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Review

Concise review: Extracellular vesicles from mesenchymal stem cells as cellular therapy

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Abstract

Extracellular vesicles (EVs) from mesenchymal stem cells (MSCs) are microvesicles produced from cells throughout their life. From research over recent years, there has been greater understanding about EVs, including their physiological characteristics and the role they play in cell targets. Indeed, EVs carry information (in the form of RNA, DNA and protein) to cell targets. Some of their main biological properties include angiogenesis and immune-modulation. Therefore, these properties can be exploited to treat various diseases, including bone disorders, spinal cord injury and diabetes mellitus. Recently, new methods have been developed to isolate and enrich EVs with high performance and low-toxicity. Thus, EVs have emerged as the new generation of stem cell therapy. This concise review aims to highlight some recent achievements of EVs in preclinical and clinical applications.

Keywords

Acellular therapy, Exosome, Extracellular microvesicle, Mesenchymal stem cell, Microvesicle, Stem cell

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Introduction

Stem cells are unspecialized cells with long-lasting self-renewal potential. After differentiation they can become specialized cells with new physiological functions (Bongso and Lee, 2005). In recent years, stem cells have been discovered to exhibit other useful functions, including secretion of cytokines

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(Kilroy et al., 2007), i.e. growth factors which help stimulate tissue regeneration (Boomsma and Geenen, 2012), and notably immune modulation of mesenchymal stem cells (Abdi et al., 2008; da Silva Meirelles et al., 2009; Prockop and Oh, 2012; Yanez et al., 2006). To date, stem cells (particularly MSCs) have been discovered to have at least 3 functionst: homing and differentiation into tissue specialized cells, production of cytokines and growth factors, and immune modulation.

Given these functions, stem cells have been tested for various diseases; in fact over the last 50 years they have been evaluated in more than 50 different diseases (Ginn et al., 2013; Squillaro et al., 2016; Van Pham, 2016). Hematopoietic stem cell transplantation has been widely used to treat hematopoietic malignancies, and MSC therapy has already been approved as routine treatment for a number of diseases in Canada, Japan, Korea, China, India and Vietnam. Compared to HSCs, MSCs play many more functions *in vivo* and possess unique characteristics, such as immune modulation and tissue healing via secreted factors (Chase and Vemuri, 2012).

One of the most well-known secreted factors is extracellular vesicles (EVs). EVs have distinct physiological characteristics and have been studied for disease applications for the past 5 years. This review aims to summarize the characteristics of EVs and their applications in the clinic.

What are extracellular vesicles?

Extracellular vesicles are nano-sized particles produced from the live cells during their lifespan. EVs can be classified into two main kinds based on their size: exosomes and microvesicles. Exosomes are about 40-150 nm in diameter whereas microvesicles are about 50nm-2000 nm in diameter. They differ in the way they are produced and, thus, exosomes and microvesicles exhibit different properties.

Generally, exosomes are produced from secretory mechanisms and are regulated by endosomal sorting complex mechanisms, which are associated with transport proteins (e.g. ESCRT), Rab proteins, tumor protein p53 pathway, tumor suppressor-activated pathway 6, and ceramide/neutral sphingomyelinase (Lespagnol et al., 2008; Ostrowski et al., 2010; Rak, 2013). Moreover, exosomes are rich in tetraspanins (CD63, CD81, CD9, etc.), gangliosides, sphingomyelin, and saturated lipids. Exosomes generally have a more rigid membrane than that of microvesicles which allow them to be more resistant to degradation and thus more stable (Pols and Klumperman, 2009; Raposo and Stoorvogel, 2013; Stoorvogel et al., 2002).

Microvesicles are directly produced from the plasma cellular membrane and therefore they contain some cytoplasmic components. Unlike exosomes,

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microvesicles contain markers of the original cells, such as common proteins of the cellular membrane like integrins, glycoprotein Ib (GPIb), and P-selectin (Kastelowitz and Yin, 2014; Raposo and Stoorvogel, 2013).

