Exosomes of human mesenchymal stem/stromal/medicinal signaling cells

Minireview

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In this review, we intend to explore the potential therapeutic effects of exosomes released from mesenchymal stem/stromal cells (MSCs). MSCs gained credibility as a therapeutic tool due to their potential to differentiate into many cell types like osteoblasts, chondrocytes, adipocytes, muscular, endothelial, cardiovascular, and neurogenic cells. They possess potent wound healing activity due to their immunosuppressive and anti-inflammatory properties. MSCs are tested in large number of clinical trials for treatment of diseases, which do not have adequate therapy at present. MSCs engineered to express suicide genes in preclinical studies have shown promising tumor targeting therapeutic tool for malignancies difficulty treatable at present. It has been increasingly observed in many different kinds of regenerative medicine and in MSCs mediated prodrug gene therapy for cancer that the intravenously administered of MSCs did not necessarily engraft at the site of injury or tumor. The therapeutic effect was exerted mainly through a paracrine action of rich secretome released from the cells. The main biocomponent of secretome are exosomes – naturally occurring membrane nanoparticles of 30-120 nm in diameter that mediate intercellular communication by delivering biomolecules like mRNA, miRNA into recipient cells. These nanosized exosomes derived from MSCs promise to be a new and valuable therapeutic strategy in regenerative medicine and cancer therapy compared with transplanted exogenous MSCs. Advantage of nanosized exosomes compared with administration of exogenous MSCs is multiple. Exosomes are easier to preserve and be transferred, have lower immunogenicity and therefore are safer for therapeutic administration.

Key words: mesenchymal stem cells, exosomes, conditional medium, secretome, regenerative medicine, yCD::UPRT gene, prodrug gene cancer therapy, yCD::UPRT exosomes

Mesenchymal stem cells (MSCs) were assigned as cells responsible for repair and maintenance of used and damaged tissues keeping cellular homeostasis in the body. They are frequently called mesenchymal stromal cells because they are supporting other stem cells in tissues, for example in the bone marrow by forming stroma support for hematopoietic stem cells. Originally they were thought to be stem cells based on their ability to differentiate to a variety of cell types *in vitro*. MSCs were regarded multipotent having the potential to differentiate into many cell types. The definition of MSCs is up to now based on an internationally approved set of criteria including plastic adherence, tri-lineage *in vitro* differentiation ability and expression of various MSC surface markers [1]. MSCs gained credibility as a therapeutic tool due of their potential to differentiate into many cell types like osteoblasts, chondrocytes, adipocytes, muscular, endothelial, cardiovascular, and neurogenic cells. In addition to their direct role in tissue regeneration, MSCs have potent wound healing activity due to their immunosuppressive and anti-inflammatory properties. MSCs can be isolated and easy expanded from tissues like bone marrow, adipose tissue, umbilical cord, placenta, dental pulp and others. Very wide range of therapeutic applications are tested in large number of clinical trials for treatment of diseases, which do not have adequate therapy at present. MSCs clinical studies are in progress around the world for clinical conditions such as multiple sclerosis, amyotrophic lateral sclerosis, stroke, acute and chronic heart failure, rheumatoid arthritis and osteoarthritis, Crohn's disease, kidney or liver chronic disease, sepsis, spinal cord contusions, critical limb ischemia and others. As of May 2017, the public clinical
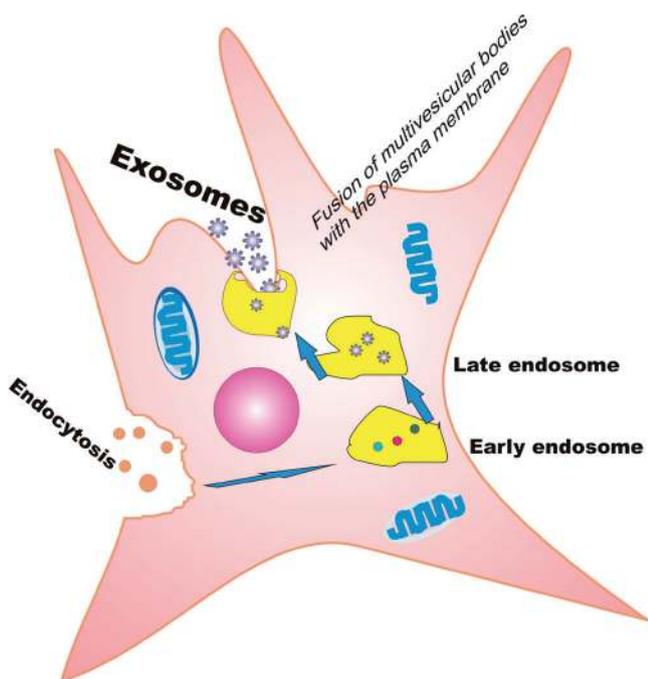
Figure 1. Schematic picture depicting MSCs exosome formation. Exosomes are secreted by MSCs upon fusion of multivesicular bodies with the plasma membrane. Their cargo contains functional biomolecules like mRNA, miRNA, DNA and proteins. Besides that, they secrete cellular waste. This behavior is used for therapeutic purposes, like for easier delivery of drugs or therapeutic gene modified MSCs release exosomes with mRNA of relevant gene. The figure is not drawn to scale.

