Mesenchymal Stem Cell-Derived Exosomes in Cartilage Regeneration

Exosomes are the small extracellular vesicles secreted by cells for intercellular communication. Exosomes are rich in therapeutic cargos such as microRNA (miRNA), long non-coding RNA (lncRNA), small interfering RNA (siRNA), DNA, protein, and lipids. Mesenchymal stem cell (MSC)-derived exosomes have been found contain miRNAs that modulate cartilage regeneration.

1. Introduction

Cartilage damage is very common and is one of the hallmarks of degenerative joint disorders such as osteoarthritis (OA) [1]. Age, gender (female), obesity, history of knee injury, joint overuse, joint abnormality, and genetics are known to increase the risk of OA [2][3]. Relying on cartilage self-repair is insufficient to restore the structure and function of the degenerated tissue as cartilage tissue has very poor regeneration potential [4]. Unlike most tissues, cartilage is avascular, alymphatic, and anueral and has low cellularity, with sparsely distributed chondrocytes embedded within the dense extracellular matrix (ECM) [5]. Thus, early intervention is critical for the management of OA. Generally, OA management can be categorized into non-pharmacological and pharmacological management modalities. Regardless of the treatment approaches, current interventions aim to prevent further damage and achieve symptoms control. Joint replacement surgery is typically prescribed when the damage is very extensive and severely affects the patient's quality of life [6].

Regenerative medicine is a new approach introduced in the last two decades to promote the regeneration of damaged cartilage. This approach is unique as it can stimulate cartilage regeneration, which cannot be achieved with conventional treatments. In recent years, instead of cells, more attention has been given to the paracrine factors secreted by cells. In particular, exosomes, a type of small extracellular vesicle (EV) secreted by cells for intercellular communication, have become the focus of recent studies. Exosomes are rich in proteins, lipids, and nucleic acids and have been documented to promote cartilage regeneration [7][8]. Previously, a battery of miRNAs was found to be involved in cartilage regeneration [9][10][11].

2. Exosomes

2.1. Categories

Exosomes and ectosomes, which are derived from endosomes and the plasma membrane, respectively, are the two subtypes of lipid-bound EVs secreted into the extracellular environment. These EVs do not possess a nucleus structure; hence, they cannot replicate. According to the latest position statement indicated in the Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018) report, exosome and ectosome terminologies should only be used if an experimental research design can establish the subcellular origin of the EV subtype being studied. Otherwise, operational terminologies for the subtypes of EVs should be adopted, which are based on (i) particle size (small EVs for particles smaller than 200 nm, and medium and large EVs for particles larger than 200 nm), (ii) surface markers expression or biochemical recognition (such as CD81+, CD63+ or annexin A5-stained), and (iii) the origin or condition of cells where the EVs are isolated (such as hypoxic EVs, apoptotic bodies, and podocyte EVs) [12].

Exosomes can be either native or bioengineered, depending on whether they have been artificially manipulated or not. Both animals and plants can produce native exosomes, hence giving rise to animal-based exosomes or plant-based exosomes. Among the animal-based exosomes, they can be further classified depending on the type of cells that are producing the exosomes—either exosomes produced by normal and healthy cells or those that are synthesized from tumor cells. Amongst the variety of normal cells that can produce exosomes, MSC-derived exosomes have been given

considerable attention due to the capability of MSCs to self-renew and undergo multilineage differentiation. Therefore, MSC-derived exosomes are being actively investigated for their tissue regeneration potential [13].

2.2. Biogenesis

Exosomes, the smallest form of EVs, with diameters ranging from 30–150 nm [14], are usually synthesized through an endosomal-sorting complex required for transport (ESCRT)-dependent or ESCRT-independent pathways [15][16][17][18]. The ESCRT-dependent pathway for exosome biogenesis usually involves a series of budding processes that are facilitated by complex protein machinery, which is primarily made up of four distinct ESCRT proteins (0 to III). This pathway is triggered when ubiquitinated proteins are recognized by ESCRT-0 and are sequestered into specific endosomal membrane domains. After further interacting with ESCRTs I/II and forming a total complex with ESCRT III, plasma membrane infolding begins, resulting in the formation of a cup-shaped early endosome that contains cell-surface proteins and soluble bioactive substances that are accumulated from the extracellular environment. As this early endosome buds inward, it matures into a late endosome that subsequently undergoes a secondary endosomal membrane invagination to form a multivesicular body containing intraluminal vesicles [16][19][20][21][22]. These intraluminal vesicles have similar membrane positioning to the cell surface, where extracellular domains of transmembrane protein face the extracellular environment while enclosing the cytosolic entities [23]. As the multivesicular body moves to the cell surface and fuses with it, the intraluminal vesicles are released into the extracellular space, giving rise to exosomes [13]. Other than ESCRT proteins, numerous other accessory proteins have been shown to be involved in the production of exosomes through the ESCRT-dependent pathway, including programmed cell death 6-interacting protein (ALIX); tumor susceptibility gene 101 protein (TSG101); heat shock cognate protein 70 (HSC70); vacuolar protein sorting-associated protein 4 (VPS4); heat shock protein 90 (HSP90); soluble N-ethylmaleimide-sensitive fusion attachment protein receptor (SNAREs); cluster of differentiation proteins 9, 81 and 63; and syndecan-1, synthenin-1, and tetraspanins [15][21][23][24]. In the alternative ESCRT-independent pathway, ceramides play an important role as their cone-shaped feature is speculated to promote microdomain-induced budding of the endosomal membrane. These ceramides are usually produced through the removal of the phosphocholine moiety by sphingomyelinases that are abundantly present in those microdomains. The presence of ceramides will also induce the lateral isolation of cargo within the endosomal membrane that leads to the formation of intraluminal vesicles within the multivesicular body, releasing exosomes once it fuses with the plasma membrane [25][26].

