Chapter 3
Functional Role of Cardiovascular Exosomes in Myocardial Injury and Atherosclerosis

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3.1 Introduction

Exosomes are bi-lipid-membranous vesicles containing protein, lipid and nucleic acid material secreted from cells. They are smaller than shedding microvesicles and apoptotic bodies (approximately 40–100 nm for exosomes compared to 100 nm–1 μm for shedding microvesicles and 1–4 μm for apoptotic bodies) and differ by their intracellular origin [19, 42]. Exosomes are identified by their components including integrins and tetraspanins (CD63, CD89, CD81, CD9, and CD82), by maturation-related proteins (flotillin and annexin), and by heat shock proteins (HSP). Together, exosomes and microvesicles are often referred to as extracellular vesicles (EVs). They are not a result of random sampling; instead they contain selective cargo assembled through dedicated packing mechanisms [40, 44], deliver these loads to targeted cells and contain unique trafficking properties. All these mechanisms depend on the individual cell, its cellular state and different physiological, pathological and stress conditions [19, 20]. EVs mediate horizontal, paracrine transfer, delivering microRNAs (miRs), mRNA and proteins between cells of different origin, resulting in silencing or activation of signaling pathways [32]. However, the underlying mechanisms of transfer and the amount of content delivered to the cells remain controversial or unclear.

This chapter will discuss the functional roles of EVs in the prevention, repair or progression of cardiovascular disease, through the communication between several cell types in the heart and vasculature, with an emphasis on signaling pathways.

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3.2   Role of Extracellular Vesicles in Intracardial Communication

3.2.1   *Cardiomyocytes, Myofibroblasts and Cardiac Injury*

The heart contains cardiomyocytes (CMs), which represent only a third of all cells in the heart, endothelial cells (ECs), immune-system-related cells (macrophages), fibroblasts (FBs), smooth muscle cells (SMCs), sympathetic and parasympathetic neuronal cells, and stem cells. A tight balance between these cell types is needed to maintain the integrity of the heart [4]. Myocardial injury disrupts this integrity by inducing CM death and destroying the vasculature, triggering several effects to repair and maintain cardiac integrity, including cardiac fibrosis by activation of myofibroblasts. This activation involves a complex signaling network containing transforming growth factor (TGF)-β, endothelin-1 (EDN-1), angiotensin II (AGTII), connective tissue growth factor (CCN2), and platelet-derived growth factor (PDGF) (Fig. 3.1a). Myofibroblasts originate from several sources including quiescent tissue FBs, circulating CD34+ fibrocytes, and the phenotypic conversion of various cell types including epithelial cells and ECs. To date, little is known about the inter-organ transfer mechanisms of cardioprotection but recent reports suggest that extracellular vesicles (EVs) release may be involved [13]. In this section we will focus on EVs released from CMs, ECs, macrophages and FBs in cardiac injury and remodeling (Fig. 3.1).

3.2.2   *Extracellular Vesicles from Cardiomyocytes, Endothelial Cells and Myofibroblasts*

