Editorial

Exosomes: scytales in the damaged heart

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Exosomes and intracellular communication

As cells in our organism are constantly sending out and receiving signals, cell-cell communication is an essential way to maintain process homeostasis while allowing adaptation to external stimuli. Disturbances in cell-to-cell communication will result in disease. Cells communicate with each other via extracellular molecules such as nucleotides, short peptides, proteins or lipids that are released to the extracellular space and bind receptors on other cells, therefore inducing signaling and modifying the molecular status of the recipient cells. In addition to such molecules, cells also release membrane vesicles, representing a rich source of small molecules such as messenger RNAs, microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), small amounts of DNA and low molecular weight lipids and proteins (including transcription factors and cytokines), all of which can also alter recipient cells that encounter such structures. Although initially thought as cellular debris and a sign of cellular death, in recent years more interest has been dedicated to extracellular vesicles (EVs) as mediators of long-range cellular communication by their presence in most body fluids. According to their size, EVs can be classified in microvesicles (MVs) (0.1 to 1 μm), exosomes (20 to 100 nm) and apoptotic bodies (ABs) (0.5 to 2 μm). (1) While MVs (and ABs) are assembled by budding from the plasma membranes, exosomes are raised from endosomal vesicles and formed as intraluminal vesicles by inward budding of the limiting membrane of the multivesicular bodies, which allows the internalization of small proteins, mRNAs, miRNAs and DNA. Once the multivesicular bodies fuse with the cell membrane, exosomes are released (2) and free to interact with target cells in different ways: (I) exosomes can fuse in a non-selective way releasing their content into the target cell; (II) hemifusion followed by a complete fusion between the exosome and the cell membrane; (III) internalization by phagocytosis; and (IV) specific interactions among exosomes and target cells mediated by extracellular matrix components (3).

It is important to underline that exosomes, although generated by different cell types expressing distinct markers, also carry common surface markers such as heat shock protein HSP70 and tetraspanins CD9 and CD63, making their characterization a difficult process (4). For research purposes, it is fundamental to purify EV populations, but despite a large body of literature describing protocols on this matter, a gold standard method for the isolation of exosomes remains to be established. This is a critical point while studying exosome-mediated cell communication since different isolation methods still result in different yield and purity populations (5).

Exosomes and intracardiac cell communication

Heart failure is one of the leading causes of morbidity and mortality worldwide (6). Cardiac remodeling is often a maladaptive mechanism that, independently of the nature of the initial insult, will ultimately cause ventricular dysfunction (7). Proper cardiac function does not only rely on cardiac muscle cells which account for one third of the total cell number of the myocardium, but rather on a balance with other cell types including smooth muscle cells, endothelial cells, fibroblasts and immune cells. These very distinct cell types do not function isolated from each other but rather interact physically and/or via different autocrine, paracrine and endocrine factors. Indeed, the myocardium secretes exosomes, which are involved in intracellular

communication in the adult heart (8). As extracellular spaces have mixed exosome populations from all kinds of cellular sources it is difficult to distinguish exosomes derived from a specific cell type from the intact organ. For this reason, *in vitro* studies have been very helpful not only providing evidence of exosome secretion but also in identifying the exosomal content and function from specific cell types. Early reports using primary cardiomyocytes and relevant cell lines have provided evidence of exosome secretion and detected nucleic acid–containing microvesicles/exosomes in the cell media, which could reprogram fibroblast gene expression (9,10). These investigations introduced a new concept in cardiac cell-cell communication, proposing that exosomes generated by cardiomyocytes are able to transfer protein or genetic information to neighboring cells of the heart.

Recent studies indicate that exosomal content is highly regulated by stress and disease conditions and despite the fact that research on cardiac exosomes is just emerging, a limited number of publications provide strong evidence that exosomes can exert pathological effects during cardiac response to stress as shown for different myocardial diseases as cardiac hypertrophy and peripartum or diabetic cardiomyopathy. Exosomes secreted from fibroblasts are enriched in miR-21* which once uptaken by cardiomyocytes will down-regulate Sorbin and SH3 domain 2 (SORBS2) or PDZ and LIM domain 5 (PDLIM5), both regulators of cardiac muscle structure and function, and induce cardiac hypertrophy (11). The fact that fibroblast-derived miR-21* induces cardiomyocyte hypertrophy also demonstrates that miRNA passenger strands can function as mature miRNAs (11). Another study demonstrates how, in women affected with peripartum cardiomyopathy (PPCM), a 16-kDa prolactin fragment (16K PRL) leads to the release of miR-146-enriched exosomes by endothelial cells (12). These exosomes can be uptaken by cardiomyocytes where miR-146a interferes with the physiological metabolism and contractile capacity of the cell, leading to the development of hypertrophy. Furthermore, levels of exosomal miR-146a were higher in plasma from patients with acute PPCM than healthy postpartum controls and patients with dilated cardiomyopathy. Maybe more important to underline is that standard heart failure therapy in PPCM patients lowered circulating exosomal miR-146a to control levels, indicating miR-146a as a strong potential biomarker for diagnosis and risk stratification of patients with PPCM (12).

