Transforming growth factor-β family and stem cell-derived exosome therapeutic treatment in osteoarthritis (Review)

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Abstract. Osteoarthritis (OA), although extensively researched, still lacks an effective and safe treatment. The only current treatment option available for advanced OA is joint replacement surgery. This surgery may pose the risks of persistent pain, surgical complications and limited implant lifespan. Transforming growth factor (TGF)-β has a crucial role in multiple cellular processes such as cell proliferation. Any deterioration in TGF-β signaling pathways can have an immense impact on OA. Owing to the crucial role of TGF-β in cartilage homeostasis, targeting it could be an alternative therapeutic approach. Additionally, stem cell-based therapy has recently emerged as an effective treatment strategy that could replace surgery. A number of recent findings suggest that the tissue regeneration effect of stem cells is attributed to the paracrine secretion of anti-inflammatory and chondroprotective mediators or trophic factors, particularly nanosized extracellular vesicles (i.e., exosomes). Literature searches were performed in the MEDLINE, EMBASE, Cochrane Library and PubMed electronic database for relevant articles published before September 2021. Multiple investigators have confirmed TGF-β3 as a promising candidate which has the chondrogenic potential to repair articular cartilage degeneration. Combining TGF-β3 with bone morphogenetic proteins-6, which has synergistic effect on chondrogenesis, with an efficient platform such as exosomes, which themselves possess a chondroprotective function, offers an innovative and more efficient approach to treat injured cartilage. In addition, multiple findings stating the role of exosomes in chondroprotection has also verified a similar fact showing exosomes may be a more favorable choice than the source itself. In the present review, the importance of TGF-β family in OA and the possibility of therapeutic treatment using stem cell-derived exosomes are described.

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1. Introduction

Osteoarthritis (OA) is the most common joint disease globally and it primarily affects the elderly. It can be defined as a degenerative disease of articular cartilage, characterized by destruction of the articular cartilage, synovial tissue inflammation, subchondral bone alterations and formation of bony outgrowths (called osteophytes), which causes joint stiffness, chronic pain and eventually disability (1). OA is a complex disease the development of which involves genetic and acquired factors. Multiple risk factors such as ageing, injury, innate genetic variations and environmental factors contribute to the progression of OA (2,3).

Articular cartilage is an avascular tissue covering joint surfaces that facilitates movement and is responsible for shock absorbance. Articular cartilage consists of chondrocytes which are embedded by an extracellular matrix (ECM) (4). ECM consists of collagen, proteoglycans, hyaluronic acid and other less common components such as gelatin, a matrix glycoprotein through which collagen imparts tensile strength and shape to the tissue (5). Type II collagen is the main structural protein

in cartilage and it is responsible for building the ECM network structure with aggrecan and other proteoglycans (6).

Disruption of healthy cartilage, which is characterized by the balance between anabolic and catabolic process of ECM production and degradation, may lead to cartilage loss (Fig. 1). Chondrocyte governs joint health by controlling the balance reaction. A number of other factors such as tensile strain, proinflammatory cytokine and growth factors are also involved in modulating chondrocyte homeostasis. The TGF-β superfamily, which involves TGF-β and bone morphogenetic proteins (BMPs), consists of anabolic growth factors (7,8). Catabolic factors such as matrix metalloprotease (MMP)-13 and inflammatory cytokines such as interleukin (IL)-6 are involved in the destruction of the collagen network and the structure of the ECM (9,10). These catabolic factors target cartilage for the degradation of types II and IV collagen, proteoglycan and aggrecan (11).

The past two decades of extensive research work has focused on unravelling the disease mechanism and associated enhancing factors; however, the full understanding of disease has not been acquired. Current understanding of the disease mechanism is insufficient with regard to early diagnosis or providing optimized treatment for OA patients. However, based on recent findings, the TGF-β signaling pathway role in OA development and progression may represent a potential therapeutic target for OA therapy.

2. TGF-β signaling and osteoarthritis

Previous studies demonstrate a crucial role of TGF-β members in multiple cellular processes such as cell proliferation; therefore, any deterioration in TGF-β signaling pathways can have an immense impact on numerous human diseases, including OA (2,3). The TGF-β family consists of 35 members, which includes TGF-βs, BMPs, activins and fibroblast growth factor (FGF)-18, all of which are essential in regulating cell proliferation, inflammation and tissue repair (12).

Three isoforms of TGF-β, TGF-β1, TGF-β2 and TGF-β3, exist in mammalian tissue and exhibit a high degree of homology; however, they have different tissue-specific expressions (13). They are generated in an inactive form by chondrocytes, which are usually bound to the ECM of cartilage. Shearing stress, a mechanical force caused by compressive loading, activates these inactive chondrocytes (13). Ligand binding stimulates type I and type II receptors complex generation, while TGF binding to the receptor complex is stabilized and facilitated by type III receptors, which recruit receptor-regulated small mothers against decapentaplegic (R-SMAD) protein (14). After R-SMAD is phosphorylated, a complex is formed with SMAD4, which is transported to the nucleus where it binds to transcription factors such as SRY-box transcription factor 9 (SOX9) and runt-related transcription factor 2 (RUNX2) and initiates transcription. SMAD2/3 signaling is associated with anti-hypertrophic and anti-inflammatory activity, whereas SMAD1/5/8 signaling is associated with pro-hypertrophic control of the ECM (15,16). The differential regulation of SMAD2/3 and SMAD1/5/8 pathways depends on the presence of active TGF-β concentration. SMAD1/5/8 signaling is stimulated with a comparatively high TGF-β concentration (>5 ng/ml), while low TGF-β concentration primarily stimulates SMAD2/3 signaling in human fibroblasts (17). This process is depicted in Fig. 2.