CMs and ECs have an intimate anatomical relationship that is essential for maintaining normal development and function in the heart. Regulation mechanisms of cardiac and endothelial crosstalk in situations of acute stress remain elusive. This cardiac and endothelial crosstalk may involve EVs, among them exosomes. Recently, HSP20-enriched EVs secreted by CMs acted as a novel cardiokine in regulating myocardial angiogenesis through activation of the vascular endothelial growth factor receptor (VEGFR) signaling cascade [54] (Fig. 3.1b). Indeed, overexpression of HSP20 in streptozotocin (STZ)-induced diabetic mice significantly decreased cardiac dysfunction, hypertrophy, apoptosis, fibrosis, and restored angiogenesis. This protective action against adverse cardiac remodeling involved p-Akt, survivin, and superoxide dismutase 1. In addition, HSP20 exosomes interacted with tumor susceptibility gene 101, an important regulator of cell cycle arrest and p-53 independent cell death [47]. Diabetes also significantly impaired angiogenesis by inhibiting proliferation and migration of mouse cardiac endothelial cells (CECs). Mechanistically, higher levels of microRNA (miR)-320 in exosomes of diabetic animals functionally down-regulated its target genes such as insulin growth factor
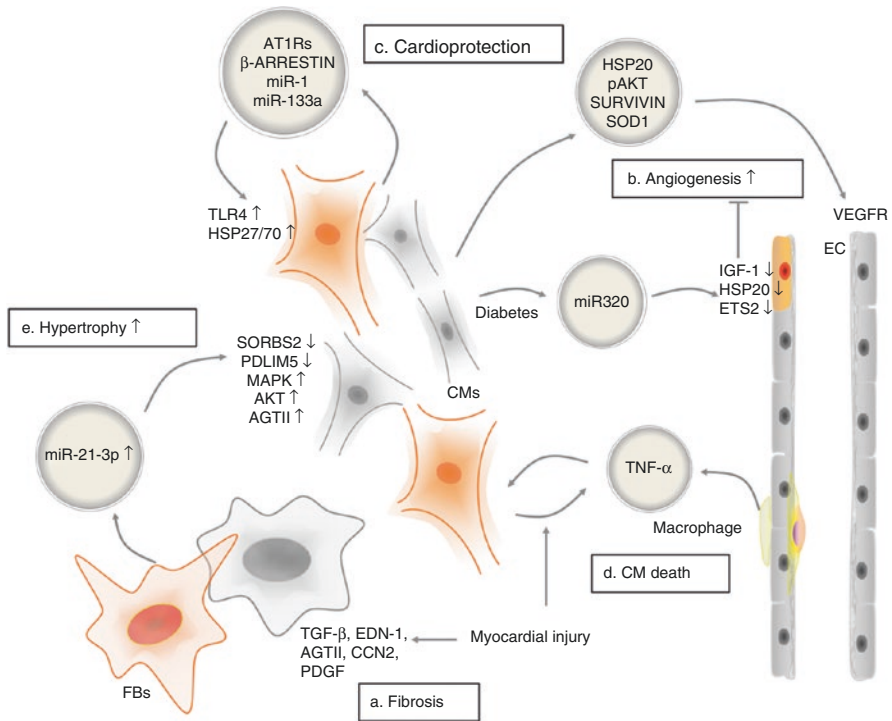
Fig. 3.1 Extracellular vesicles in cardiac remodeling: Myocardial injury induces CM death and triggers several repair mechanisms, including cardiac fibrosis. The effector cells of fibrosis are activated FBs called myofibroblasts and involves a complex signaling network containing TGF-β, EDN-1, AGTII, CCN2 and PDGF leading to fibrosis (**a**). EVs secreted from CMs were shown to be enriched in HSP20, pAKT, survivin and SOD1, and were cardioprotective and induced angiogenesis (**b**). In contrast, diabetes induces CMs to secrete EVs enriched in miR-320 impairing the expression of IGF-1, HSP20 and ETS2 in ECs resulting in impaired angiogenesis (**b**). CM-derived EVs containing A1TR, β-arrestin and miR-1 and miR133a increase TLR4/HSP27 signaling, resulting in cardioprotection (**c**). During myocardial ischemia, TNF-α is mainly released in macrophage-derived exosomes, but with persistent ischemia it can also originate from exosomes released by CMs, inducing CM death (**d**). miR-21-3p (miR-21*) in FB-derived exosomes induced CM hypertrophy by inhibiting SORBS2 and PDLIM5 (**e**). In addition, these FB-derived exosomes induced MAPK and AKT signaling resulting in intensified AGTII-induced cardiac hypertrophy. Abbreviations: *AGT* angiotensin, *AKT* AKT serine/threonine kinase 1, *A1TR* angiotensin II type I receptors, *CM* cardiomyocyte, *CCN2* connective tissue growth factor, *EC* endothelial cell, *EDN-1* endothelin-1, *FB* (myo)fibroblast, *ETS2* ETS proto-oncogene 2, transcription factor, *EV* extracellular vesicle, *HIF-1α* hypoxia inducible factor-1α, *HSP* heat shock protein, *IGF-1* insulin growth factor 1, *MAPK* mitogen-activated protein kinases, *miR* microRNA, *MQ* macrophage, *PDGF* platelet-derived growth factor, *PDLIM5* PDZ and LIM domain 5, *SOD* superoxide dismutase, *SORBS2* sorbin and SH3 domain-containing protein 2, *TGF-β* transforming growth factor, *TLR* toll like receptor, *TNF* tumor necrosis factor

