicles can be isolated from collagenase-digested articular cartilage and separated from chondrocytes by differential centrifugation and used for functional, biochemical, and ultrastructural studies [18]. Isolated MaV can incorporate calcium, hydrolyze ATP or other nucleotide triphosphates, and facilitate precipitation of hydroxylapatite [19]. Although MaV from different sources are heterogeneous [20], they are similar with respect to the capacity to mineralize matrix [12, 17]. ACEV have been shown to have a physiological function in endochondral bone development and pathologic role in calcium crystal deposition in articular cartilage [21]. The majority of the proteome was shared by EV isolated from normal and OA cartilage, but immunoglobulins and complement components were present only in OA ACEV which also contained lower levels of matrix proteoglycans [22]. Importantly, the ACEV proteome shares fewer similarities with exosomal proteomes. The heterotrimeric G proteins, HSP70 and 90 and members of the tetraspanin family such as CD9, CD63 and CD81 that are particularly characteristic of exosomes were not seen in ACEV [22]. CD9, CD63 and CD81 were previously considered to be specific markers for exosomes; however, in recent proteomics comparison, these proteins were observed in all EV including MV and apoptotic bodies [11, 23].

RNAs are also packaged in EV and are transferable genetic material from tissue to tissue and from human to human [5]. RNAs are protected from degradation by the lipid membrane of the EV. Coding and non-coding small RNAs in EV were in proportions that differed from parent cells with an enrichment of specific miRNAs suggesting that miRNAs are selectively packaged into EV. For example, small RNAs such as miRNAs were enriched in EV isolated from cultures of costochondral growth zone chondrocytes, while large RNAs such as 18S and 28S rRNA were not detected [24]. EV from normal articular cartilage contain full length mRNAs for factor XIIIA, type II transglutaminase, collagen II, aggrecan, ANKH and GAPDH. When transferred to chondrocytes, ACEV-derived RNA was internalized. This was associated with changes in the expression of alkaline phosphatase and osteopontin [25].

The mechanisms of ACEV formation include apoptosis which is increased in OA-affected cartilage [26]. Chondrocyte-derived apoptotic bodies contain alkaline phosphatase and NTP pyrophosphohydrolase activities, and can precipitate calcium [27]. A role of apoptosis in generating this type of ACEV has been demonstrated in experiments where apoptosis was induced by the nitric oxide donor sodium nitroprusside or antibody to the Fas antigen [27]. EV accumulate around apoptotic cells (Figure 1). The levels of pyrophosphate produced by apoptotic bodies were increased by pretreatment of the chondrocytes with transforming growth factor-β and decreased by IL-1β [27]. It has also been suggested that ACEV from primary articular chondrocytes can be generated through the autophagy pathway [28]. In normal but not in OA chondrocytes, rapamaycin, which induces autophagy by inhibiting mTOR signaling, increased the release of ACEV that contained LC3, a marker of autophagosomes [28]. Release of ACEV was inhibited by gene knock down of caspase-3, suggesting an involvement of apoptosis-related mechanisms [28]. Thus, ACEV include various types of EV that differ in mechanism of generation and apparently in molecular content.

More detailed information about calcium crystal deposition by ACEV is presented in a recent review [21]. A database of MaV proteins also provide comprehensive information on protein components of mineralization-related MaV [29].

EV IN COMMUNICATION AMONG JOINT TISSUES

The concept that EV can mediate communication among cells from different joint tissues has thus far only been tested in a limited number of examples. Exosomes from IL-1β stimulated synoviocytes significantly up-regulated MMP-13 and ADAMTS-5 expression in articular chondrocytes, and down-regulated COL2A1 and ACAN compared with synoviocyte derived exosomes [30] (Figure 2). Migration and tube formation activity were significantly higher in human umbilical vein endothelial cells treated with the exosomes from IL-1β stimulated synoviocytes, which also induced significantly more proteoglycan release from cartilage explants. Inflammatory cytokines, IL-6, MMP-3 and VEGF in exosomes were only detectable at low level. IL-1β, TNFα MMP-9 and MMP-13 were not detectable in exosomes. NanoString analysis showed that levels of 50 miRNAs were differentially expressed in exosomes from IL-1β stimulated synoviocytes compared to non-stimulated cells [30].