Besides exosomes and microvesicles, apoptotic bodies and oncosomes can also be found in EVs (Crescitelli et al., 2013; Meehan et al., 2016). Apoptotic bodies are products of the apoptotic process while oncosomes are larger vesicles produced from cancer cells.

Physiological functions of EVs

EVs are comprised of exosomes, microvesicles, apoptotic bodies and oncosomes, and play important physiological roles, especially in cellular communication. They are important not only in the normal physiological processes but also in pathological conditions. The roles that EVs play are dependent on the content they cargo. It was initially discovered that EVs contain siRNA molecules (Eirin et al., 2014; Kumar et al., 2015; Lai et al., 2015; Vallabhaneni et al., 2015). Nowadays, it is known that they carry many more forms of "information". In fact, exosomes have been described as "information cargos" for their transport of siRNA, DNA, peptides, and proteins (Baglio et al., 2012; Biancone et al., 2012; Camussi et al., 2010; Lai et al., 2015; Rani et al., 2015; Yu et al., 2014). All of these aforementioned molecules help regulate cell targeting by modulating gene expression and gene regulation in target cells at the level of post-transcription and translation (Camussi et al., 2011; Collino et al., 2010; Mokarizadeh et al., 2012; Zhang et al., 2015b).

EVs from mesenchymal stem cells (MSCs)

It has been known for over a decade that MSCs can produce EVs. MSC-derived EVs (MSC-EVs) contain at least 2 components: exosomes and microvesicles. Both exosomes and microvesicles express tetraspanin molecules and MSC markers on their surface; these include CD9, CD63, CD81 and CD107, and CD29, CD73, CD44 and CD105, respectively (Lai et al., 2015; Yu et al., 2014).

The components inside EVs have been the focus of many research studies (Baglio et al., 2012; Lo Sicco et al., 2017; Wang et al., 2017; Yuan et al., 2017). The main components found inside MSC-EVs are miRNAs (De Luca et al., 2016; Fafian-Labora et al., 2017; Livingston and Wei). Notably, MSC-EVs have been found to contain miR-223, miR-564, and miR-451 (De Luca et al., 2016; Nawaz et al., 2016). These miRNAs play the important roles in cell survival, cell differentiation, and immune regulation (Yanez-Mo et al., 2015)). Besides miRNAs, other RNAs can be found in MSC-EVs (Borger et al., 2017). Such RNAs include transcription factor CP2/clock homolog (which regulates transcription),

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retinoblastoma-like 1 (which also regulates transcription), small ubiquitin-related modifier 1 (which regulates cell proliferation), and interleukin-1 receptor antagonist (which regulates immune responses) (Tomasoni et al., 2013).

MSC-EVs are generally comprised of 3 main groups of proteins/molecules, including surface receptors, signaling molecules, and cell adhesion molecules. These proteins were demonstrated to regulate cell self-renewal and differentiation. Surface receptors and cell adhesion molecules are present on the surface of EVs, and likely originated from cell membrane. Conversely, signaling molecules are usually found within EVs, and likely originated from secretory processes. Some common surface receptors found in MSC-EVs include platelet-derived growth factor receptor, epidermal growth factor receptor, and plasminogen activator urokinase receptor. Some common cell adhesion proteins include fibronectin, ezrin, IQ motif containing GTPase activating protein 1, CD47, integrins, lectin galactose binding soluble 1 (LGALS1), and lectin galactose binding soluble 3 (LGALS3) (**Fig. 1**).

Other important molecules to be found within MSC-EVs include RAS-related protein/neuroblastoma RAS, mitogen-activated protein kinase 1 (MAPK1), guanine nucleotide-binding protein subunit 13/G protein subunit 12 (GNA13/GNG12), cell division control protein 42 homolog, Vav guanine nucleotide exchange factor 2, transforming growth factor beta, mitogen-activated protein kinase, and peroxisome proliferator-activated receptor (Baglio et al., 2012). Given these important aforementioned components, MSC-EVs represent a cellular therapeutic approach with great potential in regenerative medicine (**Fig. 1**).

