Cardiac vascular disease (CVD) continues to be the most common cause of death, being 47% of all deaths according to European Cardiovascular Disease Statistics. In most Western countries, the CVD death rates are declining possibly because of progress in treatment strategies, such as valvular surgery, coronary bypass surgery, balloon dilatation of coronary vessels, β-blockers in acute myocardial infarction, as well as preventive initiatives, with smoking cessation being the most effective one. Earlier (particularly surgical) treatments have aimed to restore normal anatomy, whereas future therapies increasingly will be relying on cellular, subcellular, and molecular myocardial actions. A major step forward in molecular genetics in clinical cardiology took place with the finding by the Seidman group in 1989 that hypertrophic cardiomyopathy, in part, is due to a mutation in the myosin heavy chain gene. Since then, the ambition has been to look for a possible genetic basis in hitherto unexplained cardiac diseases as well as to explore new means of diagnosis and treatment based on modification of gene expression, microRNA (miR), and protein handling of the cell (synthesis, folding/chaperones, degradation by the ubiquitine–proteasome pathway). It has been reported that misfolding of proteins can lead to proteotoxicity, which is an important pathophysiologic mechanism in many heart diseases. Exosomes are vesicles released from cells via exocytosis, and myocardial exosomes may play a major role in many of the abovementioned processes. Although knowledge about specific myocardial exosome function and importance is limited, the subject is extensively studied at present. Analogous functions of cardiac exosomes are now looked for based on knowledge about exosomes from other cell systems. The first description of exosomes (prostasomes) was in epithelial glandular tissue. Glandular epithelial structures as well as unicellular systems were those initially thought to release exosomes. The first identification of exosomes in cancer-derived cells was made in prostate cancer cell lines PC3, Du145, and LnCaP grown in monolayer.

Abstract: Exosomes are nanovesicles released from cells through exocytosis and are known to be mediators of proximal as well as distant cell-to-cell signaling. They are surrounded by a classical bilayered membrane with an exceptionally high cholesterol/phospholipid ratio. Exosomes were first described in 1977, then named prostasomes, and in 1987 the name exosome was coined. Exosomes contain surface proteins, some of which can act as labels in order to find their target cells. Exosomes also contain messages in the form of proteins and nucleic acids (RNA and DNA) that are transferable to target cells. Little is known and written about cardiac exosomes, although Gupta and Knowlton described exosomes containing HSP60 in 2007. It is now known that exosomes from cardiomyocytes can transfect other cells and that the metabolic milieu of the parental cell decides the quality of exosomes released such that they induce differential gene expression in transfected cells. Future clinical use of exosomes in diagnosis, monitoring disease progress, and treatment is promising. (Circ Res. 2014;114:315-324.)