(IGF-)1, HSP20 and ETS proto-oncogene 2, transcription factor (ETS2) in recipient CECs [48] (Fig. 3.1b).

In myocardial ischemia-reperfusion injury, both miR-1 and miR-133a in the exosome-rich fraction in plasma protected CMs by inhibiting cardiac hypertrophy [7]

(Fig. 3.1c). Therefore, they may be utilized to suppress maladapted hypertrophy when blood flow and energy supply is limited [26]. A pro-survival signaling pathway was activated in CMs involving toll-like receptor (TLR)-4 and various kinases, leading to activation of the cardioprotective HSP27.

In addition to secreting microvesicles and exosomes, CMs were found to secrete cytokines, chemokines, and factors like ANP, BNP, TGF-β, and tumor necrosis factor-α (TNF-α) [15, 46]. Excessive TNF-α is thought to be harmful to CMs in acute myocardial infarction (MI). During myocardial ischemia, TNF-α is mainly released in macrophage-derived exosomes, but with persistent ischemia it can also originate from exosomes released by CMs, induced directly by hypoxia and activation of hypoxia inducible factor-1α (HIF-1α) [53] (Fig. 3.1d).

Finally, FBs also have an important function in the pathophysiology of CM death, fibrosis and hypertrophy. MiR-21-3p in cardiac FB-derived exosomes induced CM hypertrophy (Fig. 3.1e). Sorbin and SH3 domain-containing protein 2 (SORBS2) and PDZ and LIM domain 5 (PDLIM5) were identified as miR-21-3p targets by proteome profiling. Silencing SORBS2 or PDLIM5 in CM and inhibition of miR-21-3p induced hypertrophy [5]. In addition, FB-derived exosomes induced mitogen-activated protein kinases (MAPKs) and Akt resulting in increased expression of the Renin Angiotensin System, thereby intensifying AGTII-induced pathological cardiac hypertrophy [29].

3.3 Vesicles from Stem Cells and Progenitor Cells

3.3.1 Cardiac Stem Cells

By definition, a stem cell is capable of self-renewal and can differentiate into at least one cell type. Embryonic stem cells (ESCs) are pluripotent stem cells which were first isolated as a small cluster of cells within mouse blastocysts [11], later from human blastocysts [38]. Hematopoietic stem cells (HSCs) differentiate into different blood cells and are CD34+. Mesenchymal stem cells (MSCs) are multipotent stromal cells that can differentiate into a variety of cell types like myocytes and adipocytes [39]. In contrast to HSCs, standardization of MSCs was hampered by the lack of molecular markers to discern MSCs from FBs. However, recently, specific DNA methylation patterns were used to discriminate between MSCs and FBs, and to distinguish between MSCs from bone marrow and adipose tissue [1]. In addition to ESCs, HSCs and MSCs, a population of resident cardiac stem cells (CPCs) have been identified in the heart; but they comprise less than 1% of the cells in the heart. As yet it is not known whether the CPCs actually migrate from bone marrow to the heart, or originate from remnants of embryonic cell populations in the right atrium and right ventricle. CPCs have been sub-classified into c-kit and Scal-1. C-kit cardiogenic stem cells can differentiate into myogenic, vascular endothelial and smooth muscle lineages. Sca-1 are involved in cell signaling and cell adhesion [16]. Finally, induced pluripotent stem cells (iPSC) can generate an abundance of cells without the risk of immune rejection for cell therapy.