All three isoforms of the TGF-β superfamily can induce chondrogenic differentiation of mesenchymal stem cells in adult bone marrow. Compared to TGF-β1, TGF-β2 and TGF-β3 are more efficient in stimulating chondrogenesis by aggregating glycosaminoglycan (18). Scientific evidence has confirmed embryonic lethality or various bone defects of the hindlimbs and forelimbs in mice lacking TGF-β isoforms, which indicates a key role of TGF-β in skeletogenesis (19). It has been reported, based on animal models, that the elevated expression of TGF-β1 is involved in the development of OA. Injecting multiple intra-articular doses of TGF-β into mouse joints shows similar changes in the articular cartilage that occurs in experimental and spontaneous mouse OA (20). Similar results showing a higher concentration of active TGF-β1 leading to osteoarthritic changes in the bone and cartilage has been demonstrated in mice subchondral bone (21). Synovial lining layer-induced TGF-β1 expression in the murine knee joint also shows OA-like features of chondro-osteophyte formation and hyperplasia of the synovium (22).

Various *in vitro* and animal studies indicate the involvement of TGF-β in OA but findings in human data are limited (23,24). Suarez *et al* (23) report that 11 patients with hip OA and 11 patients with femoral neck fracture had higher expression of TGF-β isoforms. Wu *et al* (24) found that the protein expression of TGF-β1 was 16-fold lower in OA cartilage than in healthy cartilage, which indicate that TGF-β1 has a joint-specific effect in OA. According to data collected from six hip OA patients and four controls, TGF-β1 may also have a role in the hypertrophy stage of the OA process (25).

Supplementary TGF-β can help in sustaining joint homeostasis in a healthy joint when it is targeted to cartilage with relatively low active levels of TGF-β. Low active levels could be advantageous only when the chondrocyte TGF receptor expression pattern supports hypertrophy inhibition to retain the differentiated chondrocyte phenotype. Failing to fulfil such conditions or exposing the whole joint to high TGF-β levels may cause osteophyte formation and synovial fibrosis and may forcefully cause articular chondrocyte hypertrophy (26). Systemic inhibition of TGF-β in osteoarthritic joints may block its pathology but may influence TGF-β's crucial role in healthy cells, which may cause unwanted adverse effects on healthy cartilage.

3. TGF-β family members in OA therapy

Multiple *in vivo* experiments have shown cartilage defect treatment by adding extra TGF-β. Injecting TGF-overexpressing fibroblasts, mesenchymal stem cells and chondrocytes into rabbits enhances cartilage injury recovery through cartilage regeneration (27,28). In the joints of rabbits with experimental OA, intra-articular TGF-β1 transfection (used for its overexpression) significantly reduces cartilage matrix degradation (29). In the OA-affected cartilage, TGF-β1 expression appears to be highly linked with SMAD3 expression, but this link is not observed in healthy cartilage. Furthermore, TGF-β1 expression appears to be influenced by age, sex and obesity. TGF-β1 switches its role from a protective agent to a damage-causing agent in human OA cartilage, possibly via