Key Words: biogenesis • myocytes, cardiac • nucleic acids • transfection

Role of Exosomes in Myocardial Remodeling
Anders Waldenström, Gunnar Ronquist

Cardiac vascular disease (CVD) continues to be the most common cause of death, being 47% of all deaths according to European Cardiovascular Disease Statistics. In most Western countries, the CVD death rates are declining possibly because of progress in treatment strategies, such as valvular surgery, coronary bypass surgery, balloon dilatation of coronary vessels, β-blockers in acute myocardial infarction, as well as preventive initiatives, with smoking cessation being the most effective one. Earlier (particularly surgical) treatments have aimed to restore normal anatomy, whereas future therapies increasingly will be relying on cellular, subcellular, and molecular myocardial actions. A major step forward in molecular genetics in clinical cardiology took place with the finding by the Seidman group in 1989 that hypertrophic cardiomyopathy, in part, is due to a mutation in the myosin heavy chain gene. Since then, the ambition has been to look for a possible genetic basis in hitherto unexplained cardiac diseases as well as to explore new means of diagnosis and treatment based on modification of gene expression, microRNA (miR), and protein handling of the cell (synthesis, folding/chaperones, degradation by the ubiquitine–proteasome pathway). It has been reported that misfolding of proteins can lead to proteotoxicity, which is an important pathophysiologic mechanism in many heart diseases. Exosomes are vesicles released from cells via exocytosis, and myocardial exosomes may play a major role in many of the abovementioned processes. Although knowledge about specific myocardial exosome function and importance is limited, the subject is extensively studied at present. Analogous functions of cardiac exosomes are now looked for based on knowledge about exosomes from other cell systems. The first description of exosomes (prostasomes) was in epithelial glandular tissue. Glandular epithelial structures as well as unicellular systems were those initially thought to release exosomes. The first identification of exosomes in cancer-derived cells was made in prostate cancer cell lines PC3, Du145, and LnCaP grown in monolayer.
finding was followed by investigations on exosomes of other cancer cells.\textsuperscript{15–18} Accordingly, subsequent investigations proved that most cells were able to produce exosomes.\textsuperscript{19–21} Gupta and Knowlton\textsuperscript{22} reported in 2007 that cardiomyocytes grown in vitro could release exosomes and that they contained HSP60. Moreover, extracellular HSP60, when not in exosomes, may be apoptosis-inducing to the surrounding cardiac myocytes because of a probable activation of Toll-like receptor 4.\textsuperscript{23} Still, it is suggested that exosomes may be involved in mediating messages to proximal and distant cells for normal events such as cardiac development/growth, normal function, as well as pathology.

### Plasma Membrane

Cardiac exosomes are derived from plasma membrane elements, and recognition of some properties of plasma membrane is important to understand extracellular vesicle formation (including exosomes) and behavior. About 100 years ago, Overton\textsuperscript{24} asserted that the barrier function of the plasma membrane could best be understood if it were attributed to lipids. Gorter and Grendel\textsuperscript{25} suggested a lipid double-layer structure and the idea was further explored by Davson and Danielli.\textsuperscript{26} They reasoned that the amphipathic nature of the phospholipid molecules and their conspicuous place as membrane components made them a dominant feature of the membrane. According to the model, the hydrocarbon sidechains were directed inwardly toward the center of the membrane, and the polar groups outwardly together with a layer of proteins made up the surfaces. In 1960, Robertson\textsuperscript{27} demonstrated the basic similarity of images of several membranes of a cell (ie, plasma membrane, nuclear membrane, and inner mitochondrial membrane) obtained by electron microscopy (unit membrane), in that many membranes of a cell are fundamentally similar in structure, consisting of a bilayer, 7 to 9 nm thick.

The fluid model is another expression for the lipid bilayer model, with emphasis on the probable mobility of protein components in the lipid matrix. Singer and Nicolson\textsuperscript{28} introduced the concept of membrane surface proteins and transversing proteins, thus allowing some of the membrane proteins to move laterally on the cell surface. This was described as the fluid mosaic membrane. The phospholipid bilayer was no longer considered a continuum because of the presence of transversing proteins. Simons and Ikonen\textsuperscript{29} launched the raft hypothesis in 1997 describing a heterogeneous organization of the membrane (including lipid composition) with functional lipid raft microdomains, described in more detail elsewhere in this review.

### History of Exosomes

In 1977, we described the extracellular presence of vesicles surrounded by a bilayer membrane in prostatic and seminal fluids.\textsuperscript{30} Electron microscopy examinations of acinar cells from surgically removed specimens of human prostatic tissue revealed that the extracellular vesicles, which we called prostasomes, corresponded to intracellular vesicles inside another larger vesicle, a so-called storage vesicle, equivalent to a multivesicular body (MVB)/multivesicular endosome of late endosomal origin.\textsuperscript{31} We also measured the diameter of prostasomes in 3 different locations: an intracellular location within storage vesicles, extracellularly in the acinar lumen, as well as (extracellularly) in isolated and purified prostasomes from prostatic and seminal fluids. We found similar mean values of ≈150 nm for all 3 locations. Moreover, the ultrastructure of the vesicles (prostasomes) was suggestive because, while inside the storage vesicles within the acinar cell, they could be released after fusion with the surrounding membrane of the storage vesicle and the plasma membrane of the acinar cell into the extracellular space, that is, exocytosis, into the glandular duct.\textsuperscript{32} Vesicles released in this way, thus, formally fulfill the criterion to be termed exosomes. Functional and beneficial effects of prostasomes on sperm cells were recognized early.\textsuperscript{33} Concomitantly, it was recognized that other cells could also be in receipt of these beneficial properties.\textsuperscript{34}