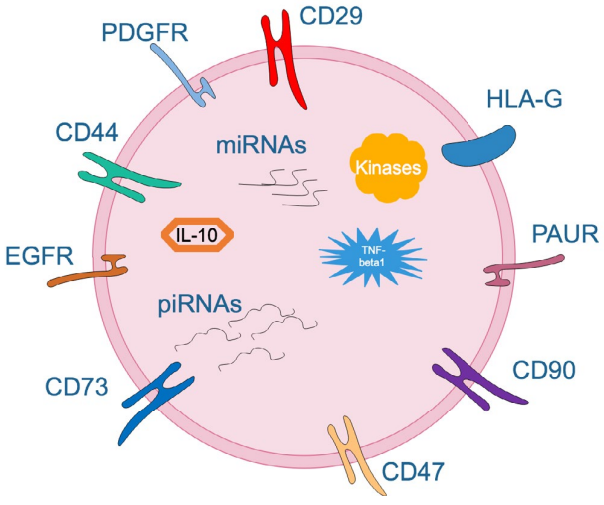

Figure 1. The components of EVs from MSCs. EVs from MSCs contain some markers included CD29, CD44, CD73, CD90, CD47; some receptors in the their surfaces, and some miRNAs, piRNAs, IL-10, TNF-beta1, some kinds of kinases… inside the EVs.

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Applications of EVs

Critical size bone defects

MSCs-EVs have been evaluated for treatment of certain bone defects. Recently, MSC-EVs were shown to stimulate bone regeneration in a bone defect model (Qin et al., 2016). MSC-EVs promoted cartilage restoration and subchondral bone regeneration in osteochondral defects (Zhang et al., 2016). They were also capable of preventing bone loss and enhancing neo-angiogenesis in a femoral head necrosis model (Liu et al., 2017). Qin et al. (2016) isolated MSC-EVs by gradient ultracentrifugation and ultrafiltration. These EVs were used to treat osteogenesis both *in vitro* and *in vivo*. The authors showed that MSC-EVs could induce bone formation in Sprague Dawley rats with calvarial defects (Qin et al., 2016). Qin et al. showed evidence that miR-196a in MSC-EVs may play an essential role in the regulation of osteoblast differentiation (Qin et al., 2016).

Zhang et al. (2016) also tested the intra-articular injection of 100 ug EVs per rat bearing osteochondral defects (n=12 adult rats); the EVs were derived from human embryonic MSCs. After 12 weeks of injection, the EV-treated group showed an histological score greater than that of PBS. Moreover, cartilage and subchondral bone were restored (Zhang et al., 2016).

EVs derived from MSCs can differentiate from induced pluripotent stem cells, according to a study by Liu et al. (2017) (Liu et al., 2017). Indeed, EVs are sometimes referred to as induced pluripotent stem cell-/differentiated mesenchymal stem cell-derived exosomes (iPS-MSC-Exos). These exosomes can stimulate endothelial cells to proliferate and migrate, and stimulate tube forming via expression of PI3K/Akt signaling pathway (Liu et al., 2017). By this mechanism, the iPS-MSC-Exos can prevent the bone loss and increase microvessel density in the femoral head compared to the placebo group in the rat model.

Epidermolysis bullosa (EB)

Epidermolysis bullosa (EB) is a rare genetic disorder of which dystrophic epidermolysis bullosa (DEB), which causes skin fragility, is one of the major forms. In this disorder, patients lack collagen type 7 (C7) and have defective anchoring fibrils at the dermal-epidermal junctions (Fine et al., 2014). Recently, this disease was treated by infusion of the MSC-EVs. EVs derived from human embryonic stem cell differentiation, from human biblical cord, and from adipose tissue were used to treat this disease in animal models. The first pre-clinical trial was conducted in a murine model; the authors injected MSC-EVs (from human ESCs) and evaluated allogenic skin grafts in the mouse model. The results showed that the infusion induced the M2 phenotype in monocytes *in vitro* and regulatory T cell polarization *in vivo*, as well as enhanced the survival of skin grafts (Zhang et al., 2014).