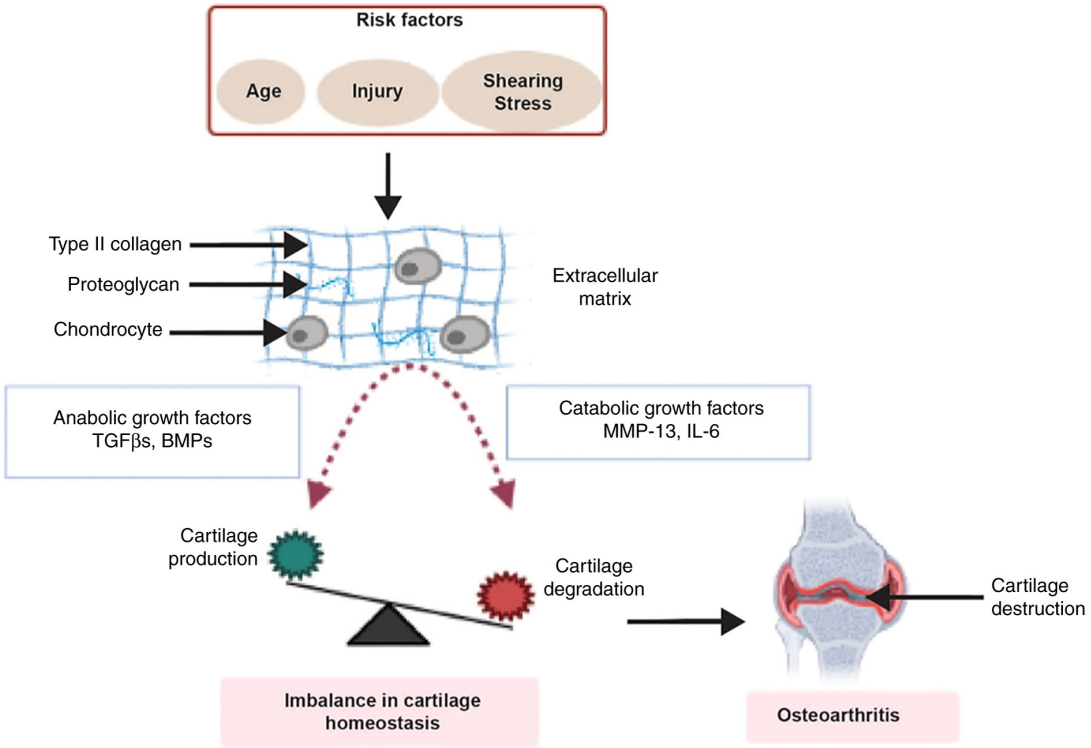

Figure 1. Explanation of the osteoarthritis process. Normal cartilage is usually controlled and maintained by the balance between anabolic and catholic process of extracellular matrix production, known as homeostasis. Osteoarthritis is generated as a result of imbalance in cartridge homeostasis by various triggering factors. Created with BioRender.com.

SMAD independent pathway by augmenting MMP-13 expression, which is a cartilage-degrading enzyme (30).

TGF-β2 also inhibits OA progression (31). It is reported to advance the expression of specific tissue inhibitor of MMP-3 (TIMP), thereby imparting cartilage protection (32). Furthermore, TGF-β2 inhibits collagenase activity and proteoglycan degradation in OA by downregulating IL-1β and tumor necrosis factor (TNF)-α (33,34). TGF-β2 regulates collagen degradation of articular cartilage by downregulating collagenase MMP-9 in OA (35). Despite having a key protective role in chondrocyte homeostasis during OA progression, a high concentration of TGF-β2 can lead to the destruction of normal cartilage (23).

In a large animal investigation that included sheep, Mrugala *et al* (36) reported a favorable result in which bovine mesenchymal stem cells (MSCs) with 50 ng of TGFβ-3 in a chitosan scaffold were utilized to fill partial thickness defects generated in the inner region of the patella. At two months, histological tests indicated the presence of chondrocyte-like cells embedded in a hyaline cartilaginous matrix that was entirely integrated into native cartilage tissue. Another finding, by Tang *et al* (37), was the clinical enhancement effect of TGF-β3 *in vitro* and *in vivo* on cartilage formation with a suitable dose and scaffold carrier. In human MSCs, TGF-β3 has shown influence on anabolic chondrogenic gene markers such 1-collagen type II and cartilage oligomeric matrix protein. For the *in vivo* study, TGF-β3 cultured with ovine MSCs in a chitosan scaffold enhances the growth of hyaline cartilage that was fully integrated into the sheep's host cartilage tissue (37).

A previous study also demonstrates dose- and time-dependent expression of TGF-β3 on the chondrogenic gene expression of cartilage oligomeric matrix protein, α1-collagen type II and alkaline phosphatase at concentrations of 10, 20 or 60 ng/ml (38). Furthermore, mode of delivery is another crucial factor for effective chondrogenesis effect of TGF-β3. *In vivo* studies have shown that hydrogels implantation containing TGF-β3 (10 ng/ul) and rabbit chondrocytes into 10 nude mice result in a substantial increase in glycosaminoglycan (GAG), collagen and chondrocyte DNA content, where continuous stimulation with them led to chondrogenesis for two weeks (39,40). TGF-β3 release from poly-lactic-co-glycolic acid (PLGA) microspheres embedded in chitosan thermo-sensitive gels is shown to be linear ≤28 days, with a concentration of roughly 3 ng/ml generating a 12-fold increase in GAG synthesis from hMSCs. This data suggested that the mode of delivery is equally important (41).

Another member of TGF-β family, BMP-6, has been linked with chondrocyte differentiation. It is also reported to be found in both normal and OA adult human articular cartilage (37). Such endogenous expression of BMP-6 in cartilage independent of the presence of OA suggests that it serves a role in joint integrity maintenance and might be used as a therapeutic molecule for cartilage regeneration (42).

Applying all these growth factors with a suitable platform for the purpose of cartilage lesion repair may represent potential therapeutics which may aid hyaline cartilage regeneration and thereby slow the progression towards OA. Most *in vitro* investigations support chondrogenesis with TGF-β3; however, very few *in vivo* studies exist and virtually no study has investigated TGF-β3 application in human OA treatment for clinical purpose (36,37,39,40). A comparative chart of different TGF-β family members are shown in Table I.