Electron microscopic studies by Pan et al\textsuperscript{35} in 1985 on maturing reticulocytes disclosed an intracellular sac filled with small membrane-enclosed structures nearly uniform in size. When these sacs fused with the plasma membrane, the vesicular contents were released,\textsuperscript{36} again an example of exocytosis. A similar finding was reported in 1984 by Harding et al\textsuperscript{37} for reticulocytes of another species. The Johnstone group\textsuperscript{38} interpreted this process to be something like reverse endocytosis, with internal vesicular contents released in contrast to extracellular molecules internalized in membrane-bound structures, and in 1987, they named the extruded structures exosomes. The functionality of exosomes in case of reticulocytes was interpreted to represent shedding of some membrane proteins, which were known to diminish during the final stage of development to mature erythrocytes.\textsuperscript{39}

In 1996, Raposo et al\textsuperscript{40} demonstrated in B-lymphocytes that both the limiting membrane and the internal vesicles contain major histocompatibility complex class II, and the released exosomes were perceived to have a role in antigen presentation in vivo. Subsequently, it was realized that many cell types release exosomes, including hematopoietic cells, B- and T-lymphocytes, dendritic cells, mast cells, platelets, intestinal epithelial cells, astrocytes, neurons, and tumor cells.\textsuperscript{15–21} In addition to exosomes, cells can also shed other types of extracellular membrane vesicles, namely, microvesicles, microparticles, and apoptotic bodies,\textsuperscript{35,36} after various biological stimuli, including induction of programmed cell death. Exosomes (especially those harvested from growth media of cells grown in vitro) have generally undergone a filtration step in their purification, and they, therefore, represent a population of membrane vesicles rather homogeneous in size (40–100 nm in diameter). Accordingly, regarding size, exosomes can be categorized into those with a narrow size range (because of filtration in the preparatory procedure) and those not filtered, with a broader size range (eg, prostasomes). In contrast, microvesicles, microparticles, and apoptotic bodies represent heterogeneous populations of extracellular vesicles (100 to >1000 nm) that are the result of a direct budding from the plasma membrane.
Extracellular vesicles, including exosomes, have potent proinflammatory effects; they can affect the function of endothelium and promote coagulation. Therefore, they may play a role in the pathogenesis of CVDs. Hence, cancer cell– and endothelial cell–derived exosomes stimulate endothelial cells by receptor activation and transfer of exosomal effectors leading to the progression of angiogenesis in vitro and in vivo.37–39 It should be kept in mind, however, that exosomes derived from mesenchymal stromal cells have been claimed to exert an anti-inflammatory effect on lung vessels. In this way, hypoxic pulmonary hypertension is inhibited via the suppression of macrophage action.40 This reasoning is relevant in revascularization of ischemic myocardium and justifies new approaches in trying to master exosome production and movement in interstitial fluid and peripheral blood.

