

Review Article

Exosome and Extracellular RNA in Stem Cell Biology

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Submitted: 24 March 2015

Accepted: 07 May 2015

Published: 13 May 2015

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Keywords

- Exosome
- Extracellular RNA
- Stem cell
- Cellular communication

Abstract

Exosome, a unique nanovesicular structure released from cells, has emerged as an important cell-free cargo of biologically active macromolecules. It carries DNA, RNA, protein and other molecules that can be transported intercellularly and mediate biological functions in the recipient cells. Detailed mechanisms for the biogenesis and biofunction of exosomes (and exosomal extracellular RNA) have not been understood, but potential functional roles of exosomes have been suggested in diverse physiological events, such as cellular homeostasis, molecular signaling pathways, immune responses and pathogenesis/progression of diseases. The maintenance of stem cell potency or the initiation of differentiation is significantly affected by stem cell niche. Stem cells, pluripotent or multipotent, are actively communicating with surrounding cellular environment and mutually influencing on the cellular fates potentially through exchanging their molecular information. Evidently exosomes may play significant roles in molecular communication between stem cells and their microenvironment. In addition, recent evidences demonstrate that stem cell-derived exosomes mediate the transfer of stemness and renewing capability to the recipient cells and induce regeneration of damaged cells. One of imminent hurdles in stem cell-based therapy in regenerative medicine is the proper settlement and sustenance of grafted cells in the newly adapted environment. In that regard, stem cell-derived exosomes may present promising potential utility of cell-free system towards regenerative therapy and suggest important avenues of future stem cell research.

INTRODUCTION

Intercellular communication is a fundamental feature of multi-cellular organisms and may involve direct physical contact between cells or the secretion of signaling molecules. However recently, a third mechanism for intercellular communication has emerged involving extracellular vesicles. Although the release of apoptotic vesicles has been long known [1], the notion that healthy cells release vesicles in intercellular communication has only recently been acknowledged. In particular, the most prominent and widely studied extracellular vesicles are exosomes, which are membrane derived nanovesicles constitutively produced by most cell types [2].

Exosomes were first discovered in maturing reticulocytes where their secretion into the extracellular environment by multi-vesicular bodies was reported [3,4]. The term "exosome" for these vesicles was subsequently coined, although the term had previously been used to describe other membrane fragments [5] as well as the ribonuclease complex [6]. Exosomes are derived from the endocytic pathway, whereby endocytic vesicles

form on the cell surface, and fuse to form early endosomes that mature to become late endosomes [2]. This vesiculation allows for the accumulation of various proteins and nucleic acids specific to the cell of origin to become encapsulated. Exosome-encapsulated RNA is protected from RNA degrading enzymes, making exosomes a viable vehicle for intercellular RNA exchange. Potential biological roles of exosomes and exosomal RNA have been documented. In particular, the horizontal transfer of genetic information including both coding and non-coding RNAs may influence physiological and pathological processes across cells.

Exosomal extracellular RNA in intercellular communication

A major breakthrough was the demonstration that exosomes contained functional RNA. Valadi *et al.*, demonstrated exosomes from murine and human mast cell lines (MC/9 and HMC-1), as well as primary bone marrow-derived mouse mast cells contained RNA [7]. It was identified that exosomes contained about 1300 unique gene mRNAs that were not found in the cytoplasm of the producing cells [7]. The mRNA present in exosomes was further

found to be functional in the recipient cells and translatable to proteins in the presence of functional protein machinery. Micro RNAs (miRNAs) were also first identified within the exosomes and suggested a novel mechanism of intercellular regulation [7]. Subsequent experiments confirmed these initial findings, and furthermore elucidated exosomal processes [8,9]. Exosomal transport of miRNAs was demonstrated to regulate target gene expression and recipient cell function. Zhang *et al.*, reported miR-150 to be selectively packaged into exosomes in human blood cells and cultured THP-1 cells [8]. THP-1-derived exosomes can enter and transfer miR-150 to human HMEC-1 cells where elevated exogenous miR-150 levels reduced c-Myb expression and promoted cell migration in HMEC-1 cells [8]. More recently, Montecalvo *et al.*, have now directly tested this hypothesis of functional transfer of miRNA using dendritic cells (DC) [9]. Using a GFP-linked marker incorporated into exosomes, it was shown that DC exosomes were transferred to recipient DCs and activated CD4 T cells. Exosomes were also confirmed to fuse to recipient DC membranes in two independent assays and functional transfer of two exosomal miRNAs was visualized by employing cells transfected with vectors encoding luciferase-coupled complementary targets [9]. In addition, recent analysis of RNA from exosomes using unbiased deep sequencing revealed that in addition to mRNA and miRNA, exosomes also contain a variety of non-coding RNA species including RNA transcripts overlapping with protein coding regions, repeat sequences, tRNA fragments, Y RNA, structural RNAs, and small interfering RNAs [10,11]. Through the protection and transport of miRNA molecules, exosomes have been shown to be involved in homeostasis, RNA and protein complex shuttling, post-translational modification of gene expression, and tumor suppression. The stability of secretory miRNAs through exosomes is significant as it provides protection from RNase enzymatic degradation in the bloodstream and extreme temperatures and pH values during handling [12].