2.3. Therapeutic Cargos of Exosomes

Upon secretion into the extracellular space, exosomes will migrate and deliver their contents to their target cells, resulting in the alteration of gene expression as well as the modification of physiological and biological functions. The functional modification relies on the type of exosomal contents present within the exosomes [27]. Invariably, most exosomes are rich in proteins and lipids. To date, about 8000 proteins and 194 lipids have been found to be related to exosomes [28]. Along with these, exosomes are known to carry nucleic acid cargos, including mRNAs and miRNAs, for intercellular communication [29]. Since exosomes are secreted by numerous cell types and the exosomal content is heavily associated with the cell of origin, each exosome may have different roles in intercellular communication for varying physiological effects. For example, platelets secrete exosomes containing prostaglandins that modulate inflammatory activities [30]. Meanwhile, antigen-presenting cells, such as dendritic cells, release exosomes that carry functional major histocompatibility complexes class I and II (MHC I and MHC II) to attenuate or stimulate antigen-specific B-cell or T-cell responses [31]. Similarly, many other studies have also demonstrated the involvement of exosomes in various biological processes, including coagulation, blood vessel formation, red blood cell maturation, and removal of unwanted RNAs and proteins [32].

In view of exosomes' capability to carry their cargo to the target cells for participation in both normal and pathobiological mechanisms through intercellular communication, extensive investigations have been performed to exploit their therapeutic purposes to restore the diseased tissue to its normal phenotype. One of the approaches involves the isolation of these endogenous exosomes from body fluids so that they can be artificially enriched with therapeutic miRNAs before these bioengineered exosomes are reintroduced back into the patient's body [29][32]. Exosomes have increasing popularity as a preferred drug delivery vehicle as they are non-immunogenic. In addition to that, other advantages of exosomes over MSCs are their homing ability to the site of injury and passing through tight junctions such as the blood–brain barrier. Since exosomes are very heterogeneous, they can also carry different proteins on their surface that can be delivered into the cell through receptor-mediated endocytosis upon interacting with the target cells [13]. Therefore, the exosome-assisted delivery approach has been utilized by various research groups to deliver a variety of therapeutic compounds to their target cells, including short interfering-RNA (siRNA), recombinant proteins, miRNA, and antagomirs, as well as anti-inflammatory and chemotherapeutic drugs [25]. For instance, exosomes equipped with siRNAs against the mRNA of Huntington's disease have been successfully introduced into the cortical neurons of mice, resulting in an improvement of the murine Huntington's disease condition [33]. Through the use of exogenous siRNAs, the MAPK gene in target

lymphocytes and monocytes could be silenced. Hence, this proves the feasibility of bio-engineering isolated exosomes for the targeted delivery of therapeutic materials to a specific tissue to exert its function [34]. Alternatively, donor cells can be bio-engineered to contain the therapeutic materials, which will then be synthesized and encapsulated in the exosomes produced. These enriched exosomes can then be used for the treatment of specific diseases. An example would be a study done by Shimbo et al. that introduced miR-143 into MSCs, where this miRNA was then secreted and packaged into the exosomes. Upon delivery to osteosarcoma cells, the miR-143 present within the exosomes were able to significantly inhibit the migration of the cancerous cells [35]. In view of these and many other successful examples in utilizing exosomes as a delivery vehicle for therapeutic purposes, exosomes have been accepted as drug carriers for the treatment of various diseases in clinical trials [15].