**Exosome Biogenesis**

Better understanding of the biogenesis of exosomes has led to more insight during the past decades into their function.41–44 Exosomes are a subgroup of extracellular vesicles generally ranging in size from 40 to 200 nm, which are released extracellularly through exocytosis.30,33,34 Interactions between cells, which are mediated by exosomes, lead to a much more complex event compared with those mediated by a single ligand with a single receptor. Most cells are capable of releasing exosomes. The plasma membrane of mammalian cells is organized heterogeneously, that is, there is no bilayer continuum. Instead, the bilayer is interrupted by so-called transversing proteins, which extend across the biological membrane. The plasma membrane also contains specific microdomains known as detergent-resistant membrane domains, lipid rafts, or caveolae.55–58 More than 20 years ago, we observed an extraordinary composition of the membrane of prostasomes.49 The prostasomal membrane contains a high amount of saturated phospholipid acyl chains and sphingomyelin, which in turn results in an affinity for cholesterol. Hence, the cholesterol/phospholipid ratio was strikingly high (≈2, contrasting to most other biological membranes with a corresponding ratio of ≈0.8), and electron spin resonance studies revealed that lipids in the prostasome membrane were highly ordered.49 The implications of these findings were not clear at first but were explained some years later when Simons and Ikonen29 formulated the raft hypothesis. According to this hypothesis, sphingolipid–cholesterol microdomains are involved in numerous cellular functions, from membrane trafficking and cell morphogenesis to cell signaling. These rafts recruit a specific set of proteins and exclude others. The exact mechanisms of selection are not known. Endocytic vesicles arise at the lipid raft domain of the plasma membrane through clathrin- or nonclathrin-mediated endocytosis, leading to the intracellular formation of early endosomes. These early endosomes are subjected to a maturation process that includes an interaction with the Golgi complex to become late endosomes. The bilayer membrane surrounding late endosomes can in turn display invaginations, giving rise to intraluminal vesicles completing the formation of MVBs/multivesicular endosomes (or, in case of prostasome nomenclature, the so-called storage vesicles).30,50,52 Rendering the membrane surrounding the intraluminal vesicles a right-side-out position in relation to the plasma membrane (Figure 1).52 The components of the endosomal sorting complex required for transport pathway are critical for the formation of MVBs (Figure 2). However, the relationship between the endosomal sorting complex required for transport pathway and the secretion of exosomes remains unclear. The fusion of MVBs with the plasma membrane results in the release of their cargo, the exosomes, to the extracellular space. This process completes the exocytosis event.

The protein composition of exosomes produced in vitro has been studied in various ways, including Western blotting,33,54 flow cytometry of exosome-coated beads,39 and mass spectrometry.36–38 These studies have demonstrated that exosomes from different cellular origins share some common characteristics. Such characteristics are the lipid bilayer with exceptionally high cholesterol/phospholipid ratio, size, density, and a basic collection of lipid and protein composition. Among these proteins are those derived from the cytoplasm or some that are membrane-bound, such as tubulin, actin, actin-binding proteins, annexins and Rab proteins, and some glycolytic enzymes. Others represent those responsible for signal transduction, such as protein kinases and heterotrimeric G-proteins.59–64 Many exosomes typically contain major histocompatibility complex class I and class II molecules65,66 and heat shock proteins62,63 that are involved in antigen binding and presentation. However, the protein family most characteristically associated with exosomes would seem to be the tetraspanin and integrin proteins (targeting and cell adhesion), including CD9, CD63, CD81, and CD8264–70 (Figure 3).

Although differential centrifugations, including preparative ultracentrifugation, do not discriminate between exosomes and other small vesicles, or large protein aggregates, exosomes float on sucrose gradients, and their densities range from 1.13 g/mL (B-cell–derived exosomes) up to 1.19 g/mL (epithelial cell–derived exosomes). Accordingly, contaminating protein material can be separated from exosomes by floatation on sucrose gradient.67 Other modes of enrichment of prostasomes/exosomes include size exclusion chromatography,14,30,37 filtration,69 immunoaffinity purification,70 and specific isolation kits.72

