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Literature Review

UNVEILING THE POTENTIAL OF SALIVARY EXOSOMAL BIOMARKERS IN CARDIOVASCULAR DISEASE:INSIGHTS AND FUTURE DIRECTIONS

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Abstract

Cardiovascular disease (CVD) is the primary cause of mortality world wide, necessitating innovative diagnostic approaches due to the limitations of current tools. The growing significance of salivary exosomes as biomarkers for CVD detection and treatment is explored in this narrative review. Salivary diagnostics offer a non-invasive alternative, with exosomes-small extracellular vesicles containing proteins, lipids, and nucleic acids—serving as potential indicators of cardiovascular health. The review highlights how salivary exosomes reflect the physiological states of their parent cells and can indicate systemic inflammation, oxidative stress, and endothelial dysfunction, all critical factors in CVD progression. Furthermore, it discusses specific exosomal biomarkers linked to different cardiovascular diseases such as valvular heart disease, coronary artery disease, cardiac fibrosis, arrhythmias, and heart failure. By integrating salivary exosome analysis into clinical practice, there is potential for enhanced early detection, risk stratification, and monitoring of cardiovascular diseases, ultimately paving the way for more personalized treatment strategies. This exploration underscores the transformative potential of salivary exosomes in improving cardiovascular disease management and patient outcomes.

Keywords: *Cardiovascular diseases; Exosomes; Micro RNA; Salivary biomarkers.*

INTRODUCTION

Cardiovascular disease (CVD) refers to a group of conditions affecting the heart and blood arteries, including disease of coronary artery, cardiomyopathy, congenital heart disease, arrhythmias and heart failure. CVD is the primary cause of death worldwide, accounting for 18.6 million deaths, which emphasizes the need for more sophisticated and easily available diagnostic tools.[1,2] The pathogenesis of CVD is multifactorial, involving complex interactions between genetic, environmental, and lifestyle factors.[3] Traditional risk factors include hypertension, hyperlipidemia, diabetes, smoking, and obesity, which contribute to the development of atherosclerosis and other cardiovascular conditions.[4,5] Early detection and risk stratification are crucial for implementing preventive

strategies and improving patient outcomes.[2,3]

In recent years, the field of salivary diagnostics has gained momentum as a promising approach for detecting and monitoring various diseases, including CVD.[6–8] Saliva, as a biological fluid, offers several advantages over traditional blood-based tests, such as ease of collection, non-invasive nature, and potential for frequent sampling.[6,8] Within the salivary milieu, extracellular vesicles (EVs), particularly exosomes, have emerged as potential biomarkers for CVD. Exosomes are small extracellular vesicles released by multiple cell types, including those in the cardiovascular system, and they carry a cargo of nucleic acid , lipids and proteins that represent the physiological condition of their parent cells.[9,10] The analysis of salivary exosomes offers a non-invasive method for obtaining valuable information about systemic health, including cardiovascular status. Studies have shown that specific molecular signatures within salivary exosomes can indicate the presence of cardiovascular risk factors, such as inflammation and oxidative stress, which are critical in the pathophysiology of CVD.[10,11]

The integration of salivary exosome analysis into routine clinical practice could revolutionize the approach to cardiovascular disease management. By providing insights into the molecular mechanisms underlying CVD, salivary exosomes may aid in early diagnosis, risk stratification, and monitoring of disease progression.[9,10] Furthermore, the ease of saliva collection compared to blood draws enhances patient compliance and facilitates frequent monitoring.

This review serves to highlight the importance of salivary exosomes in the context of cardiovascular disease, emphasizing their potential to transform current diagnostic practices. By exploring the biochemical composition and functional implications of salivary exosomes, we aim to contribute to the growing body of knowledge that supports their use as reliable biomarkers.

Exosomes

The word "exosomes" was initially used by Johnstone et al.,[12] and Pan et al.[13] who found exosomes in sheep reticulocytes. Exosomes are tiny extracellular vesicles that are essential for communication between the cells and are generated through a specific biogenesis process. Early-sorting endosomes (ESEs) are produced when the plasma membrane invaginates inward, which is the first step in the synthesis of exosomes.[14] These ESEs can mature into late-sorting endosomes (LSEs) and ultimately develop into multivesicular bodies (MVBs), which contain intraluminal vesicles (ILVs). The MVBs can either fuse with lysosomes for degradation or merge with the plasma membrane to release the ILVs as exosomes, typically ranging from 40 to 160 nm in diameter.[14,15] These tiny vesicles, which typically have a density of 1.10 to 1.19 g/mL, are made up of proteins, lipids, messenger RNA (mRNA), microRNA (miRNA) circular RNA (circRNA), long non-coding RNA (lncRNA), and DNA.[16,17] They are encased in a lipid bilayer. Numerous cell types, including B lymphocytes, platelets, glial cells, dendritic cells, mast cells, adipocytes, endothelial cells, smooth muscle cells, and stem cells, can create exosomes. It's interesting to note that some microbes can also produce exosomes, which increases their biological relevance.[10]