3. Promoting Cartilage Repair Using Exosomes

Plastic and reconstructive surgery can be used to restore the structure and function of cartilage defects. However, these treatments are more successful for minor defects. When it concerns a sizable cartilage defect, such treatment options still pose some limitations. Therefore, autologous or matrix-assisted implantation of chondrocytes could be a more effective treatment. Unfortunately, the use of these therapeutic approaches is also restricted by the lack of donor sites as a result of possible post-donation adverse effects as well as risks associated with the deterioration of graft tissue [36]. Recently, researchers have resorted to utilizing exosomes derived from stem cells as an alternative therapy due to the ease of accessibility, unlimited supply, and better stability. Besides that, exosomes are secretory products enriched with active molecules that could result in the desired therapeutic outcomes [37]. The following paragraphs describe the composition of exosomes that are desirable for therapeutics.

Firstly, exosomes contain various types of growth factors and microRNAs that can stimulate cartilage regeneration. This notion arose through a study that demonstrated the successful repair of injured cartilage tissue when it was co-cultured with MSCs. It was eventually discovered that the cartilage tissue regeneration was achieved due to the presence of different growth factors secreted by MSCs, including fibroblast growth factor (FGF) 2, interleukin (IL)-6, and insulin-like growth factor (IGF). These biological factors possess the capability to induce the proliferation and matrix synthesis of chondrocytes, thus assisting in the healing of the injured cartilage [38]. In agreement with this, Jiang et al. observed enhanced osteochondral regenerative activity in the presence of exosomes derived from human umbilical cords. These exosomes were able to induce the proliferation and migration of chondrocytes, hence repairing the osteochondral knee injury in a rabbit model. The regenerative potential of the exosomes in this study was attributed to the presence of about 20 miRNA types that may exert positive effects in the regulation of the joint microenvironment [39]. Similar findings were observed in another study in which complete recovery of osteochondral defects in a rat model was achieved after treatment with MSC-derived exosomes. The restored cartilage and osteochondral bone displayed normal structural characteristics that resembled their normal counterparts, such as hyalinized cartilage with ideal surface regularity and good attachment to the adjoining cartilage, with well deposited ECM [40].

Other than their regenerative capacity, exosomes were also found to possess chondroprotective effects. For instance, treatment of synovial explants with MSC-conditioned media was found to inhibit the expression of matrix degradative enzymes, including matrix metalloproteinase (MMP)-1, MMP-12, and IL-1b, thus facilitating the repair of cartilage tissue [41]. A separate in vitro study also pointed towards the aptitude of MSCs to protect chondrocytes in cartilage via the upregulation of type II collagen production [42] to resynthesize the matrix. Apart from restoring the cartilage matrix, enhanced expression of type II collagen could also prevent the hypertrophy of chondrocytes, thus avoiding the progression of cartilage degeneration [43]. Moreover, this chondrogenic protective effect has been demonstrated by Cosenza et al., who showed the ability of MSC-derived exosomes to exert protective effects on chondrocytes in a murine model with induced joint disease. This chondroprotective outcome was achieved through an increased expression of chondrocyte markers (such as aggrecan and type II collagen) while suppressing catabolic genes (including ADAMTS5 and MMP-13) to prolong the survival of OA-like chondrocytes induced with IL-1β [44]. Additionally, MSC-derived exosomes potentially assisted in cartilage repair by increasing the number of chondrocytes by preventing chondrocyte apoptosis through the upregulation of anti-apoptotic proteins, including Bcl-2 and survivin [45].

Post-traumatic inflammation almost always occurs alongside cartilage injury, and it is a huge obstacle for cartilage repair. This is because cartilage is an avascular tissue; hence, they do not have the capability to resolve an inflammatory response, resulting in the cartilage being easily assaulted by the proinflammatory mediators [46]. Thus, it becomes a critical therapeutic obstacle to suppress inflammation prior to the repair of injured cartilage via regeneration. In this aspect, MSC-derived exosomes have proven to be valuable as they can exert an anti-inflammatory response. For example, adipose MSC-derived exosomes could decrease levels of pro-inflammatory mediators, reducing stimulation of NF-κB and activator protein 1 while increasing expression of anti-inflammatory cytokines and the expression of miR-100-5p, which

binds to 3′UTR of mTOR to augment autophagy activity [8][47]. With similar mechanisms, exosomes isolated from bone marrow-derived MSCs [48] and umbilical-cord-derived MSCs [49] were able to relieve the pathological characteristics in diseased joints through decreasing the infiltration of inflammatory cells as well as repressing levels of inflammatory factors [50].