The sorting behavior of lipids in MVBs/storage vesicles is not known. It has been claimed that it might be determined by the nature of their hydrophobic tails.73 We have noted the unusual distribution of phospholipids in prostasomes with sphingomyelin being predominant and with relatively high amounts of phosphatidylethanolamine and phosphatidylserine as well as with lysophospholipids, whereas phosphatidylcholine and especially phosphatidylinositol were found at consistently low levels.49 This prostasome/exosome-specific phospholipid pattern was subsequently confirmed by others.74,75 Exosomal lipids might be biologically active,57 and we reported in 1993 the presence of prostaglandin E in prostasomes.76 Exosomal prostaglandins have been shown to be able to trigger prostaglandin-dependent intracellular signaling pathways within the target cells.77 The local enrichment of prostaglandins in prostasomes/exosomes may favor a more efficient biological activity as compared with what can be achieved with prostaglandins in soluble form.78 This reasoning also holds true for other soluble molecules.

**Exosome–Target Cell Interaction**

Intercellular communication is essential for multicellular organisms to maintain vital functions. Direct cell-to-cell contact
or transfer of secreted molecules can accomplish this communication. A third mode of contact between cells is the release of extracellular vesicles such as exosomes, with interaction and uptake by another cell. Using free-zone electrophoresis, we found that both prostasomes and spermatozoa (presumed target cells) had a net negative surface charge (prostasomes less negative than spermatozoa), favoring repulsive forces. Nevertheless, spermatozoa and prostasomes interacted strongly with each other, and the interaction site most probably had a hydrophobic character. This type of interaction enables prostasomes to act in close vicinity to spermatozoa. Not only prostasomes, but also other exosomes will certainly display net negative surface charges that facilitate the solubility and integrity of exosomes in body fluids such as blood plasma. The transfer of a message to distant cells could occur by 3 possible mechanisms: by direct contact between the exosomal membrane and the plasma membrane of the target cell, by fusion of the 2 membranes, or by target cell internalization of the exosome (Figure 4). By these means, a parental cell is able to communicate with a target cell in its vicinity or at a distance through an amplification process.

**Cardiac Remodeling and Exosome Function**

Modern therapy of acute myocardial infarction has resulted in early increased survival, although heart failure due to loss of contracting myocardium is still a problem in survivors. The remaining myocardium has to adapt to meet the work requirement performed earlier by a larger myocardial mass.

Many different stress factors can lead to heart failure. Most often, such stressors lead to events in the myocardium, including certain adaptive measures. When these adaptations are insufficient, a state of maladaptation occurs. Obvious adaptation is hypertrophy (increased wall thickness and decreased wall stress) concomitantly with change in cellular metabolism and contractility to increase cost-effectiveness (switch to the fetal gene program). The stress may be pressure-induced (hypertension, aortic stenosis), volume-induced/increased blood flow (valve insufficiency), or induced by the loss of contractile myocardium (myocardial infarction or dilated cardiomyopathy). The heart strives for the maintenance of adequate cardiac output, that is, through increased sympathetic tone, which in the short term is adaptive, but in the long term is deleterious. The adaptive measures are orchestrated by different signal substances, such as chemokines, growth factors, miRs, many of which are mediated via exosomes from cardiac cells.

In 1998, Fire et al described short, ≈19 to 23 nucleotides, noncoding ribonucleic acid molecules (miRs) that play important gene regulatory roles. They bind to complementary sequences on target mRNA, causing translational repression or target degradation and gene silencing. Several cellular processes such as proliferation, differentiation, and apoptosis...
are regulated by miRs. Recent studies revealed that miRs are aberrantly expressed in the cardiovascular system under some pathological conditions. Hence, complex changes in miRs occur during prolonged CVDs, such as cardiac hypertrophy. Myocardial miRs are downregulated in the early stage of hypertrophy. miR-21 reaches 8 times upregulation by day 14 of induction of experimental cardiac hypertrophy. miR-23 has a similar pattern of expression and function. miR-21 and miR-26a also regulate the expression of matrix metalloproteinase (MMP)-2, known to be important in extracellular matrix remodeling during hypertrophy. miR-26 and miR-133a/b are highly expressed in muscle and heart, but are only expressed in late-stage hypertrophy. miR-133 regulates the IP3 channel receptor gene, leading to prohypertrophic calcium signaling. miR-499 is embedded in the myosin heavy chain gene and is important in its regulation during hypertrophy. miR-30b-5p is downregulated in cardiac hypertrophy and regulates Ca²⁺/calmodulin-dependent protein kinase A. Such complex changes in miRs discussed for long-standing CVDs are also valid during acute events such as myocardial infarction.