EV IN SYNOVIAL FLUID AS POTENTIAL OA BIOMARKERS

EV are abundant in synovial fluid, and can be derived from resident cells in joint tissues and from leukocytes that infiltrate arthritis-affected joints. Synovial fluid EV modulate the release of chemokines and cytokines in synoviocytes [31, 32]. The expression patterns of miRNAs in synovial fluid of OA were similar to miRNAs secreted by synovial tissues [33]. A recent microarray analysis of miRNAs in synovial fluid exosomes showed that in samples from female OA patients miR-16-2-3p was up-regulated and miR-26a-5p, miR-146a-5p and miR-6821-5p were down-regulated. In synovial fluid from male OA patients miR-6878-3p was down-regulated and miR-210-5p was up-regulated. Thus, synovial fluid exosomal miRNA content is altered in patients with OA and these changes are gender specific [34]. This is the first study to analyze exosomal molecules as biomarkers in OA. Future studies need to address the possibility of detecting joint-derived EV in blood and of identifying the cellular origin of EV. This has potential to detect tissue specific changes as biomarkers for OA.

EV IN THERAPEUTIC APPLICATIONS

Mesenchymal stem cells (MSC) have been used successfully in tissue engineering approaches to treat cartilage lesions and OA in animal [35] and human studies [36, 37]. These beneficial functions of MSC are at least in part mediated by paracrine effects of cytokines and growth factors that decreased inflammation, enhanced progenitor cell proliferation, improved tissue repair. In a mouse model of myocardial ischemia/reperfusion injury it was first demonstrated that the protective paracrine effect was mediated by secreting exosomes [38].

Since then additional studies have demonstrated that EV derived mesenchymal stem/stromal cells (MSC) have therapeutic effects [39–41]. We reported that MSC-derived EV promote

skeletal muscle repair and bone fracture healing in mouse models through acceleration of biological function such as angiogenesis and cell differentiation [32, 42].

Exosomes can be used in therapeutic approaches, either from specific cell types such as MSC or from cells that are transfected with genes that have therapeutic potential to enrich for RNA levels for the gene of interest. Exosomes derived from synovial membrane MSC promoted chondrocyte proliferation and migration but inhibited their secretion of extracellular matrix (ECM). Wnt5a and Wnt5b carried by exosomes activated the alternative Wnt signaling pathway and enhanced proliferation and migration of chondrocytes but significantly decreasing ECM secretion. We previously reported that miRNAs, in particular miRNA-140, one of the most abundant miRNAs in chondrocytes are important regulators of cartilage homeostasis [43, 44]. Exosomes were prepared from cells that were transduced with lentiviral miR-140-5p enhanced the proliferation and migration of articular chondrocytes and reduced OA severity in a rat model [45]. Human embryonic MSC exosomes promoted cartilage regeneration in a rat osteochondral defect model [46]. In that study, MSC exosomes accelerated neotissue filling and enhanced matrix synthesis of type II collagen and sulphated glycosaminoglycans. By the end of 12 weeks, exosome-treated rats displayed complete restoration of cartilage and subchondral bone.

Exosomes from conditioned culture media of embryonic stem cell derived MSC (ESC-MSC) maintained the chondrocyte phenotype by increasing collagen type II synthesis and decreasing ADAMTS5 expression in the presence of IL-1β. Intra-articular injection of ESC-MSC alleviated cartilage destruction and matrix degradation in the DMM model. Immunocytochemistry revealed colocalization of the exosomes and collagen type II-positive chondrocytes. Subsequent intra-articular injection of exosomes derived from ESC-MSC successfully impeded cartilage destruction in the DMM model. The exosomes from ESC-MSC exert a beneficial therapeutic effect on OA by balancing the synthesis and degradation of chondrocyte ECM, which in turn provides a new target for OA drug and drug-delivery system development [47]. This study demonstrated the utility of MSC exosomes as a ready-to-use and ‘cell-free’ therapeutic alternative to cell-based MSC therapy.