4. Exosomal miRNAs

The cargo of stem cell-derived exosomes has been widely studied since 2010 when the therapeutic potential of MSC-derived exosomes was first described [51]. Although exosomes are enriched in many bioactive molecules, such as lipids, proteins, RNA, and mtDNA [52], the therapeutic potential of MSC-derived exosomes is usually rationalized with the presence of biologically relevant miRNA and proteins [53]. To date, more than 1000 proteins have been identified and mapped in MSC-derived exosomes, suggesting that the proteome of MSC-derived exosomes play a vital role in various biological processes such as cellular communication, exosome biogenesis, and tissue repair [54][55][56]. Similar to the MSC-derived proteins, many studies have suggested that MSC-derived miRNAs have the potential to modulate cell–cell communication as well as influence the progression of various diseases by regulating the signaling pathways of the recipient cells.

The miRNAs are a group of small, non-coding single-stranded RNAs, averaging 19–24 nucleotides, that regulate post-transcriptional gene expression. miRNAs are essential for physiological development and are involved in a variety of biological processes [57]. Abnormal expression of miRNAs is associated with several human diseases [58]. In addition to intrinsic cellular functions, miRNAs are secreted into extracellular fluids via EVs as signaling molecules mediating intercellular communication [59][60][61][62]. Hypothetically, each functional miRNA can interact with up to 200 mRNAs [63]. Intercellular communication can occur by several means, including receptor-mediated chemical interaction, direct cell–cell communication, and cytosolic synapses. These intercellular gene communications may occur not only in the microenvironment but can also occur at a distance through the secretion of exosomes into systemic circulation. Indeed, exosomes may be a more effective intercellular communication option, compared to proteins or small biochemical molecules such as mRNAs and miRNAs, that can regulate recipient cell protein production and gene expression. The ability of exosomes to deliver genetic material into cells at a distance also makes them ideal candidates for cell-free therapy. Over the decades, many studies have been conducted to study the potential of MSC-derived exosomes for treating OA and to identify the miRNAs that play a vital role in maintaining a healthy joint (**Table 1**).

Table 1. List of miRNAs in MSC-derived exosomes related to chondrocytes and cartilage regeneration.

miRNA	Target	Physiological Role	Ref.
miR-23b	PKA	Induce chondrogenic differentiation of human MSCs by inhibiting PKA signaling	[64]
miR-92a	Noggin3	Targets Noggin3 and activates the PI3K/Akt/mTOR pathway to positively regulate the proliferation and matrix synthesis of chondroprogenitors	[65][66]
miR-125b	ADAMTS-4	miR-125b overexpression suppresses IL-1-induced upregulation of ADAMTS-4 in human OA chondrocytes	[67]
miR-320	MMP-13	Downregulates MMP-13 expression in both the ATDC5 cell model of chondrogenesis and IL-1-treated primary mouse chondrocytes	[68]
miR-145	Sox9	miR-145 inhibition upregulates Sox9 expression and promotes MSC chondrogenesis	[69]
miR-221	MDM2	Downregulates MDM2 to prevent slug protein degradation that, in turn, negatively regulates chondroprogenitor proliferation	[70]
miR-22	PPARA, BMP-7	miR-22 inhibition upregulates BMP-7 and PPARA expression, inhibits IL-1 expression, and suppresses MMP-13 expression in OA chondrocytes	[71]
miR-92a-3p	Wnt5a	Regulate cartilage development and homeostasis by targeting Wnt5a	[72]
miR-135b	Sp1a	Promote chondrocyte proliferation and cartilage repair in OA by downregulating Sp1a in chondrocytes	[73]
miR-100-5p	mTOR	Inhibit mTOR signaling pathway to enhance chondrocyte autophagy	[74]
miR-140-5p	YAP	Enhance ECM secretion and induce proliferation and migration of articular chondrocytes via activating YAP as well as prevent osteoarthritic joint damage	[75]

miRNA	Target	Physiological Role	Ref.
miR-26a-5p	PTGS2	Promote the survival of synovial fibroblasts and reduce synovitis	[76]
miR-136-5p	ELF3	Inhibit cartilage degeneration in traumatic osteoarthritis	[77]
miR-127-3p	CDH11-mediated Wnt/β-catenin pathway	Inhibit CDH11, thereby blocking the Wnt/β-catenin pathway in chondrocytes and reducing the chondrocyte damage in osteoarthritic joints	[78]
miR-9-5p	Syndecan-1	Has anti-inflammatory and cartilage protective effects on osteoarthritis	[79]

5. Summary

In summary, exosomes contain miRNAs that can modulate cartilage repair and regeneration by enhancing proliferation, attenuating apoptosis, promoting chondrogenesis, increasing cartilage matrix secretion, and subsiding inflammation. Thus, exosome therapy has great potential for treating cartilage diseases and promoting cartilage regeneration,

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