Signaling between endothelial cells, endothelial progenitor cells, and stromal cells is crucial for the establishment and maintenance of vascular integrity and involves exosomes, among other signaling pathways. van Balkom et al. showed that miR-214 (a miR that controls endothelial cell function and angiogenesis) plays a dominant role in exosome-mediated signaling between endothelial cells. Endothelial cell-derived exosomes turned out to stimulate migration and angiogenesis in target cells, whereas exosomes from miR-214-depleted endothelial cells failed to stimulate these processes. Results from another research group revealed that atheroprotective stimuli induced communication between endothelial cells and smooth muscle cells through a miR- and extracellular vesicle-mediated mechanism, suggesting a promising strategy to combat atherosclerosis. Another possible antiatherosclerotic exosome-mediated mechanism is already commented upon.

Although subjected to debate, a recent investigation established that the majority of miRs in blood plasma (and saliva) are enclosed in exosomes. Few years ago, Ji et al. applied an analogous experimental approach to identify circulating miRs that might accurately reflect myocardial injury in vivo. Accordingly, miRs apparently circulate in an exosome-shielded form in body fluids such as blood plasma, and this established a basis for the idea that circulating miRs could serve as a new generation of biomarkers for CVDs.

With the rapid expansion in stem cell research (including cardiosphere-derived stem cells), hope arose that stem cells could be seeded in myocardial cell-deficient tissue. Despite great efforts with seeding of different types of stem cells, this has not led to the expected positive result. Moreover, it was observed that despite a measurable increase in cardiac function, this could not be attributed to an abundance of stem cell invasion alone. Another explanation was that a paracrine function of stem cells and other cells could induce remodeling and cell protection, as well as improved function and facilitate cell transformation to cardiomyocytes. Recent findings suggest that a surprisingly high number of different miRs can independently trigger cardiomyocyte mitosis in the border zone of an infarction, emphasizing the superiority of exosomal miRs over stem cell implantation in restitution of infarcted myocardium. This opens up for a new treatment strategy replacing stem cell implantation.

The paracrine functional capacity of cardiac exosomes has been shown indirectly by our group as well as by others. Cardiac cells can release exosomes that contain heat shock proteins, among others, and nucleic acids. The population of such cardiomyocyte-derived exosomes is not homogeneous. They differ in size from 40 to 300 nm, and some are electron-lucent, and others electron-dense. When characterized by surface proteins, 80% contain flotillin-1 and 30% are positive for caveolin-3. Moreover, exosomes released from cultured HL-1 murine cardiomyocytes contained 1595 different mRNAs, of which 1520 also were detected in cardiac myocytes and 423 could be directly connected to a biological network. Accordingly, 35 genes coding for proteins in the small and large ribosomal subunit and additional 8 genes could be connected to a network. Finally, 33 genes coded for proteins in the mitochondria. These exosomes were internalized by fibroblasts when cocultivated, and it could be demonstrated that exosomes were forwarded to the nucleus where both exosomal DNA and RNA colocalized. Exosomes were functional in that they induced a gene response of the transfected cells, giving rise to 333 differentially expressed genes (175 upregulations and 158 downregulations) compared with controls. Moreover, 343 different chromosomal DNA sequences were identified. The question arises whether these unequivocal effects are applicable to other cardiomyocyte cell lines. This relevant question cannot be answered until proper comparisons are made.