FUTURE PERSPECTIVES

The role of EV in joint homeostasis and OA pathogenesis is of great interest and further research on this topic has potential implications for the discovery of novel biomarkers and therapeutic approaches. Currently this field is at an early stage and the topic that seems most advanced is the use of EV as therapeutic vehicles. Key questions that need to be investigated are: regulation of the types, amounts and compositions of EV that are generated and released by cells; stability of EV in the various joint tissue environments and transport of EV through dense ECM structures such as in cartilage; mechanisms of recognition and internalization by cells; the role of EV in joint homeostasis and pathogenesis. Markers of EV in synovial fluid or blood that allow tracking their cellular origin and thus profiling the status of these cells.

CONCLUSION

EV are present in articular cartilage and synovial fluid and represent a heterogeneous mixture that varies in regard to mechanism of generation and molecular content. Synovial Fluid EV are potential new OA biomarkers. Composition of EV from OA cartilage appears to be altered and may contribute to abnormal mineral and crystal deposition. EV are released from synovial fibroblasts and affect gene expression in chondrocytes. The pattern of mRNAs and miRNAs in EV can be altered by stimulation or gene transduction of cells and thus be designed to specifically change the function of target cells for therapeutic use.

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KEY POINTS

- EV are released from chondrocytes through various mechanisms, including autophagy and apoptosis.
- EV from chondrocytes can play a role in abnormal articular cartilage mineralization.
- EV from mesenchymal stem cells can be enriched for certain bioactive molecules, such as miRNAs and used for tissue repair and in the treatment of OA.

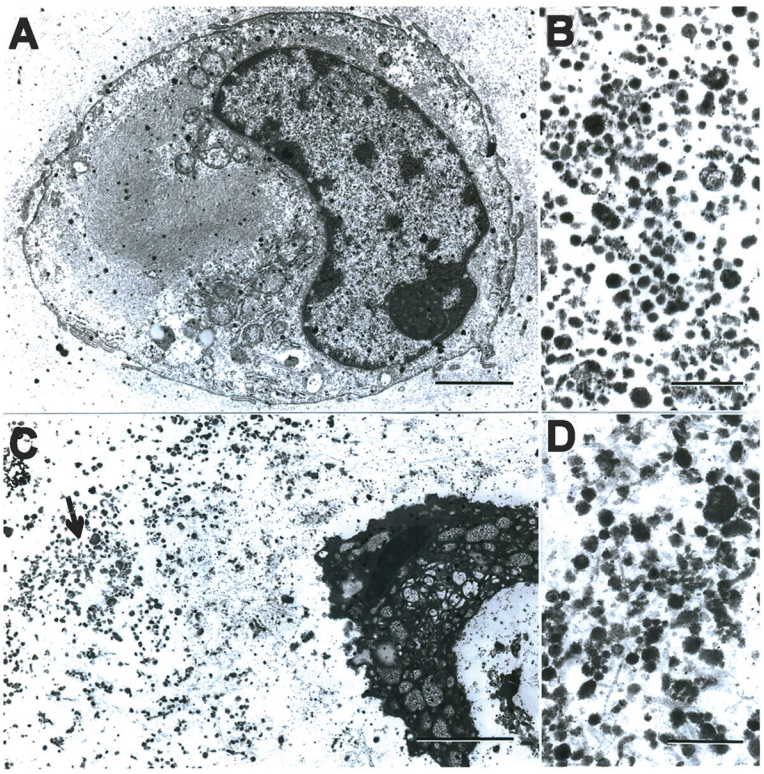

Figure 1. Electron microscopy of apoptotic chondrocytes, apoptotic bodies and matrix vesicles

(A) Electron micrograph of a chondrocyte from normal articular cartilage. Bar represents 2 μM.

(B) Isolated matrix vesicles from normal cartilage. Bar represents 0.5 μM.

(C) Electron micrograph of an apoptotic chondrocyte in cartilage treated with the NO donor SNP. The area indicated by the arrow is shown at higher magnification in (D). Bar represents 2 μM.

(D) High magnification view of the area indicated by the arrow in (C). Bar represents 0.5 μM.