3.3.2 Stem Cell Derived Vesicles

MSCs have been considered to be one of the most promising candidates for regeneration of cardiac cells lost upon injury. But with age, dysregulated MSCs differentiate into dysfunctional inflammatory FBs leading to pathologic fibrosis. The phenotypic change is specific to the heart since MSCs originating from bone or FBs derived from MSCs were free of these defects [43]. In contrast with the original hypothesis that the regenerative capacity of MSCs was due to their potential to engraft, differentiate and replace damaged cardiac cells, recent studies suggested that this was primarily due to paracrine factors released from MSCs in exosomes [35]. For example, GATA binding protein 4 (GATA-4) enriched in exosomes released by MSCs at the border of an ischemic region significantly restored cardiac contractile function and reduced infarct size (Fig. 3.2). Mechanistically, these exosomes increased miR-19a in CMs, resulting in decreased expression of Phosphatase and Tensin Homolog (PTEN), a predicted target of miR-19a, and in the activation of the Akt and ERK signaling pathways [52] (Fig. 3.2). In addition to miR-19, miR profiling analysis revealed that cardiac stem cells exposed to MSC-derived exosomes secreted more miR-147, let-7i-3p, miR-503-5p, and miR-362-3p, and less miR-326-5p, miR-328a-5p, miR-207, miR-760-3pn, and miR-702-5p, associated with activation of target genes involved in angiogenesis and positive regulation of cell proliferation, cell migration, cell differentiation, and response to hypoxia [55]. They activated several signaling pathways important in wound healing (Akt, ERK, and STAT3) and induced the expression of a number of growth factors [hepatocyte growth factor (HGF), IGF1, nerve growth factor (NGF), and stromal-derived growth factor-1 (SDF1)] [37], ultimately leading to preserved cardiac performance after MI [6] (Fig. 3.2). Levels of ATP, NADH and phosphorylated-Akt and phosphorylated-inosine/guanosine kinase (GSK)-3β were increased, while phosphorylated-c-JNK was reduced, thereby decreasing oxidative stress and inflammation in ischemic/reperfused hearts [3]. In addition, the proteome of MSCs and MSC-derived exosomes, from cells cultured under expansion conditions and under ischemic tissue simulated conditions, was shown to contain key angiogenic paracrine effectors and, potentially, differentially expressed in these conditions. In total, 6342 proteins were identified in MSCs and 1927 proteins in MSC-derived exosomes. They included PDGF, epidermal growth factor (EGF), fibroblast growth factor (FGF), and most notably nuclear factor-kappaB (NFkB) signaling pathway proteins. The latter was identified as a key mediator of MSC exosome induced angiogenesis in ECs [2] (Fig. 3.2).

Exosomes derived from iPSC-derived mesenchymal stem cells (iMSC), expressing CD63, CD81, and CD9 at their surface, enhanced microvessel density and blood perfusion in mouse ischemic limbs, consistent with an attenuation of ischemic injury [18], possibly by delivery of cardioprotective miRs, including nanog-regulated miR-21 and HIF-1α-regulated miR-210 [49] (Fig. 3.2).