It is notable that the milieu of the parental cell may influence the quality of exosomes released. Thus, when HL-1 cells...
were cultured with different growth factors, namely, transforming growth factor (TGF)-β2 or platelet-derived growth factor BB, individual responses were induced concerning the quality of exosomes released. Exosomes were isolated from each group of the treated cardiomyocytes and were cocultivated with fibroblasts. In the 3 groups of transfected fibroblasts (with exosomes derived from cells treated with TGF-β2, or platelet-derived growth factor BB, or controls), a common pool of 235 transcripts was found in all 3 groups where 14% were ribosomal and 5% were connected to the energy supply system. Apart from this, there were 138 transcripts unique for controls. Cardiomyocyte-derived exosomes contain a basic stock of transcripts common for exosomes derived from controls as well as growth factor–stimulated myocytes. The products are involved in intracellular transport, mitogen-activated protein kinase signaling pathways, and the nucleus. TGF-β2–derived exosomes induced 201 platelet-derived growth factor BB 74–specific transcripts, apart from the 138 in untreated controls.110 It may be concluded that cardiomyocyte-derived exosomes carry a basic package of transcripts and growth factor stimulators of cardiomyocytes, resulting in the alteration of the transcriptional content in a specific way. Thus, the conditions under which the parental cell maintains life is decisive for the quality of the exosomal message sent by the cell. It is of fundamental interest to know that cardiomyocytes can send messages with specific content according to the need or will of the sending cell. Examples of this are outlined below when the myocardium adapts to the external stimuli (such as different forms of stress), inducing hypertrophy and changes in contractility. Such adaptation to increased workload (valvular disease, hypertension, loss of myocardium/infarction) can generally be described as remodeling.

Literature on cardiac exosomes is limited, but certain assumptions can be made based on the knowledge about exosomes from other tissues. Remodeling of the heart involves interplay mainly between cardiac cells, myocytes, fibroblasts, endothelial cells, smooth muscle cells, and extracellular matrix (and inflammatory cells). In particular, myocyte–fibroblast interaction is of importance.112 This interplay enables myocardial cells to grow or proliferate or be substituted by stem cells/fibroblasts transformed into cardiomyocytes. This includes angioneogenesis for regeneration of scarred myocardium or poorly perfused myocardium.109 This complicated and only partly known interplay is to some extent governed by signals delivered by exosomes.

In 2008, a series of important articles from Utrecht demonstrated that a mesenchymal stem cell–conditioned medium, when given intravenously just before reperfusion after ischemia, induced limited infarct size in both pigs and mice.113 Injection of isolated exosomes into a tail vein before reperfusion of coronary ligated mice resulted in limitation of myocardial infarct size.114 This article also suggests an explanation for the paracrine effect of mesenchymal stem cell implantation on tissue repair. Moreover, it was shown that exosomes derived from cardiomyocyte progenitor cells stimulate the migration of

Figure 3. Cartoon of an exosome. The exosomal contents comprise mRNA, DNA, microRNAs, proteins (ie, enzymes, growth factors, and cytokines), and transmembrane proteins of different kinds, including tetraspanins, annexin, and intracellular cell adhesion molecule. For further information, see text. Reprinted from Hu et al124 with permission of the publisher. Copyright © 2012, Frontiers in Genetics.
endothelial cells. Human cardiomyocyte progenitor cells were cultivated, and exosomes were isolated from the medium. A major factor for endothelial cell migration was the exosomal contents of MMPs. A membrane-bound MMP activator is also found in these exosomes, which induces MMP and vascular endothelial growth factor release from neighboring cells. Progenitor cell–derived medium, including exosomes, modulates cell differentiation, proliferation, and survival of cardiac progenitor cells, cardiomyocytes, and fibroblasts. It was reported recently that when the mechanism of protection was further studied in this mouse ischemia reperfusion model, ventricular dilatation was prevented and myocardial function improved. It was suggested that exosomes activated the survival pathways and restored ATP depletion. These exosomes also carry CD73 (5′-nucleotidase) that can produce extracellular adenosine, a well-known mediator of protection in ischemic preconditioning. Finally, the inflammatory reaction to ischemia was shown to be reduced. It is still not known which exosomal factors are most important for the effects described above, but this body of results so far supports the interpretation that certain growth factors and cytokines, such as basic fibroblast growth factor, tumor necrosis factor-α, TGF-β, epidermal growth factor, heat shock proteins (HSP20, 60, and 70), and miRs known to be carried by exosomes, are, at least in part, responsible for the response.