Exosomes function in intercellular communication has drawn a lot of interest, especially when it comes to disease processes. They are involved in a number of pathological and physiological processes, such as tissue healing, tumor growth, and immunological responses.[10,12,17] Exosomes help move bioactive molecules from one cell to another, changing the behavior of the receiving cell and assisting in the control of inflammation and immunological responses. Because of their ability to mirror the chemical changes in their parent cells in response to a variety of stimuli, including disease conditions, exosomes are promising candidates for biomarker identification and therapeutic applications.[10,16,17]

Exosomes and CVD

The molecular content of exosomes represent the pathological and physiological states of their parent cells, making them potential indicators of oxidative stress, endothelial dysfunction and systemic inflammation—key factors in the development and progression of CVD.[10,17] Exosomes play a crucial role in cardiovascular signal transduction, serving as effective disseminators of biological signals related to myocardial function. These nano-sized extracellular vesicles mediate local communication in the heart through crosstalk between different cell types, such as cardiac fibroblasts, endothelial cells and cardiomyocytes.[17]Exosomes secreted by these cells can influence the same cells that release them (autocrine signaling) or target other cell types (paracrine signaling). Additionally, exosomes facilitate remote communication, enabling functional crosstalk between the heart and other organs like the kidneys, brain, and bone marrow. This exchange of proteins, lipids, and nucleic acids through exosomes regulates transcription and post-transcriptional processes in target cells, contributing to the maintenance of cardiac function under normal and pathological conditions.[17]

Exosomes derived from various cardiac cell types exhibit distinct functions. Cardiomyocyte-derived exosomes regulate angiogenesis and cardiac hypertrophy through the transfer of specific miRNAs, such as miR-222, miR-143, and miR-217. Cardiac fibroblast-derived exosomes induce cardiomyocyte hypertrophy by delivering miR-21-3p and promoting the production of angiotensin II.[18,19] Endothelial cell-derived exosomes play a crucial role in regulating endothelial senescence, angiogenesis, and cardiomyocyte resistance to ischemia/reperfusion injury. These exosomes modulate various signaling pathways, including ERK1/2 MAPK and the inhibition of autophagy and glucose metabolism in cardiomyocytes. [20,21]Circulating exosomes also contribute to cardiovascular signal transduction, carrying bioactive molecules like angiotensin II receptors and miRNAs.[17] Exosomes derived from cardiomyocytes can be detected in the circulation and mediate functional crosstalk between the bone marrow and ischemic heart. These circulating exosomes regulate the number of progenitor cells and facilitate systemic responses to cardiac injury. Additionally, exosomes from patients with myocardial ischemia initiate angiogenesis through the miR-939-iNOS-NO pathway. [22]The ability of exosomes to modulate various aspects of cardiovascular pathophysiology highlights their potential as biomarkers and therapeutic targets for cardiovascular diseases.

Exosomes facilitate intercellular communication by transferring their cargo to recipient cells. This transfer can have both beneficial and detrimental effects. In the context of CVD, exosomes from injured or stressed cells may transfer harmful molecules that promote inflammation, apoptosis, and fibrosis in neighboring cells. Conversely, exosomes from healthy or therapeutic cells can carry molecules that counteract these pathological processes.

Salivary exosomes with cardiovascular disease biomarkers

Cardiovascular exosomes are extracellular vesicles formed in cardiovascular cells, such as endothelial cells and cardiomyocytes.[15] Salivary exosomes are derived primarily from salivary glands and contain a rich array of bioactive molecules. Their composition can change in response to pathological conditions, making them promising candidates for biomarker discovery.