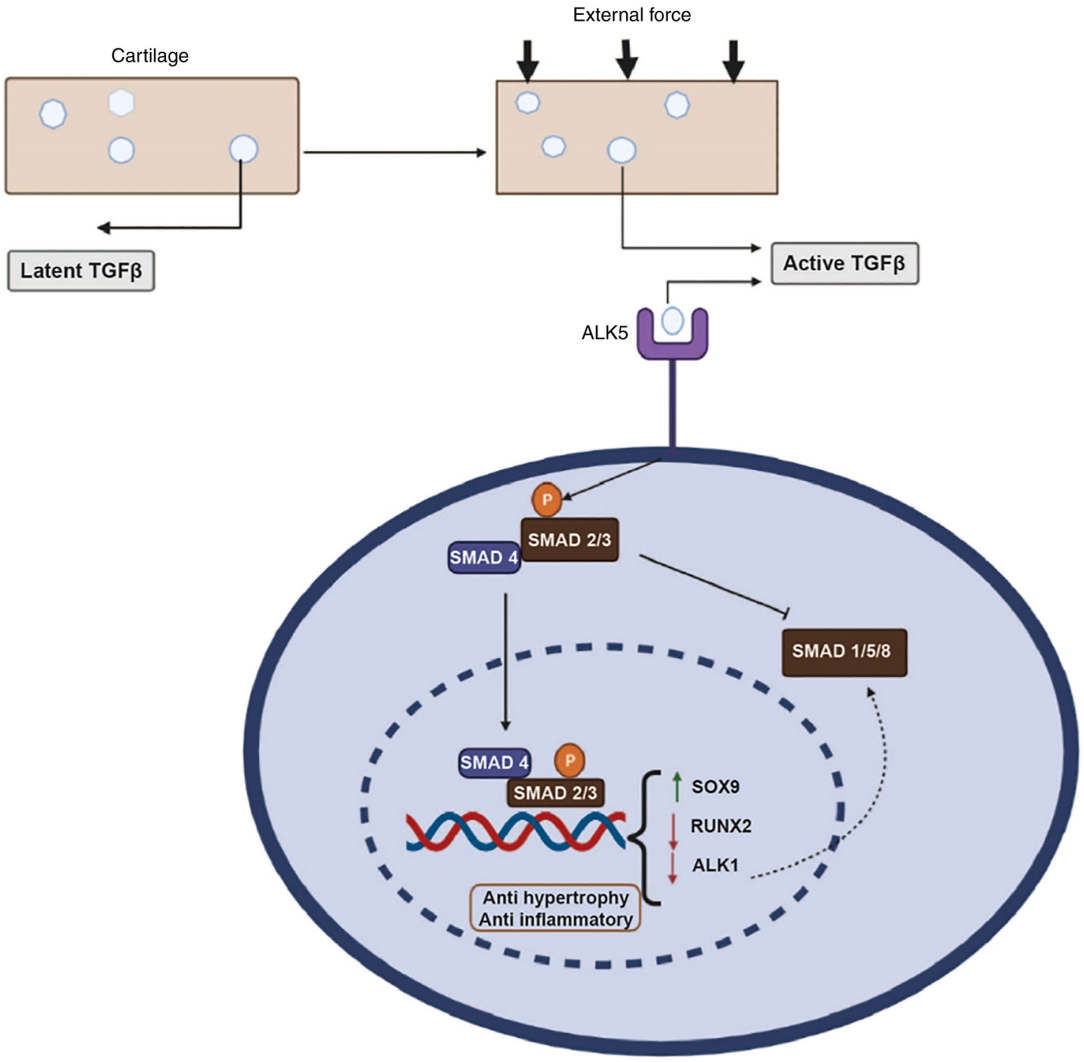

Figure 2. Process of synthesizing extracellular matrix of cartilage chondrocytes generated and regulated by binding TGF-β superfamily with SMAD protein. Created with BioRender.com. TGF, transforming growth factor; SMAD, small mothers against decapentaplegic.

4. Stem cell and stem cell-derived therapeutics in OA

Existing conventional treatments for OA include physical therapy, chondroitin sulphate supplementation or surgical therapy such as microfracture and abrasion arthroplasty which aim to improve joint function and relieve pain. However, these therapies have the limitation of being but poorly effective (43).

Adult stem cells, particularly adipose-derived stem cells (ASCs) and bone marrow-derived mesenchymal stem cells (BMSCs), are extensively used for cartilage tissue engineering due to their potential to differentiate into a chondrogenic lineage and their ability to be matched to the patient (44,45). However, these cell lines have the drawback of a limited number of cell passages. Adult stem cells with a high passage number will have short telomeres. Telomere shortening causes senescence and loss of function. This limitation is circumvented by over-expressing human telomerase reverse transcriptase (hTERT), a well-known approach for *in vitro* chondrogenesis which prevents telomere shortening and makes ASCs immortal (46).

MSCs are non-hematopoietic multipotent stem cells which are commonly employed in laboratory research. These cells can be obtained from tissues such as bone marrow, Wharton's jelly, spleen, liver and adipose tissue (47,48). Due to the ability of MSCs to develop into mesodermal tissues such as cartilage bone, muscle and ligament under certain conditions (49), MSC therapy has been proven as effective for treating OA (50). Human adipose-derived mesenchymal stem cells (ADMSCs), are multipotent stem cells and easier to harvest than are BMSCs. They can be easily isolated from subcutaneous adipose tissue by using lipoaspirates after enzymatic digestion (50). Moreover, their high abundance makes cell culture expansion easy (51,52). Findings from a comparative study on human adult MSCs derived from bone marrow, adipose tissue and dermal tissue reveal that human adipose-derived stem cells (hASCs) secrete the highest level of paracrine factors involved in tissue regeneration; this feature makes the cell line more favorable for regenerative therapies (53). In addition, low immunogenicity, self-renewal potential, ability to differentiate on multiple lineages and high rate of proliferation give this cell type further advantage over other types of stem cells (54-56). hASCs have also been linked to tissue regeneration through immunomodulation and paracrine activity (57). Spasovski *et al* (58) first reported using adipose tissue as a source of MSCs. Their findings regarding the use of

Table I. Comparative role of different members of TGF-β superfamily.