Ischemic preconditioning was first described by Murry et al in 1986. Several factors have been shown to function in this phenomenon, including adenosine, ATP-dependent K⁺ channels, mitochondrial transition pores, and some other factors. It was recently found that this complex system is also governed by factors that are known to be exosome-borne, much the same way as in ischemia/reperfusion injury described above. They are likely to be important especially in the late phase of preconditioning, as first described by Bolli. Similarly, miRs that are involved in ischemic injury and preconditioning and known to be carried by exosomes include miR-1 (post-transcriptional regulation of HSP60 and 70), miR-21 (control of cell survival and expression of MMPs), miR-29 (regulates p53 and induces apoptosis), miR-133a (regulates hypertrophy and apoptosis), and miR-499 (inhibits apoptosis). MMPs (regulated by miRs) induce degradation of scar tissue and substitution by invading transformed cells. Dying or apoptotic cells convey emergency signals, and rescue packages are sent back from stem cells, which to a certain extent are mediated via exosomes.

Exosomes with their narrow spectrum of molecules (nucleic acids, proteins, and lipids) may well be mediators of all these processes, thereby modulating cellular responses and signal transduction. It should be kept in mind that protein and RNA sorting into exosomes is highly regulated, and this allows cells to produce tailormade exosomes with different functional characteristics, in turn governed by the nature of signals triggering their production and release. After release, exosomes are targeted specifically to conjugated and distant cells as well. Accordingly, exosome release and cellular uptake are tightly controlled, and if we are able to govern this release and understand the mechanisms involved, we might be able to control the process.

**Future Aspects**

The era of clinical use of exosomes is just beginning. With the increased rate of publications, it can be expected that the field of use will also develop into unexpected areas. Exosomes in peripheral blood most probably reflect the status of the whole organ of exosomal origin and can, therefore, be expected to be used for:

- Diagnosis, for example, acute myocardial infarction
- Screening, for example, silent ischemia and hypertrophic cardiomyopathy
- Prognosis, for example, myocardial infarction
- Monitoring disease progression; exosome number and quality would reflect progression
- Monitoring treatment efficacy, for example, normalization of exosome number and quality
- Treatment, for example, as vehicle for gene therapy

Because the status of the parental cell decides the quality of exosomes released, it is assumed that exosomes can be used to diagnose organ disease. The methods for diagnosis and prognostication of prostate cancer are under development. This method will have advantages over biopsies because exosomes reflect the whole part of sick tissue, whereas biopsies might miss the diseased part. The quality and quantity of exosomes might tell severity of the disease as, for example, in cancer but also in congestive heart failure and hypertrophic cardiomyopathy depending on which miR, mRNA, and DNA are found. Moreover, the treatment effect could be monitored by these means. Many monogenic diseases do not start at a given time, for example, the onset of hypertrophic cardiomyopathy ranges from childhood to adulthood. The monitoring of the quality of circulating exosomes might give a hint of when to start therapy.
like β-blockers or implantable cardioverter defibrillator, and they will also be excellent for screening purposes. Finally, exosomes loaded with therapeutic contents (DNA, RNA, and miRs) will probably be possible to generate in the future.

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