Furthermore, CPC-derived EVs had the same beneficial effects as their parent cells in the treatment of chronic heart failure in mice [23]. Like MSCs, CPCs
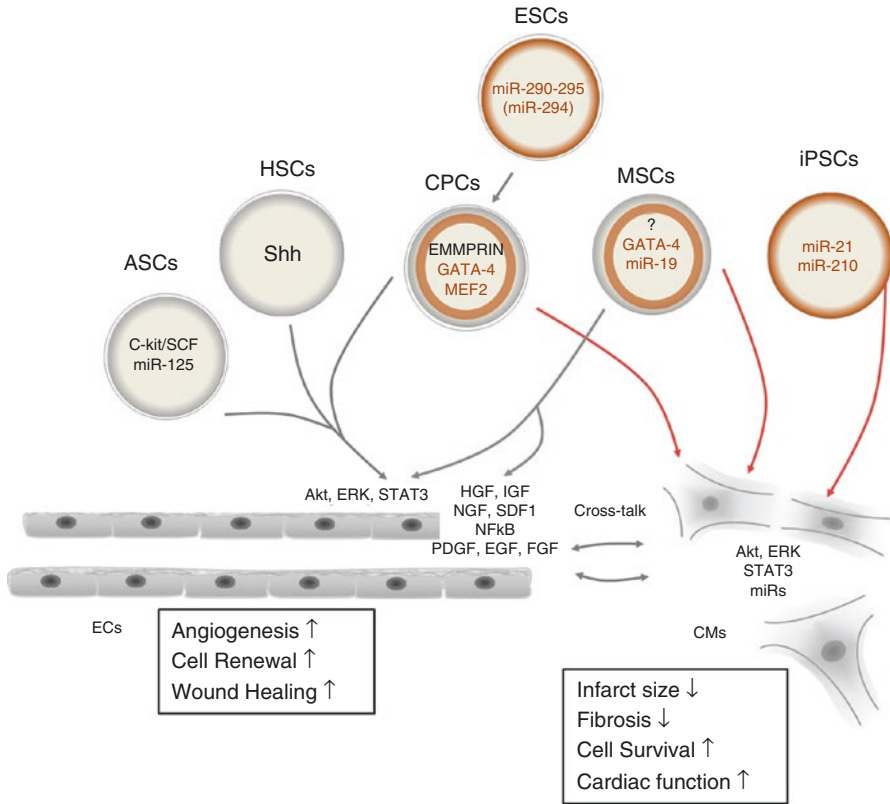
Fig. 3.2 Stem and progenitor cells secrete EVs which protect CMs. EVs released by MSCs overexpressing miR-19 and GATA-4 at the border of an ischemic region following ligation of the left anterior descending coronary artery significantly reduced infarct size and restored cardiac contractile function. Exosomes from iPSC protected against myocardial ischemia/reperfusion injury most likely by delivery of cardioprotective miRs, including nanog-regulated miR-21 and HIF-1α-regulated miR-210. CPCs release EVs containing GATA-4 and MEF2, which are important in the development of pre-cardiac cells. ESC-derived exosomes deliver ESC-specific miR-290-295 cluster and more particular miR-294 to CPCs and CMs, promoting increased survival, cell cycle progression, and proliferation in ECs and CMs. On aggregate, these EVs reduced infarct size and fibrosis and increased cell survival and cardiac function. ASC-derived exosomes carry c-kit and stem cell factor, which play a role in angiogenesis. Exosomes secreted by ASCs also promote EC angiogenesis by transferring miR-125a repressing the NOTCH ligand delta-like 4. HSCs induce angiogenesis by secreting exosomes with increased expression of Shh. CPCs also secrete exosomes with pro-angiogenic properties mediated via ERK/AKT-signaling. They contain high levels of EMMPRIN. MSCs exposed to ischemia secrete exosomes enriched in PDGF, EGF and FGF, and most notably, NFkB signaling pathway proteins inducing angiogenesis in ECs. In addition, MSC-derived exosomes activated several signaling pathways important in wound healing (AKT, ERK, and STAT3) and induced the expression of a number of growth factors (HGF, IGF1, NGF, and SDF1). On aggregate, these EVs induced angiogenesis, cell renewal and wound healing in endothelium. Abbreviations: *AKT* AKT serine/threonine kinase 1, *ASC* adipose-derived mesenchymal cell, *CPC* cardiac progenitor cell, *EGF* epidermal growth factor, *EMMPRIN* extracellular matrix metalloproteinase inducer, *ERK* extracellular signal–regulated kinase, *ESC* embryonic stem cell, *FGF* fibroblast growth factor, *GATA-4* GATA binding protein 4, *GSK* inosine/guanosine kinase, *HGF* hepatocyte growth factor, *HIF-1α* hypoxia inducible factor-1α, *HSCs* hematopoietic stem cell, *IGF-1* insulin growth factor 1, *MEF2* myocyte enhancer factor 2, *MSC* mesenchymal stem cell, *NFkB* nuclear factor-kappaB, *NGF* nerve growth factor, *PDGF* platelet-derived growth factor, *SCF* stem cell factor, *Shh* sonic hedgehog, *SDF1* stromal-derived growth factor-1, *STAT3* signal transducer and activator of transcription 3

secreted exosomes with pro-angiogenic properties mediated via ERK/Akt-signaling (Fig. 3.2). Analysis of pro-angiogenic factors revealed high levels of extracellular matrix metalloproteinase inducer (EMMPRIN) [45]. Also mouse ESC-derived exosomes augmented function in infarcted hearts by enhancing neovascularization and CM survival and by reducing fibrosis, most likely by delivery of ESC-specific miR-294 to CPCs, promoting increased survival, cell cycle progression, and proliferation [24] (Fig. 3.2).