As a result, the discovery of exosomal mediated intercellular communication has elucidated another important mechanism behind physiological and pathological processes. As exosomes are a potent source of information transfer to local and distant cells, dysregulation of these vesicles allow for the transfer of micro RNAs and pathogenic proteins that may be linked to tumor pathogenesis. For example, exosomal-mediated gene transfer has resulted in the spread of neurodegenerative diseases through the transfer of specific miRNAs and other pathogenic proteins [2]. Skog *et al.*, reported glioblastoma exosomes can enter recipient human brain micro-vascular endothelial cells and translate reporter mRNA delivered via the exosomes [13]. Such findings suggest that tumour-derived exosomes can modify normal, healthy cells by altering their translational profile to promote tumor progression. Moreover, tumor-derived exosomes may stimulate tumor growth and metastasis by promoting endothelial migration, invasion, and tube formation and may be an effector in tumor-induced angiogenesis [14]. Tumor-derived exosomes were shown to influence the microenvironment in tumors by acting on the phenotype of stromal cells to support tumor metastases and escape immune recognition. The mechanisms behind micro-vesicular interaction with target cells are still unclear, however some studies suggest ligand-receptor interactions or micro-vesicle-cell fusion [15]. Taken together, exosomes are quickly

emerging as powerful sources for genetic transfer between cells for the mediation of both beneficial and pathological processes.

Exosomes in stem cell maintenance and regulation

Stem cells have the ability to remain undifferentiated and undergo self-renewal [16]. Recently, extracellular micro-vesicles (EVs) including exosomes have been implicated in the ability for stem cells to mediate genetic transfer to maintain plasticity and induce cell phenotype modulations [2]. Exosomes released from stem cells may be involved in reprogramming cells and coordination of repair, providing an avenue of exploitation for gene therapy. Among diverse constituents carried within exosomes the role of RNA transport through exosome was implicated in the preservation of pluripotency and maintenance of stem cells *in vitro*. Exosomes from murine embryonic stem cells (ESCs) were highly enriched in mRNA for known pluripotent transcription factors and expression of Wnt-3, which was shown to enhance survival and expansion [17]. It was demonstrated that molecular components of ESC-derived EVs including exosomes were transferred to the neighboring cells [17]. Recent studies have demonstrated that ESC-derived EVs contain enriched miRNAs that could be transferred to recipient cells and induce cellular and molecular alterations [18,19].

Potential roles of adult stem cell-derived extracellular micro-vesicles have been demonstrated. Aliotta *et al.*, reported the ability for micro-vesicles isolated from lung, brain, heart and liver to transfer tissue-specific mRNA and induce phenotypic changes in bone marrow cells, which suggests the potential for micro-vesicle-mediated phenotype change to be a universal phenomenon [20]. It has also been demonstrated that mesenchymal stem cells (MSCs)-derived micro-vesicles contain unique miRNAs that are involved in the intracellular trafficking of RNAs, which suggest stem cells may deliver RNA contents and modulate the neighboring cells during the biogenesis and dynamic transport of MSC-derived extracellular vesicles [2,22]. In addition, exosomal transport of mRNA from endothelial progenitor cells (EPCs) have been implicated in reprogramming quiescent endothelial cells towards angiogenic phenotypes. Through the interaction of alpha4 and beta1 integrins on the micro-vesicle surface and transfer of mRNA associated with the PI3K/AKT signaling pathway, EPCs may be able to activate angiogenesis in endothelial cells [23]. Other studies have also shown that exosomes mediate bi-directional informational exchange between stem and injured cells to influence functional and phenotypic changes. It was identified that tumor-derived micro-vesicles carrying several surface determinants of tumor cells, such as HLA class I, CD29, epithelial cell adhesion molecule (EpCAM) and mRNA growth factors, exerted anti-apoptotic effects and activated AKT kinase in monocytes [24]. It has been shown that exosomes may provide explanation for the bone marrow stem cell plasticity during tissue repair, suggesting that exosomes play an important role in the continuum model of stem cell biology [25,26]. In hematopoietic stem cells, the entry of micro-vesicles varies with cell cycle phase and results in the reset of cellular potentials. This continuum between intra- and extra-hematopoietic cells result in a cell system with a continually changing potential [27]. By further investigating these pathological changes, exosomes can be better understood and utilized for regenerative medicine.