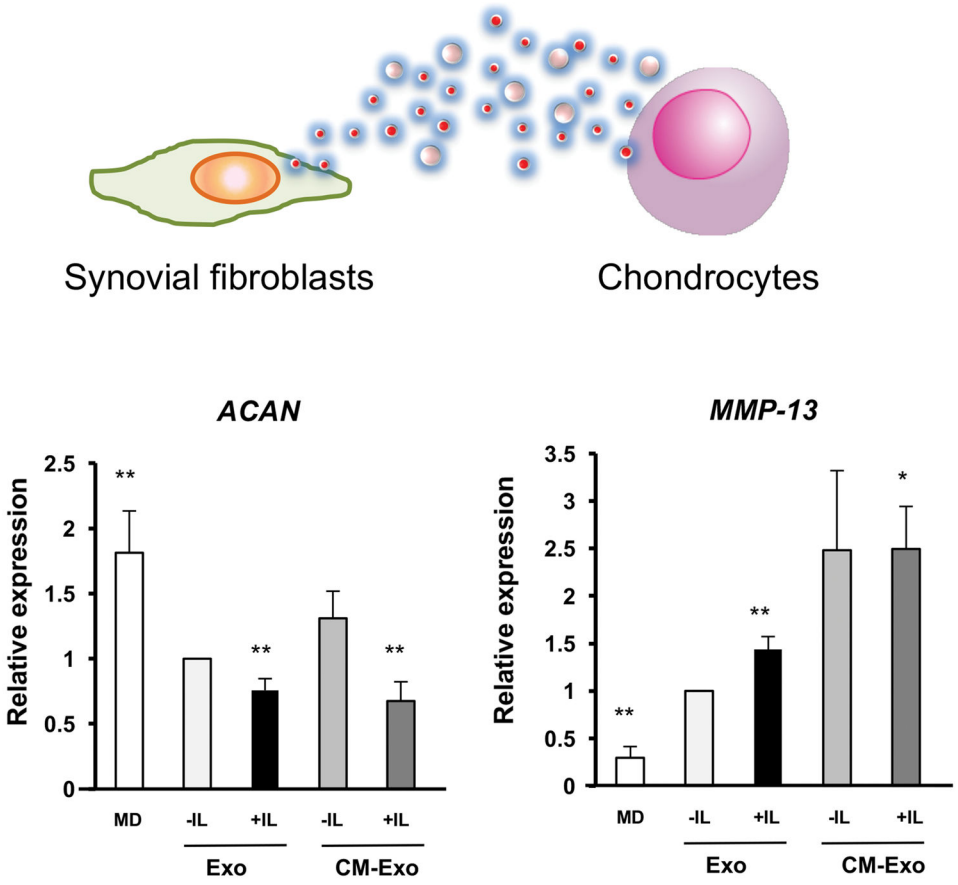

Figure 2. The effect of EV from IL-1β stimulated synovial fibroblasts on normal articular chondrocytes

Articular chondrocytes were treated with fresh-DMEM, exosomes from non-stimulated synovial fibroblasts (SFB) or exosomes from IL-1β stimulated SFB. The expression of OA-related genes was analyzed by real-time PCR. *MMP-13* was significantly up-regulated, and *ACAN* was significantly down-regulated by exosomes from IL-1β stimulated SFB. **P < 0.01 versus Exo -IL. MD; fresh-DMEM with 10% FBS, Exo -IL; exosomes from non-stimulated SFB, Exo + IL; exosomes from IL-1β stimulated SFB.

Table 1

Characteristics of Articular Cartilage-derived Extracellular Vesicle (ACEV).

	Exosome	Matrix vesicles/Microvesicle	Apoptotic body
Origin	Endocytic pathway Autophagic pathway	Budding off/fusion from the Plasma membrane Autophagic pathway	Plasma membrane in apoptotic cell
Size	30–150 nm	100 – 1000 nm	100 nm <
Marker	CD9, CD63, CD81, Flotillin-1, Alix, Tsg101, LC3 etc. No specific markers		
Content	mRNA, non-coding-RNA (microRNA etc), protein, lipid, DNA		
Isolation method	Differential centrifugation/Density gradient centrifugation Commercial kit		