Growth factors	Characteristics	Effect on each other	Function	Relation to OA
TGF-β1	Most abundant and widely expressed isoform. TGF-β1 and TGF-β2 share 71% sequence identity.	TGF-β1 and BMP-2 have a synergistic effect in the production of hyaline-like cartilage in serum-free chondrogenic differentiation of mesenchymal stem cells. TGF-β1 and BMP-7 have a Synergistic Effect on chondrogenesis and ECM synthesis.	Promotes cartilage synthesis, articular chondrocyte growth, and cartilage repair. Stimulates chondrocyte proliferation. Upregulates essential glycolytic factors to promote maintenance of healthy articular chondrocytes phenotype.	In mouse experimental OA model, increased expression was found in developing osteophytes and articular cartilage. Increased MMP-13 expression causes cartilage destruction.
TGF-β2	Expressed by neurons in the embryonic and nervous system TGF-β1 and TGF-β2 share 71% sequence identity	Bone Morphogenetic Protein-7 shows antagonistic behavior with TGF-β2 in human trabecular meshwork cells.	Promotes chondrogenesis in interstitial cells. Controls chondrocyte differentiation, induce ECM formation and chondrocyte proliferation. In the progress to TGF-β1 induced chondrogenic differentiation, TGF-β2 alters from type I to type II collagen.	Promotes the expression of TIMP-3 imparting cartilage protection.
TGF-β3	Found in lung adenocarcinoma and kidney carcinoma cell lines. High expression level in umbilical cord. TGF-β1 and TGF-β2 share 80% sequence identity.	TGF-β3 shows synergistic effects with FGF-18 in chondrogenic differentiation. TGF-β3 and BMP-6 has shown improved chondrogenicity compared with TGF-β3 alone.	Promotes *in vitro* and *in vivo* cartilage formation; both stimulate chondrogenic differentiation of cells, synthesize glycosaminoglycan sulfate and increase extra chondral matrix components.	During mouse experimental OA, enhanced expression was found in articular cartilage and osteophytes development
BMP-2	Found mainly in lung, pancreas, kidney and spleen.	BMP-2 and TGF-β1 have shown synergistic behavior on rabbit bone marrow-derived chondrogenesis.	Induces ECM production and proliferation and bone formation. Skeletal repair and regeneration. Supports expansion of the chondrogenic phenotype of human articular chondrocytes.	Overexpressed in osteoarthritic chondrocytes. Direct injection helps cartilage and subchondral bone regeneration to treat large weight-bearing osteochondral defects. Stimulates chondrocyte maturation and hypertrophy in *in vitro* mesenchymal stromal cells.
BMP-7	Expressed in liver, brain, kidney, lung, heart, and pancreas.	TGF-β1 and BMP-7 show synergistic effect on extracellular matrix synthesis and chondrogenesis. BMP-7 shows antagonistic behavior	Promotes ECM synthesis and diminishes cartilage degradation through decreasing expression of a number of ILs and MMPs	OA cartilage has decreased levels of BMP-7. Intra-articular injection of rhBMP-7 inhibits articular cartilage degradation and blocks

Table I. Continued.

Growth factors	Characteristics	Effect on each other	Function	Relation to OA
		with TGF-β2 in human trabecular meshwork cells.	Function in postnatal maintenance of articular cartilage.	the synovial membrane's production of inflammatory cytokines.
FGF-18	Expressed mainly in heart, skeletal muscle and pancreas.	Shows synergistic behavior with TGF-β3 on the chondrogenic differentiation.	Stimulates cartilage development, promotes regeneration of hyaline articular cartilage potency and delays articular cartilage degeneration. Acts chondroprotectively via regulating TIMP-1 expression.	In rats, promotes repair of damaged cartilage in progressive OA.

TGF, transforming growth factor; OA, osteoarthritis; BMP, bone morphogenetic protein; FGF, fibroblast growth factor; IL, interleukin; MMP, matrix metalloprotease; ECM, extra cellular matrix; TIMP, tissue inhibitor of MMP.

ADMSCs showed that nine patients diagnosed with OA were treated with a single injection of ADMSCs. After six months of follow up, their clinical examination using radiography showed improvements, including the restoration of the hyaline articular cartilage, with no significant adverse effects.

A number of recent findings reveal that the therapeutic properties of stem cells have been attributed to the paracrine secretion of anti-inflammatory and chondroprotective mediators or trophic factors; in particular, small extracellular vesicles (EVs) (59-61). An exosome, a major category of EVs, is a nano-sized vesicle that is surrounded by a phospholipid membrane. It has a diameter of 30-200 nm and is present in various biological fluids such as synovial fluid, saliva, blood, urine and pleural fluid, or released by most cells, including joint cells (60). Exosomes are characterized by the presence of endosomal markers such as CD9, TSG101, CD61 and CD83. They were previously considered to serve as a means of removing undesired materials from the cell. However, subsequent research has revealed that they have an important part in intercellular signaling and infectious disease pathogenesis, which indicates they have the paracrine nature of signaling (61). Exosomes are discharged via exocytosis from multivesicular bodies (i.e. late endosomes), which fuse into the plasma membrane of target cells such as chondrocytes and transfer their packaged cargo into the cytoplasm (62). Exosomal cargo includes lipids, transcription factors, ECM proteins and nucleic acids, among other substances (e.g. mRNA) and noncoding RNA and trigger a number of physiological processes, including epigenetic changes (63).