trials database (as shown on http://www.clinicaltrials.gov with "mesenchymal stem cells" used as the search term) showed 719 clinical trials related to MSCs involvement [2].

Reparatory process is induced by action of MSCs' secretome

It has been increasingly observed in many different kinds of regenerative medicine that the transplanted MSCs did not necessarily engraft and differentiate at the site of injury. When MSCs were intravenously administered, up to 80 % of the injected cells were found in the lungs within a few minutes after injection with a half-life of about 24 hours and practically complete disappearance after 4 days in animal model [3, 4]. The observed biodistribution patterns were confirmed by studies in humans. In patients with mammary carcinoma [5], the therapeutic effect was exerted by inducing the endogenous natural reparatory processes with noticeable improvement 6-8 weeks later. This was found not compatible with direct action of transplanted cells in tissue regeneration as it was believed previously. MSCs biodistribution data confirmed the importance of secreted factors from the cells into conditional medium (CM), some acting mainly through a paracrine action. It has been recognized that MSCs release a rich secretome containing massive amounts of cytokines,

chemokines and growth factors, together with extracellular exosomes that might be responsible at least to some degree for transfer of regulatory gene products needed for the paracrine and endocrine induction of reparatory processes. It is now well recognized that exosomes from various cells including tumor cells because of their easy cells internalization can act in trans-tissue manner [6]. MSCs exosomes act mainly as tissue stromal support and help maintain tissue homeostasis, Timmers et al. have shown the reduction of myocardial infarct size by human MSCs conditioned medium (CM) administered intravenously and intracoronary in a porcine model at first [7]. It was proved that purified exosomes were responsible for the reduced infarct size in an animal model of myocardial ischemia/reperfusion injury [8]. Therefore, MSCs act through paracrine mechanisms to trigger regenerative processes [9]. The main component within the secretome are exosomes, which transfer bioactive molecules (mRNAs and microRNAs) as a cargo between cells [10, 11]. It was suggested by Caplan [12, 13] that the name of MSCs should be changed to Medicinal Signalling Cells to more accurately reflect the fact that these cells home in on sites of injury or disease. These cells make therapeutic drugs *in situ* by secretion of bioactive factors that suppress the local immune system, inhibit scar formation and apoptosis, enhance angiogenesis, and stimulate mitosis and differentiation of tissue-intrinsic reparative stem cells. It is, indeed the combined action of MSCs's secretome and secreted exosomes triggering the patient's own site-specific and tissue-specific resident stem cells to construct a new tissue and/or repair the damaged one. The content of exosome's cargo, specifically mRNA, in the induced reparatory process plays an important role.