Finally, adipose mesenchymal stem cell (ASC)-derived EVs induced in vitro vessel-like structure formation by human microvascular endothelial cells (MECs) (Fig. 3.2). Treatment of ASCs with PDGF stimulated secretion of EVs, carrying c-kit and stem cell factor, SCF, regulated by HIF-1α, inducing angiogenesis [28]. Exosomes secreted by ASCs also promoted EC angiogenesis by transferring miR-125a, which repressed the NOTCH ligand delta-like 4 [27]. Similarly, neovascularization was induced by transplantation of human HSCs to ischemic tissues in preclinical models (Fig. 3.2). These cells secreted exosomes with increased expression of the angiogenic factor sonic hedgehog (Shh), to offset age- and health-related angiogenic declines [36]. They also reduced infarct size and increased border zone capillary density compared with unmodified CD34+ cells [30].

3.4 Role of Extracellular Vesicles in Endothelial to Mesenchymal Transition

Recent studies demonstrated that the phenotypic transition of ECs into MSCs, called Endothelial to Mesenchymal Transition or EndMT, plays an important role in the pathogenesis of fibrotic disorders. During EndMT, resident ECs acquire a mesenchymal phenotype characterized by an increased ability to migrate and invade, thereby contributing to tissue remodeling and fibrosis [25, 31].

Hypoxia was found to efficiently induce human coronary artery endothelial cells (CAECs) to undergo EndMT, resulting in EndMT-derived FBs (Fig. 3.3). This process was mediated through a HIF-1α-dependent pathway, TGF/SMAD signaling pathways and DNA (cytosine-5)-methyltransferase 3A (DNMT3a)-mediated hypermethylation of Ras-Gap-like protein 1 (RASAL1) promoter and direct zinc-finger transcription factor Snail (SNAIL) induction [50]. Ultimately, this resulted in increased expression of extracellular matrix proteins such as collagen COLI and COLIII [51]. Focal myocardial fibrosis is also a structural hallmark of diabetic cardiomyopathy resulting from hyperglycemia-induced endothelial injury leading to EndMT associated with reduced expression of EC markers, such as CD31 and CD34, and increased expression of multiple mesenchymal markers, such as COLI and COLIV, and vimentin. Mir-200b reverted diabetes-associated EndMT by directly interacting with VEGF, SMAD2/3 and regulating p300-dependant histone acetylation and expression of for example extracellular matrix proteins [12].