As with healthy cells, apoptotic cells secrete exosomes, called 'apoptotic exosomes'. They have an endosomal origin, produced in a caspase 3- and 9-dependent manner. Apoptotic exosomes share the same structural features in shape and size as those produced by healthy cells; in addition, apoptotic exosomes have the functional characteristic of being an intercellular communicator. They also exhibit exosome-specific marker proteins such as CD63, LAMP1, HSP70 (a stress-associated marker released during apoptosis). However, what sets apoptotic exosomes apart is the presence of sphingosine-1-phosphate receptors 1 and 3 (S1PR1 and S1PR3, respectively), a distinguished protein marker (64,65). Previous studies have confirmed their role in inflammation, immunomodulation, cell signaling and apoptotic cell clearance (64,66).

5. Adipose-derived stem cell (ASCs)-derived exosome: A therapeutic and safe approach towards OA treatment

As stated previously, exosome-based therapy has recently sparked interest in the scientific world due to its well-known role in a number of pathobiological processes. A number of studies have confirmed the stimulatory effect of BMSC-derived exosomes on injured tissues, thereby causing cartilage and subchondral bone regeneration and repair (67,68).

Cosenza *et al* (69) first demonstrated that EVs produced by different cellular pathways have identical *in vivo* functions in OA. They showed that microparticles and exosomes isolated from BMSCs of adult mice have a similar chondroprotective impact in a collagenase-induced OA model. Exosomes from BMSCs which have been pre-treated with TGF-β3 markedly elevate anabolic marker gene expression, while decreasing catabolic marker gene expression in osteoarthritic chondrocytes.

Injecting intra-articular BMSCs exosomes results in a decrement in articular cartilage impairment and subchondral bone deterioration in a collagenase-induced mouse model (70). This study evaluates the effect of exosomes and microparticles on OA-like murine chondrocytes and both were able to restore the expression of anabolic chondrocyte markers (e.g. aggrecan and type II collagen) in OA-like chondrocytes while suppressing catabolic markers (e.g. ADAMTS5 and MMP-13) and inflammatory markers (e.g. inducible nitric oxide synthase). The two EVs also protect chondrocytes from apoptosis and suppress macrophage activation.

Furthermore, exosomes generated from BMSCs may influence the biological phenotype of other OA-related cells such as synovial fibroblasts (SFBs) or macrophages. Findings by Jin *et al* (71) demonstrate that human BMSC-derived exosomes reduce the proliferation of IL-1-treated SFBs and increased their apoptosis through an miRNA-26a-5p-mediated reduction in PTGS2. Their data show that hBMSC-derived exosomes overexpressing miR-26a-5p reduce inflammation, proliferation and migration while promoting apoptosis. Thus, these exosomes could attenuate OA damage by repressing prostaglandin-endoperoxide synthase 2 (PTGS2). Mao *et al* (72) demonstrate that, among BMSC-derived exosomes overexpressing miR-92a3p, the MSC-miR-92a-3p-exosome significantly upregulates the levels of aggrecan, SOX9, COL9A1, COL2A1 and COMP and downregulates the expression of COL10A1, RUNX2 and MMP13. This finding suggests that BMSC-derived exosomes promote chondrogenesis and prevent cartilage matrix degradation in a miR-92a-3p-dependent manner.

ASC-derived EVs have a potent function in OA modulation. In Tofiño-Vian *et al* (73), MVs and exosomes were both primarily responsible for the paracrine activity of AMSCs on osteoarthritic osteoblasts. In IL-1-treated osteoblasts, EVs from human AMSCs dramatically reduced IL-6 and PGE2 levels, increased the release of IL-10 and downregulated mitochondrial membrane potential. The findings of another study on the ADMSC-exosome by Tofiño-Vian *et al* (74) suggest that it had a chondroprotective function by using anti-inflammatory effects. That study reports that ADMSC-exosomes can reduce the secretion of proinflammatory cytokines such as IL-6, TNF-α and IL-10 in OA chondrocytes. In addition, they also revealed a decline in the cyclooxygenase-2 (COX-2) expression level, which is an OA marker, and in the generation of prostaglandin E2 (PGE2), which is a proinflammatory factor in the OA joint. The intra-articular injection of ASC-EVs may efficiently protect the cartilage from degradation and diminish OA progression in subacute and chronic arthritic models, based on findings discussed in a recent paper by Woo *et al* (75). In their findings, hASC-EVs therapy effectively inhibits the IL-1β-mediated expression of MMP-1, MMP-3, MMP-13 and ADAMTS-5, while increasing the expression of type II collagen in chondrocytes. Their findings suggest that hASC-EVs therapy increases chondrocyte proliferation and migration while also mediating the balance between catabolic and anabolic metabolism, thereby resulting in cartilage regeneration. In a study by Zhao *et al* (76), in which they extracted exosomes from donor adipose tissue by using elective liposuction surgery, the investigators discovered that adipose-derived stem cell (ADSC)-exosomes may reduce the expression of proinflammatory genes while increasing the expression of anti-inflammatory cytokines in activated SFBs and improving periosteal cell proliferation and chondrogenic potential via increasing miR145 and miR221. These findings add to the growing body of evidence suggesting that AMSC-derived exosomes could offer a novel perspective for the development of an efficient and optimized OA therapy.

6. Safety perspective

The safety parameter of any product meant for human therapeutic use has a vital role. It refers to the minimization of the risk/benefit ratio associated with the product being employed for patient treatment. ADSC-based therapy has a number of applications in regenerative medicine involved in bone regeneration, neurodegenerative diseases and autoimmune and restoring wound defects, based on its efficiency and efficacy (77). However, it also has the adverse effect of blindness in SVF-treated patients, which puts the credibility and accountability of these therapies at question (78). Henceforth, for any therapy, before entering the clinical setting, checking the safety criteria of the product and the source is imperative.