As miRNA composition may reflect the metabolic or differentiation state of the exosome-producing cells, circulating exosomes found in body fluids such as plasma and serum are becoming an attractive tool for analytical studies and subsequent disease diagnosis (13). In agreement, the recent work of Chistiakov *et al.* (14), very elegantly underlines that differential exosomal content can lead to new cardiac-specific diagnostic markers by emphasizing the central role of exosomes in cardiac regeneration of the infarcted heart.

Exosomes and cardiac repair

The type of stress to which myocardial tissue is exposed seems to determine the content of the secreted EVs. In myocardial infarction (MI) where the heart is subjected to ischemic stress signals such as hypoxia, inflammation and injury, cardiomyocytes increase the secretion of MVs and/or exosomes. In addition, the distinct content between exosomes deriving from the border zone of the MI and the ones originating from the healthy myocardium suggests an adaptive response to injury. Indeed, circulating miRNAs are markedly altered after MI. miR-1 and mir-133 are elevated in the serum of patients with acute coronary syndrome, correlating with the levels of the clinical biomarker troponin T (15). Together with miR-499, these cardiac specific miRNAs are released from the infarcted and peri-infarcted myocardium and regulate the expression of sarcomeric genes and ion channels (15,16). miR-1 is also elevated, together with miR-208, in the urine of acute MI patients (17) indicating that circulating miRNAs released from the injured myocardium can travel to distant organs via exosomes as they are stable and protected from degradation by RNases present in the different body fluids. Many of these miRNAs are released immediately after an insult and could therefore, be used as markers for early detection of acute MI. In fact, circulating miR-126 is an important indicator of damage and repair mechanisms in acute MI patients exemplifying that monitoring of exosomal contents after MI can be a factor of prognosis evaluation and prediction (18,19).

Stem cells have been used in an attempt to regenerate damaged tissue when injected in the injured heart region, by engrafting, proliferating, differentiating and repopulating the myocardium. The therapeutic effects of stem cells more likely result from the secretion of molecules such as growth factors, antioxidants, cytokines, chemokines and miRNAs with a wide-range of physiological effects (20). The review by Chistiakov *et al.* (14) emphasizes the contribution of cardiac progenitor cells (CPCs) as source of exosomes with

regenerative properties. Under hypoxic conditions, CPCs are able to secrete pro-regenerative exosomes capable of inducing tube formation, proliferation and migration of endothelial cells (21,22). Elevated levels of miR-132 and miR-146 have been found in exosomes derived from hypoxic CPCs and infusion of these exosomes in a rat model of ischemia-reperfusion injury reduced fibrosis and enhanced heart function (22). These findings demonstrate how hypoxia is able to re-enforce the regenerative capacity of CPCs via exosomes. Also critical is the type of parent cell secreting exosomes. While delivery of CPCs-secreted EVs to infarcted rat hearts resulted in decreased cardiomyocyte apoptosis, reduced collagen deposition, increased blood vessel density, and ultimately in improved cardiac function, treatment with fibroblast-derived EVs did not have any effect (21). The differences observed may relate to elevated paracrine secretion of miR-210, miR-132 and miR-146a-3p from CPCs and not from fibroblasts (21). Cardiosphere-derived cardiac stem cells were reported to employ a similar mechanism via transfer of miR-146a to mediate cardioprotection by inhibiting apoptosis, promoting cardiomyocyte proliferation and inducing angiogenesis (23). Whether this cardioprotective effect is progenitor/cardiac stem cell preparation-dependent or whether it is mediated by a specific combination of miRNAs and other factors is not clear. Nevertheless, these results suggest progenitor and cardiac stem cells as unlimited sources of cardioprotective exosomes with promising therapeutic potential for post-MI cardiac repair.

Future perspectives/limitations

Only recently, the potential of using exosomes as therapy for MI and other cardiac pathologies has started to be evaluated in preclinical animal models. Among all types of EVs, exosomes are the richest in (micro)RNAs and could constitute a tool for cell- or tissue-specific delivery of RNA molecules of interest after modulating exosomal membrane receptors. One of the main problems in delivering short interfering RNAs (siRNA) or miRNAs *in vivo* is targeting specific tissues and avoiding non-specific delivery. Exosomes, as natural RNA carriers, would also bypass the issue of immunogenicity of siRNA or miRNA or their cargo vehicle. In fact, different strategies have been used to manipulate exosomal content (24) for specific delivery in the brain and targeting of neurons, microglia and oligodendrocytes (25). Exosomes are versatile carriers of both protective and pathological molecular signals and while they appear to be safe and efficient much effort should be directed to identifying their bioactive molecular content as well as understanding how such content will affect specific signaling pathways and cellular mechanisms in recipient cells.

Besides their promising clinical relevance in the identification of novel biomarkers of cardiac injury in ischemic or other cardiac diseases, the clinical and scientific benefits of studying exosomes as extracellular communicators in cardiac disease are multifold. Such studies will reveal novel mechanisms of how cells and organs communicate among each other, aid in understanding and developing new cell therapies for ischemia as well as providing insights for the development of novel therapeutics, and finally to reveal mechanisms of cell targeting for the discovery of novel candidates and delivery of therapeutic compounds for cardiac diseases. But again, issues such as biosafety and tolerance of exosome-based therapies, which could render their translational power, deserve special attention in the future.

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Footnote

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