Cardiac fibrosis does, however, not only result from a decrease in microvessels and oxygen supply but also from increased oxygen consumption by activated immune and inflammatory cells and fibroblasts leading to localized tissue hypoxia predominantly within inflammatory lesions ("inflammatory hypoxia"). Herein, HIF-1α, an oxygen-sensitive transcription factor that allows adaptation to hypoxia environments, plays an important role. Recent data suggest that the HIF-1 α-mediated metabolic shift and fibrosis is not only related to cardiovascular diseases but also to immune-related disorders [10] (Fig. 3.3). A common direct target of HIF-1α in hypoxia-induced EndMT is SNAIL [51] (Fig. 3.3).
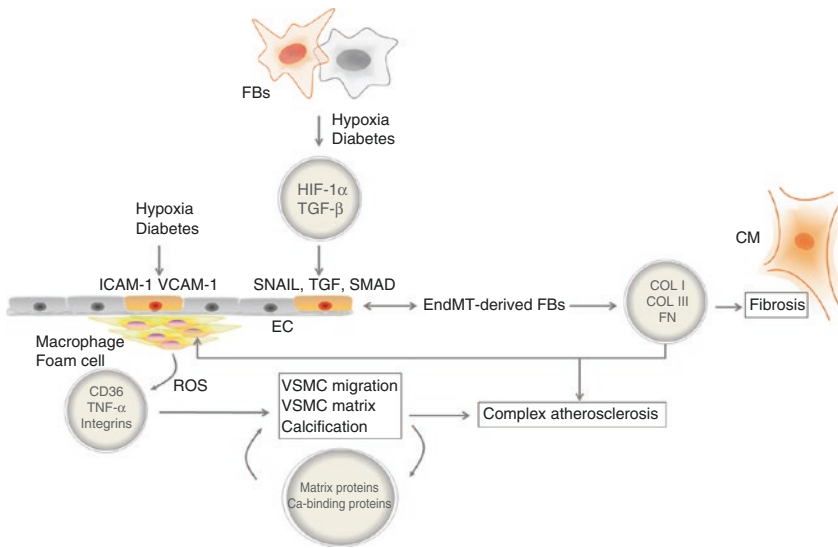

Fig. 3.3 Endothelial to Mesenchymal Transition, fibrosis and atherosclerosis. Hypoxia and diabetes induce secretion of EVs by FBs enriched in HIF-1α and TGF-β, respectively. They induce endothelial cells to undergo endothelial to mesenchymal transition (EndMT) through direct induction of SNAIL, a target of HIF-1α, and through TGF/SMAD signaling pathways and DNMT3a-mediated hypermethylation of RASAL1 promoter. EndMT results in the generation of EndMT-derived FBs with increased expression extracellular matrix proteins such as COLI, COLIII and FN. Hypoxia and diabetes cause endothelial dysfunction with increased secretion of leukocyte adhesion molecules leading to increased macrophage accumulation and oxidative stress. Macrophages secrete EVs enriched in CD36 and TNF-α which induce VSMC migration. Stimulated VSMCs secrete extracellular vesicles inducing matrix accumulation and calcification, resulting in complex atherosclerotic plaques. Collagen and fibronectin in extracellular vesicles secreted by EndMT-derived FBs exacerbate the growth of atherosclerotic plaques by inducing macrophage infiltration and making the atherosclerotic plaques more complex due to extracellular matrix deposition. Abbreviations: *COL* collagen, *DNMT3a* DNA (cytosine-5)-methyltransferase 3A, *EC* endothelial cell, *EV* extracellular vesicle, *FB* fibroblast, *FN* fibronectin, *HIF-1α* hypoxia inducible factor-1α, *ICAM-1* intercellular adhesion molecule 1, *RASAL1* Ras-Gap-like protein 1, *ROS* reactive oxygen species, *SMC* smooth muscle cells, *SNAIL* zinc-finger transcription factor Snail, *TGF-β* transforming growth factor, *TNF* tumor necrosis factor, *VCAM-1* vascular cell adhesion molecule-1

3.5 Role of Extracellular Vesicles in Atherosclerosis

Hypoxia and diabetes are known to cause endothelial dysfunction, with increased secretion of leukocyte adhesion molecules leading to increased macrophage accumulation, reactive oxygen species (ROS) and oxidative stress, ultimately leading to atherosclerosis (Fig. 3.3). The circulation of atherosclerotic patients has been shown to contain more leukocyte-derived EVs promoting vascular SMC adhesion and migration, than those of healthy participants. In addition, macrophage-derived foam cells secrete EVs which promote increased levels of vascular SMC migration and adhesion, regulating the actin cytoskeleton and focal adhesion pathways to a greater extent than macrophage-derived EVs. Western blotting revealed that foam cell-derived EVs could also promote the phosphorylation of ERK and Akt in SMCs in a time-dependent manner. Foam cell-derived EVs could enter the SMCs and transfer integrins to the surface of these cells [33].