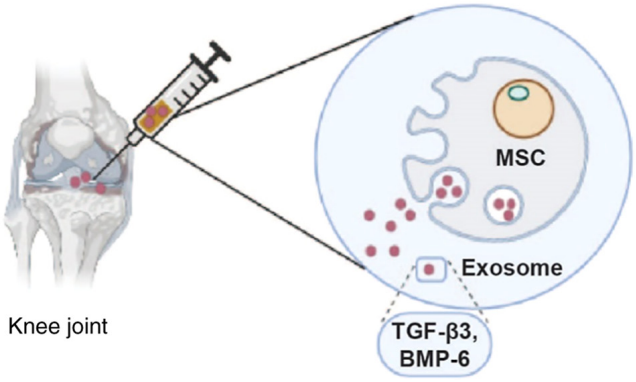

Figure 3. Schematic of Targeted therapy of exosomes into injured joint. Direct injection of exosomes carrying TGF-β3 with BMP-6 can repair cartilage injury and induce cartilage regeneration. Created with BioRender.com. TGF, transforming growth factor; BMP, bone morphogenetic protein; MSC, mesenchymal stem cell.

A number of publications of ASC-derived cell therapies to the construction of immortalized human adipose-derived MSCs vouch for its safety to be used for public health. Vériter *et al* (79) assesses the safety and efficacy of ASC-derived cell therapies and demonstrates the safety of autologous ASC transplantation in 17 patients without any serious adverse events in grafted patients. Atat *et al* (80) reveals that passaged ADSC expansion has no effect on stem cell differentiation and does not provide a malignant potential to the cells *in vitro*. Tátrai *et al* (81) demonstrate that transfecting BMI1 and TERT simultaneously into human adipose-derived MSCs results in the production of successful immortalized human adipose-derived MSCs without significantly affecting their phenotype or biological behavior. The latest research by Zhang *et al* (82), which used the same method, indicates that immortalized MSCs are safe by using *in vitro* and *in vivo* testing to confirm that immortalized MSCs are not tumorigenic. They explore the efficacy and safety of immortalized MSCs as a cellular drug carrier in brain tumor treatment.

7. Regenerating cartilages by engineered ASCs

As stated previously, BMSCs represent an appealing cell source in cartilage tissue engineering. BMSCs can accomplish chondrogenesis when stimulated with suitable growth factors such as TGF-βs and BMPs, as indicated by the overexpression of SOX9, Col2A1 and ACAN (83). However, chondro-induction of BMSCs is frequently accompanied by osteogenesis and hypertrophy, which can lead to apoptosis and calcification (84).

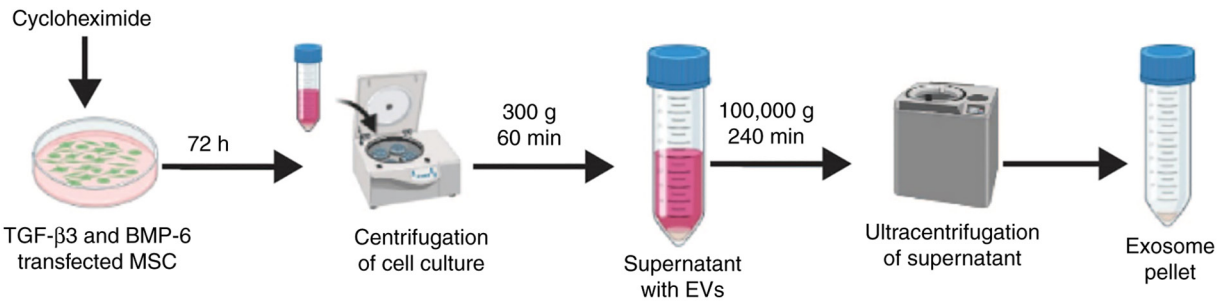

Figure 4. Schematic diagram of ultracentrifugation-based exosome isolation (Korean patent application no. 10-2020-0062365) for the mass production of exosomes. Created with BioRender.com. TGF, transforming growth factor; BMP, bone morphogenetic protein; MSC, mesenchymal stem cell; EV, extracellular vesicle.

To date, ASCs have become the more desirable stem cells used for cartilage regeneration due to the ease of production and they can initiate chondrogenesis when stimulated in chondrogenic media with TGF-β1, TGF-β3 and BMP-6 (85-87). BMSCs are superior to ASCs in the chondrogenic potential; therefore, selecting the appropriate combination of growth factors to induce ASCs chondrogenesis is essential (88).

TGF-β3 is a robust chondrogenesis inducer, while BMP-6 can synergistically induce the chondrogenesis potential of TGF-β3. The combination of TGF-β3 and BMP-6 has an exceptional potent chondrogenic effect on ASCs and will offer an ideal OA therapy. Ude *et al* (89) confirm the chondrogenic potential of ADSCs and BMSCs by using the combination of TGF-β3 and BMP-6. They compared the effectiveness of cartilage regeneration by chondrogenically induced ADSCs and BMSCs by using a combination of TGF-β3 and BMP-6. On evaluating the recovery of treated joints after 12 months, they discovered cartilage regeneration in OA in the sheep knee after injecting them with the combination of TGF-β3 and BMP-6.