Nanoparticles released by MSCs

Most cell types release extracellular vesicles as membrane-surrounded structures. Three main classes, exosomes (30-120 nm), shedding microvesicles (100–1000 nm) and apoptotic bodies (50–4000 nm) have been recognized. Exosomes, unlike the other extracellular vesicles are the only secreted vesicles to have an endosomal biogenesis [14]. They are micro membrane vesicles that possess several properties related to the stabilization of cellular homeostasis. Originally, they were recognized as nanoparticles secreting cellular waste [15] and this behavior can be used for therapeutic purpose [16]. MSCs exosomes are useful tools for easier delivery of drugs [17]. We have proved that they can include ferromagnetic material into their cargo forming thus nanoparticles suitable for hyperthermia tumor therapy [unpublished]. Exosomes are secreted by most cell types upon fusion of multivesicular bodies with the plasma membrane. Schematic picture of exosome formation is depicted in Figure 1. Intercellular communication mediated by transfer of functional biomolecules like mRNA, miRNA, DNA and proteins is the main function of exosomes [18]. Ability to transfer gene informative molecules especially exosomes released from MSCs is the basis of many applica-

tions in regenerative medicine. Exosomes of MSCs together with their secretome have been identified as tools behind the immunomodulatory effects [19-21], induction of angiogenesis [22-25], cell proliferation [26], antiapoptotic effect [27, 28] and anti-inflammatory effect [29]. Experimental data suggest that MSCs exosomes might be therapeutically useful in such lethal medical complications as the acute kidney injury [30]. Despite the exact mechanism of *in vivo* action of exogenously administered stem/stromal mesenchymal cells-derived exosomes are not fully elucidated [reviewed in [31], several clinical studies with MSCs extracellular vesicles (Evs) are in progress. Analysis of results of the older phase I clinical trials of exosomes-based therapies revealed that no serious acute events have been associated with EVs administration [32, 33].

MSCs induced mechanisms of regenerative process

The involvement of MSCs exosomes and components released from their cargo in the recipient cells rest in modulating multiple cellular pathways. The activation of regenerative process is rather a complex mechanism. Differential expression analysis in porcine adipose tissue-derived MSCs revealed 4 miRNAs, 255 mRNAs, and 277 proteins enriched in exosomes versus cells [34]. Perhaps the interactions between mRNA and miRNA targeting transcription factors and proteins capable of modifying multiple cellular pathways may be a selective mechanism driving so many various MSCs-based repairs. In addition, MSCs exosomes may in the inflammation microenvironment support extracellular matrix remodeling and angiogenesis. Regenerative medicine experience suggests that MSCs are naturally found as pericytes localized on vein walls of the vascularized tissues. In order to heal injured tissues without scarring, MSCs are released at sites of the injury, where they secrete large quantities of bioactive factors and exosomes that are immunomodulatory thus preventing autoimmunity and inhibit lymphocyte surveillance of the injured tissue. The exosomes in concord with MSCs secretome target tissue intrinsic progenitor cells. Consequently, ischemia-caused apoptosis is prevented, and angiogenesis is stimulated. Healing process continues through cell division of the natural intrinsic regenerative cells [12]. The consecutive steps in wound healing

process triggered by presumptive coordinated actions of MSCs secretome through paracrine/autocrine/endocrine manner are listed in Table1. To the human MSCs healing activity is contributing the antibacterial effect mediated in part by the secretion of human cathelicidin hCAP-18/LL-37 [35]. MSCs exosomes are partly involved in antibacterial activity through the expression of keratinocyte growth factor by mRNA in the site of injury [36]. Recently it was reported that MSCs can communicate with their microenvironment through bidirectional exchange of mitochondria. The apoptosis of damaged cells was prevented through delivery of their own mitochondria [37]. It was established that MSCs use tunneling nanotubes as the means to transfer mitochondria to injured cell [38].