Although the therapeutic effect of exosomes have been discovered in a wide range of cellular-injury models, the underlying mechanisms of function have yet to be fully elucidated. An *in vivo* study discovered that human induced pluripotent stem cell-derived mesenchymal stem cells (hiPSC-MSCs) release exosomes that promote wound healing through stimulation of collagen synthesis and angiogenesis. This study found the therapeutic effects of hiPSC-MSCs exosomes to promote tissue repair in a dose dependent manner by increasing the proliferation and migration of fibroblasts, and the secretion of type I, III collagen and elastin [28]. Another study involving MSC-derived exosomes showed the promotion of hepatic regeneration through activation of proliferative and regenerative responses. With exosomal treatment, hepatocyte proliferation was increased along with priming-phase genes involved in liver regeneration and expression of proliferation proteins, including proliferating cell nuclear antigen (PCNA) and cyclin D1. These studies highlight a shift from the analysis of regenerative stem cell genomes to their paracrine modulatory effects, which are in part mediated through exosome-derived mechanisms.

Potential utility of stem cell-derived exosomes in regenerative medicine

Research has begun to investigate the potential therapeutic use of exosomes by utilizing the natural mechanisms by which they transfer genetic information [2]. In contrast to traditional cell-based therapies, exosome-based therapies provide an alternative that is relatively easier to manufacture and safer, due to a lack of intrinsic functions and inability to form tumors. Recent studies have shown that stem cell-derived exosomes naturally mediate tissue repair and regeneration through epigenetic reprogramming with miRNAs [26,29-32]. The protective effect of exosomes in a renal ischemia injury model has been demonstrated, where the administration of MSC-derived exosomes inhibited apoptosis stimulated proliferation and prevented the development of chronic renal disease [29]. In another study, human liver stem cells-derived exosomes shuttled mRNA to accelerate morphological and functional recovery of the liver in a model of 70% of hepatectomy in rats [26].

EPC-derived micro-vesicles containing miRNAs (miR-126 and miR-296) were able to induce hypoxic renal cells to regenerate or activate angiogenic pathways in islet endothelium to sustain revascularization after transplantation [30]. A study suggested that exosomes might be harnessed for use in human genetic therapies through the introduction of exogenous genetic cargoes such as siRNA for gene-specific silencing in the brain. The injection of a neuron-specific RVG-targeted exosomes in wild-type mice delivered GAPDH siRNA to neurons, microglia, and oligodendrocytes, resulting in the knockdown of BACE, a therapeutic target in Alzheimer's disease [31]. In another study, plasma exosomes derived from peripheral blood were able to deliver heterologous siRNA to human blood mononuclear cells to selectively silence mitogen-activated protein kinase 1. This effectively demonstrated that human exosomes can be utilized as a method for delivering RNA interference-based therapy [32].

The use of mesenchymal stem cell derived exosomes has been found to increase miRNA-133b levels when exposed to brain tissue to stimulate neurite outgrowth and improve functional

recovery after stroke [33]. CD34+ stem cell-derived exosomes have been shown to induce angiogenic activity in endothelial cells, a beneficial therapy for recovery following ischemic injuries [30,34]. Similarly, the injection of exosomes from MSCs into swine and murine models of ischemic injury has been shown to reduce infarction size [35]. In addition, exosomes from human bone marrow stem cells have been shown to accelerate the repair of acute kidney injuries in mouse models while those from human liver-derived stem cells have induced proliferation and apoptotic resistance in rat hepatocytes [25,26].

These early findings have laid the groundwork for future studies to further develop the use of stem cell-derived exosomes as targeted gene therapeutic agents and for the delivery of therapeutic macromolecules including oligonucleotides and proteins. However, many more functional studies are required to discover other candidate proteins and their therapeutic potential.

RESULTS

The role of exosomes in cell-free cellular communication has gained great deal of attention in scientific community. It has been implicated in diverse molecular and cellular processes and importantly its active role in intercellular transfer of molecular information and further mediation of biological function has been demonstrated. Exosomal transfer of pathologic information has also been demonstrated such as in the progression of cancers. Cell-free transfer of biologic function via exosome should have great merits in translational applications where cell based systems pose practical difficulties. Recent evidences suggest the functional importance of exosome and exosomal molecular transfer in stem cell maintenance and differentiation. Utilization of cell-free exosomal transfer of stem cell properties will hold great promise in stem cell research and further regenerative therapy.

ACKNOWLEDGEMENT

This work was partly supported by an NIH award from NIAAA (R01AA21301) to YK.

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Cite this article

Hoang M, Roubal I, Kim Y (2015) Exosome and Extracellular RNA in Stem Cell Biology. *Arch Stem Cell Res* 2(1): 1008.