The calcification of SMC is also mediated by regulated exosome secretion. Comparative proteomics showed that vascular SMC-derived exosomes shared components with osteoblast-derived EVs including calcium-binding and extracellular matrix proteins (Fig. 3.3). Elevated extracellular calcium was found to induce sphingomyelin phosphodiesterase 3 and the secretion of calcifying exosomes from vascular SMCs in vitro. Chemical inhibition of sphingomyelin phosphodiesterase 3 prevented SMC calcification. In vivo, EVs containing exosomes were observed in vessels from chronic kidney disease patients on dialysis, and CD63 was located where there was calcification. Importantly, factors such as TNF-α and PDGF-BB were also found to increase exosome production, leading to increased calcification of SMC [22]. Comparison between exosomes from quiescent and activated SMCs showed evidence of 29 differentially expressed proteins which are involved in cytoskeleton organization, chaperones, cell adhesion, cell signaling, metabolic pathways, vesicle trafficking and extracellular matrix production [9] (Fig. 3.3). Foam cell formation and enhanced VSMC and extracellular matrix accumulation with calcification resulted in the generation of complex atherosclerotic plaques (Fig. 3.3). The extracellular matrix proteins COL and fibronectin, induced in EndMT, were associated with increased luminal endothelial expression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). Mechanistically, the relation between EndMT and atherosclerosis also depends on loss of endothelial fibroblast growth factor receptor 1 (FGFR1) expression and activation of endothelial TGF-β signaling [8].

3.6 Research Limitations and Future Outlook

Facing a shortage of human data we focused our review to studies containing in vivo data obtained in relevant preclinical models. Even with these restrictions, it proved to be difficult to compare the outcome of selected in vivo studies because of

differences in isolation procedures of EVs (for example ultracentrifugation, size exclusion chromatography, or immunoadsorption) which may yield EVs with different sizes (not necessarily purified exosomes), use of different sets of surface markers which may yield information about cell origin of EVs and differences in extent of evaluation of cargo (miRs, proteins or none of them). Therefore standardization of separation procedures and protocols to analyze biogenesis, composition and function are needed to improve our insight in the mechanistic role of EVs [34]. Novel isolation procedures may involve microfluidics devices for on-chip isolation and quantification of circulating micro-particles [21], and microchip-based RNA extraction, amplification and RT-PCR analysis [41].

A major shortcoming of many of the previous studies is that the full content of EVs was not all analyzed or that the selection of compounds to which functional roles were attributed was biased. Indeed, many of the reviewed studies focused on one of a few numbers of compounds without displaying information about the complete cargo or specifying the reason why these compounds were selected. Furthermore, data on the signaling pathways involved in the mechanisms of action of EVs are rare and again incomplete. Therefore, an unbiased systems biology approach is needed to generate and test hypotheses about the effect of context (e.g. spatial organization of endothelial cells in relation to other cell-types like macrophages or myofibroblasts and interaction through paracrine factors) dependent on state of the disease on the functional role of specific EVs. To this end Gray and colleagues [14] proposed cue-signal-response studies using partial least square regression (PLSR) methods that study how signals (exosome content or cargo) translate cues from the secreting cell (its gene/protein expression state) to elicit a specific response in the recipient cells.

In reviewing the role of EVs in cardiovascular diseases it became obvious that, although it is generally accepted that macrophages play a crucial role in the development of cardiac fibrosis and atherosclerosis, information about the role of their precursor cells, monocytes, and exosomes derived from monocytes is limited. Recently, we found that low mitochondrial cytochrome oxidase-1, a marker of mitochondrial dysfunction, in monocyte-derived exosomes predicted the risk of future cardiovascular events in the same way as low mitochondrial cytochrome oxidase-1 in monocytes. Therefore, the role monocyte-derived exosomes should be investigated further [17].

3.7   Conclusions

The studies reviewed present evidence that extracellular vesicles secreted by CMs, ECs, immune-system-related cells (macrophages), fibroblasts (FBs) and stem cells play an important role in the regulation of endothelial cardiomyocyte and endothelial function in relation to cardiovascular diseases. However, knowledge of the underlying signaling pathways is still too sparse to identify targets for EV-mediated treatment of these diseases.

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