Tumor trophic behavior of MSCs engineered to express suicide genes

MSCs recognize tumor as a not-healing wound [39]. MSCs migrate to it and frequently became a part of tumor stroma with consequences like tumor growth modification, confer of drug resistance and transition to tumor associated fibroblasts [40, 41]. We used MSCs tumor trophic behavior to develop two prodrug suicide gene therapy systems for cancer mediated by MSCs. We have shown that MSCs transduced with yeast cytosinedeaminase::uracil phosphoribosyltransferase gene (yCD::UPRT) by retroviral infection can convert nontoxic 5-fluorocytosine (5-FC) to the effective cytotoxic compound 5-fluorouracil [42]. MSCs engineered to express thymidine kinase of Herpes simplex virus is the second therapeutic system we have developed [43]. In this system the prodrug ganciclovir is converted by cellular enzymes to ganciclovir triphosphate that inhibits DNA synthesis of recipient cells. Suicide gene transduced MSCs have the advantage of being stable with an effective production of the prodrug-converting enzyme under the control of a strong retroviral promoter from the DNA provirus integrated into the cellular DNA. Vector construction allows for antibiotic selection of the transduced cells yielding pure populations of transduced cells [44]. Elimination of non-transduced cells is rather important, MSCs can potentially support tumor cell growth by secreted

Table 1. MSCs involvement in natural wound healing

	The prominent paracrine factors involved:	Reference
Pericytes present on the walls of veins of vasculature are activated to act as MSCs	Activation of platelet-derived growth factor-beta receptor	[55]
MSC-secretome suppress the local immune system and apoptosis	Prostaglandin E2 and IL-6	[56]
Secreted bioactive factors inhibit fibrosis (scar formation)	Platelet-derived growth factor, insulin-like growth factor-1, IL-8, hepatocyte growth factor	[57]
Angiogenesis is enhanced	Vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), monocyte chemotactic protein-1 (MCP-1), angiopoietin-1.	[58]
MSCs exosomes internalize cells surrounding wound		[26]

cytokines and growth factors. The attractiveness of these therapeutic systems lies in the conversion of a not toxic prodrug to a cytostatic drug directly within the tumor mass, thus avoiding systemic toxicity [45]. In addition, the genetically modified MSCs designated as Therapeutic Stem cells (ThSc) have sustained tumor-tropism and the prodrug administration not only eliminates tumor cells, but consequently kills the more resistant therapeutic cells as well, thereby eliminating them from the host [46]. Compared with conventional chemotherapy, yCD::UPRT/5-FC MSCs mediated therapeutic system exhibited no significant systemic adverse effects.

MSCs transduced with yCD::UPRT gene release exosomes with mRNA of the suicide gene in their cargo

In preclinical studies with human melanoma cells [46], prostate cancer cells [47] implanted subcutaneously to immunocompromised nude mice and with intravenously injected ThSc significant tumor growth inhibition was observed. These data were not compatible with the known biodistribution of intravenously administered cells, where 80 percent of MSCs end in lungs [3]. Biodistribution measurements of intravenously injected labeled MSCs revealed that cells are immediately entrapped in lung tissue and then clear to the liver within one day [48]. The high tumor inhibiting activity of intravenously administered ThSc was explained when we found that suicide gene transduced MSCs release exosomes with mRNA of suicide gene in their cargo [49, 50]. Analysis of CM from yCD::UPRT-AT-MSCs revealed the presence of exosomes with mRNA of the yCD::UPRT gene and free translated enzyme. We have proved that all yCD::UPRT gene transduced human MSCs derived from various tissues like adipose, bone mar-