Lu *et al* (90) designed genetically engineered rabbit ASCs (rASCs) with sustained TGF-β3/BMP-6 expression using baculovirus. They note that continued expression of TGF-β3/BMP-6 for two weeks enhances chondrogenesis, reduces osteogenesis/hypertrophy and results in the development of cartilaginous constructions with improved maturity and mechanical qualities. Choi *et al* (91) show the significance of stem cells as a cell source for chondrogenesis on induction with TGF-β3 and BMP-6. The two growth factors exhibit a strong synergistic effect of ≤281% when compared with control. In comparison with not only controls, but also TGF-β3 or BMP-6 single treatments, the combined therapy significantly boosts Sox9, aggrecan and collagen II expression. All these findings validated the hypothesis that TGF-β3 and BMP-6 possess a strong chondrogenic potential for OA treatment, compared to TGF-β3 alone.

As Cosenza *et al* (69) show, pre-activation of MSCs with TGF-β can boost the anti-osteoarthritic potential of exosomes and this approach of utilizing MSCs with growth factors would be an efficacious platform which exploits the benefits of stem cells, TGF-β3 and BMP-6, thus maintaining cartilage homeostasis and joint health. To bring all these facts together, incorporating MSC-derived exosomes along with the chondrogenic potential of TGF-β3 and BMP-6 would be a superior approach to halt OA progression and this would be a novel means to provide exceptional treatment for OA patients.

8. Bench to bedside

Exosomes, isolated from MSCs which themselves possess chondroprotective function, besides the characteristic of being a natural cargo carrier with the inherent property of low immunogenicity, excellent specificity and high penetrability, present an excellent candidate for targeted delivery of both TGF-β3 and BMP-6 into an injured joint for cure and treatment at the cellular level without any side effects (Fig. 3).

As exosome-based therapy seems to be the improved substitute for stem cell-based therapy, recently a number of companies are developing stem cell derived exosomes-based therapeutics for OA and joint injury. Exopharm is one such Australian based company that has developed a stem cell derived exosome-based drug for the treatment of osteoarthritis (Cevaris), which is under pre-clinical development phase. (https://exopharm.com/). Similarly, another company based in the Republic of Korea (Exostemtech) is conducting clinical trials for exosomes produced from adipose derived stem cells. (http://www.exostemtech.co.kr/).

Similarly, CK-Exogene is a Republic of Korea based company which recently launched its exosome based Covid-19 vaccine against SARS Covid-2 infection (under the process of approval from the Ministry of Food and Drug Safety) for its commercialization (92). Following a similar strategy, this company(CK-Exogene) is also planning to launch exosome-based targeted therapy for osteoarthritis treatment. With the company's patented technology of mass production of exosome using apoptosis (Fig. 4), they plan to develop targeted therapy for osteoarthritis using exosome as a drug delivery vehicle by overexpressing both TGF-β3 with BMP-6.

9. Conclusion

OA, although extensively researched, still lacks an effective and safe treatment. The only current treatment option available for advanced OA is joint replacement surgery. This surgery may pose the risks of persistent pain, surgical complications and limited implant lifespan. Existing therapy of joint treatment has multiple adverse effects and ~20% of patients receiving this surgery are dissatisfied with the outcome. In addition, scientific efforts remain far behind with the development of therapy that could slow the progression of OA or could reverse the disease phenotype.

Of note, stem cell-based therapy is useful as an alternative treatment before surgery. As stated previously, the paracrine

activity of MSCs and ADSCs has been attributed to exosome. Multiple findings stating the role of exosomes in chondroprotection have also shown exosomes may be a more favorable choice than the source itself.

Additionally, considering the crucial role of. TGF-β in the cartilage homeostasis, targeting it could be an alternative therapeutic approach. Studies have confirmed TGF-β3 as a promising candidate which has the chondrogenic potential to repair articular cartilage degeneration. Combining TGF-β3 with BMP-6, which has synergistic effect on chondrogenesis, with an efficient platform such as exosomes, which themselves possess a chondroprotective function, offers an innovative and more efficient approach to treat injured cartilage. Investigating such strategies for use in clinical practice for OA therapeutics would provide optimized treatment without posing any adverse effects and would open a novel avenue for targeted OA therapy in the future.

However, despite the findings of a number of studies supporting the fact of MSC-/ASC-derived exosomes as a treatment tool for OA, the lack of sufficient evidence makes their usage challenging. More clinical studies using exosomes for OA treatment are required to determine the repercussions and potential adverse effects.

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Availability of data and materials

Data sharing is not applicable to this article, as no data sets were generated or analyzed during the current study.

Authors' contributions

KHY, NT, YJC and JK substantially contributed to the conception and the design of the study. SHY, BJK, JOL and YNJ were contributed to data acquisition and data analysis and interpretation. And KHY, NT, YJC, SHY, BJK, JOL, YJ and JK were involved in manuscript drafting and revision and critically revised the manuscript for important intellectual content.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The purification strategy for the mass production of highly purified and concentrated exosomes is subject to Korean patent application no. 10-2020-0062365 (Fig. 4), associated with CK-Exogene, Inc. NT and JK are employees of CK-Exogene, Inc. However, the other authors (KY, YC, SY, BK, JL and YJ) are not associated with CK-Exogene, Inc. and declare that they have no competing interests.

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