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EVs from umbilical cord-MSCs have demonstrated to activate the WNT4 signaling pathway in deep second degree burn injury in rats. Therefore, these EVs can accelerate skin regeneration (Zhang et al., 2015a). EVs from ADSCs can recruit fibroblasts to the wound areas, increase collagen type I and III, and reduce scar formation (Hu et al., 2016).

Spinal cord injury (SCI)

Spinal cord injury is a condition related to a disconnection of axons which direct signals from the brain to peripheral organs. Injection of EVs was shown to be an effective treatment for SCI in animals. Both EVs from MSCs and embryonic neurons successfully reduced inflammation and promoted neuro-regeneration in rats after SCI (Doeppner et al., 2015; Han et al., 2015; Rivero Vaccari et al., 2016). The mechanisms of action are likely a weakening of TLR4 mediated signaling and reduction of the IL-1beta and TNF-alpha axes (Teixeira et al., 2015).

GVHD in hematopoietic stem cell transplantation

Graft versus host disease (GVHD) is a common condition that arises in almost all cases of hematopoietic stem cell transplantation (HSCT), especially allo-graft transplantation. GVHD arises when the new immune system that comes from the HSCs attack to the owner cells. Recently, EVs from MSCs can contribute to improving the allo-HSCT allograft. Indeed, the MSC-EVs can modulate the immune system (Blazquez et al., 2014; Budoni et al., 2013; Chen et al., 2016; Conforti et al., 2014), therefore, they can be used to prevent or reduce the immunoreactions as GVHD. In the clinical study, Kordelas et at. showed that bone marrow MSC-EVs could alleviate the GVHD symptoms in grade IV GVHD patients with no side effects (Kordelas et al., 2014). This study also showed that MSC-EVs contained some anti-inflammatory factors included IL-10, TGF-beta and HLA-G.

In another study in animal model, Wang et al. also showed that umbilical cord blood derived MSCs-EVs can prevent the acute GVHD in mouse model of allo-HSCT (Wang et al., 2016).

Acute renal injury

Acute renal failure (ARF) is characterized by the loss of renal function with concurrent accumulation of creatinine and nitrogen metabolism products (e.g. urea). This condition is associated with ischemia, reperfusion injury, and/or exposure to nephrotoxic agents. The effects of EVs in ARF have been investigated in some models of ARF, including models of kidney injury induced by glycerol, cisplatin, and gentamicin. In these models, high inflammatory reactions were observed, with an increase of interstitial infiltrate, apoptosis and tubular necrosis. MSC-EVs have been evaluated as treatment in these models. In almost all cases, injection of EVs decreased inflammation and inhibited

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apoptosis. To date, there are 3 clinical trials using MSCs to evaluate the efficacy and safety of ARF: NCT01275612, NCT00733876 and NCT01602328. However, there has not been any clinical trial using EVs for the treatment of ARF.

Diabetes mellitus

The first documented study showing the application of MSC-EVs for treatment of diabetes type 1 (T1D) was reported this year; Shigemoto-Kuroda et al. (2017) demonstrated that MSC-EVs effectively prevented the onset of disease in T1D. In this study, the authors showed that the effects MSC-EVs were similar to that of MSCs in terms of immune modulation potential. EVs have been shown to be capable of inhibiting antigen presenting cells, and Th1 and Th17 cells (Shigemoto-Kuroda et al.).

Conclusion

EVs from MSCs contain some biological components such as DNA, RNA and proteins. These molecules help EVs exhibit particular physiological activities and functions, similar to those of MSCs, such as stimulation of tissue regeneration and immune modulation. Therefore, EVs from MSCs have become increasingly popular to study in recent years. Importantly, primary investigations have indicated the promise of EVs in applications of regenerative medicine.

Abbreviations

ARF: Acute renal failure
EB: Epidermolysis bullosa
EVs: Extracellular vesicles
GVHD: Graft versus host disease
HSCT: Hematopoietic stem cell transplantation
MSCs: Mesenchymal stem cells
SCI: Spinal cord injury
T1D: Diabetes type 1

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