row, dental pulp, umbilical cord and menstrual blood derived endometrial regenerative cells release exosomes with mRNA of yCD::UPRT gene into CM. Thus the efficacy of prodrug gene therapy for cancer mediated by MSCs in the presence of 5-FC was found to act not only through bystander effect, but is potentiated by internalized exosomes as well. The exosomes inhibit growth of human tumor cell lines and human primary glioblastoma cells in a dose dependent manner *in vitro*. Growth of tumor cells with CM additions with and without prodrug 5-FC monitored in real time was found very informative. It revealed that for translation of suicide protein from mRNA delivered by exosomes present in the CM was needed about 30 hours. In addition, the tumor cell growth comparison of cells influenced by control medium versus control medium with CM additions has shown the growth stimulation caused by secretome in a dose dependent manner. Tumor cell killing by 5-fluorouracil formed intracellulary form the prodrug was a dose dependent manner as well (Figure 2). Therapeutic exosome involvement was likely responsive for curative therapy of rat glioblastoma treated with intracerebral administration of human yCDy-UPRT cells [51]. Accumulating evidence indicates that cancer therapy using MSCs exosomes have multiple advantages over cell therapy. CM or exosomes are stable after intravenous administration and exhibit a superior safety profile. Since MSCs have the remarkable tendency to home to tumors, exosomes produced by MSCs may retain the homing properties of their parent cells. Dental pulp derived MSCs being of neural crest-derived cells might serve as an example. We have recently shown that dental pulp-derived MSCs can migrate to intracerebral glioblastomas after intranasal administration [50]. Number of studies of regenerative medicine field has shown that nanoparticles produced by MSCs exert their therapeutic effects in several diseases, suggesting that MSC-derived
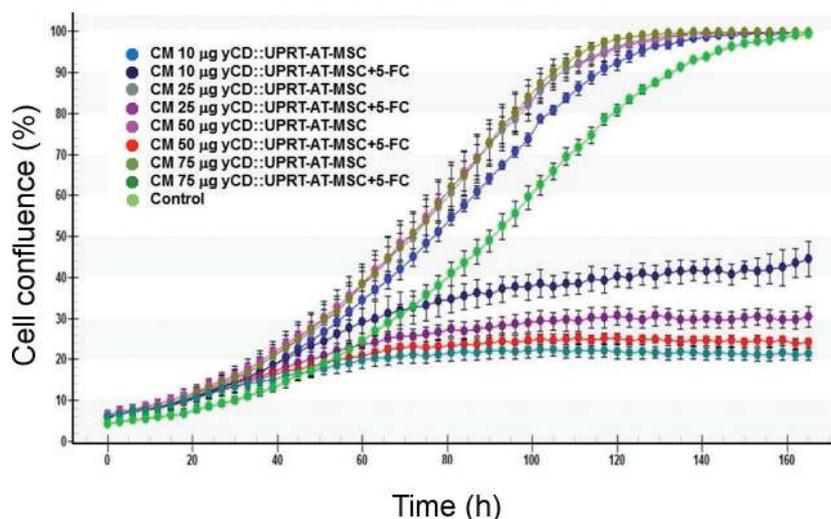

Figure 2. Exosomes in conditional medium kill prostate cancer cells PC3 in a dose dependent manner. Growth curves of PC3 cells treated with CM from yCD::UPRT-AT-MSCs in presence and/or absence of 5-FC. PC3 cells (3×10^3) were plated in wells of the 96 well plate. Next day, indicated μg of CM were added to growth medium in each well either with prodrug 5-FC or without 5-FC. The course of growth/inhibition was monitored by the Incucyte system.

nanoparticles – exosomes may be a promising alternative to cell therapy. MSCs engineered to express suicide genes release exosomes. Their cargo contains suicide gene mRNA. Internalization of these exosomes in recipient cancer cells was found responsible for the growth inhibiting effect. Thus the prodrug suicide gene therapy mediated by MSCs is converted to the prodrug cancer suicide gene therapy mediated by therapeutic MSC exosomes. Nanoparticles released from tumor cells possess many diverse biological functions. Tumor cells secreted exosomes might support neoplastic growth, invasion, and metastasis [52]. Moreover; exosomes from human lung-, liver- and brain-tropic tumor cells are organ specific, preferentially penetrate resident cells at their predicted destination [53]. The tumor-derived exosomes up taken by organ-specific cells prepare the pre-metastatic niche [53, 54]. All these findings might have application in prodrug gene cancer therapy mediated by exosomes targeted to organ specific metastases.

Advantage of nanosized exosomes compared with administration of exogenous MSCs is multiple. Exosomes are easier to preserve and transferred have lower immunogenicity and therefore are safer for therapeutic administration.

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