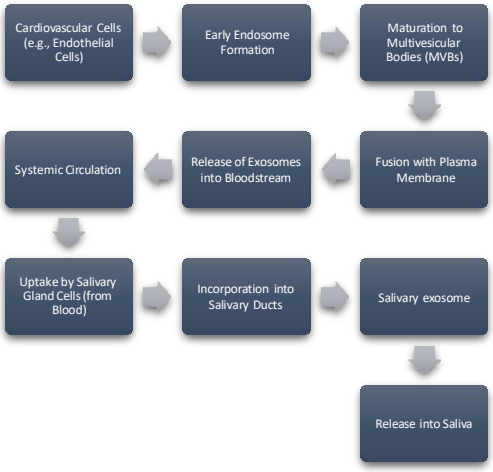

Figure1. Release of cardiovascular exosomes in saliva

Salivary exosomes, originating from cardiovascular cells, undergo a multi-step formation and transfer process. Cardiovascular cells, including cardiomyocytes and endothelial cells, first create exosomes by budding the plasma membrane inward to create early endosomes. These endosomes then develop into multivesicular bodies (MVBs) that contain intraluminal vesicles (ILVs). These MVBs then fuse with the plasma membrane, releasing ILVs as exosomes into the extracellular space.[10,11,15] The exosomes enter the bloodstream and circulate systemically. Notably, extracellular vesicles are known for their ability to traverse epithelial barriers, such as the blood-brain barrier, through a process called transcytosis.[23,24] This capability facilitates the movement of RNAs and other cargo from the blood into saliva. In the salivary glands, cardiovascular exosomes are taken up by glandular cells from the blood, incorporated into saliva, as these cells release the exosomes into the salivary ducts, thus becoming part of the saliva. As a result, salivary exosomes contain cardiovascular biomarkers that can be detected and analyzed for diagnostic purposes(Figure 1).

As we explore the potential of exosomal biomarkers, it becomes clear that their application in cardiovascular diseases is both promising and transformative. So far, we have examined the link between exosomes, cardiovascular diseases (CVD), and salivary exosomes, including their production mechanisms. Now, we will focus on the various exosomal biomarkers identified for diagnosing different cardiovascular conditions. Notably, many of these biomarkers can also be found in saliva, positioning them as promising candidates for non-invasive diagnostics. This emerging field holds significant potential for early detection and monitoring of cardiovascular diseases, enhancing our ability to provide tailored therapeutic approaches.

Coronary artery disease (CAD)

Coronary artery disease (CAD) is a significant public health issue defined by the narrowing or blockage of coronary arteries due to atherosclerosis.[25,26] Ischemic heart diseases are a group of diseases characterized by reduced blood flow to the heart due to obstruction of coronary vessels by atherosclerotic plaque formation. Hence ischemic heart diseases and coronary artery diseases are two terminologies which go hand in hand.[27] This condition can lead to various clinical manifestations, including myocardial infarction (MI), ustable angina, stable angina, and sudden cardiac death. CAD is commonly classified into acute coronary syndrome (ACS) and stable CAD (SCAD) based on the severity and clinical characteristics.

Exosomal Biomarkers in CAD Diagnosis

1. **Growth Arrest-Specific 5 (GAS5):** In the context of atherosclerosis, exosomal lncRNA GAS5 plays a regulatory role in the death of macrophages and vascular endothelial cells, indicating its potential as a CAD biomarker.[28]
2. **SOCS2-AS1:** Liang et al.[25] identified that exosomal SOCS2-AS1 levels are downregulated in CAD patients. This lncRNA may serve as a specific indicator for CAD.
3. **miR-126, miR-21, and PTEN:** Ling et al.[26] demonstrated distinct differences in the levels of these exosomal biomarkers in ACS patients compared to healthy individuals. miR-21 was found to be downregulated, while miR-126 and PTEN were upregulated, suggesting their roles as specific serum markers for ACS .
4. **miR-208a:** Bi et al.[29] reported that exosomal miR-208a levels are elevated in ACS patients, highlighting its potential as both a diagnostic and prognostic marker for ACS
5. **miR-146a:** Li et al.[30] found that the levels of exosomal miR-146a were raised in ACS patients, suggesting its utility as a novel diagnostic biomarker and its involvement in inflammatory responses . It also regulates autophagy in hypoxic cardiomyocytes, reducing apoptosis and fibrosis.[31,32]
6. **Cysteine-Rich Protein 61 (Cyr61):** Increased exosomal Cyr61 levels in plasma were linked to ACS, indicating its potential use for diagnosis and prognosis in ACS patients .[33]
7. **miR-942-5p, miR-32-5p, and miR-149-5p:** These exosomal miRNAs were found to be significantly elevated in SCAD patients, suggesting their potential as novel diagnostic and prognostic indicators.[34]
8. **miR-19a:** Research demonstrated that miR-19a can reduce oxidative stress-induced apoptosis in myocardial ischemia-reperfusion injury, making it a potential therapeutic target for CAD.[35]
9. **miR-210:** Huang et al.[36] highlighted that exosomal miR-210, upregulated by hypoxia-inducible factor 1 (HIF-1), enhances cardiac progenitor cells' tolerance to oxidative stress, suggesting its therapeutic potential for CAD.
10. **miR-133a:** This miRNA, abundant in the heart, has shown to improve cardiac function in myocardial infarction models, indicating its role as a CAD biomarker.[37]
11. **miR-25-3p:** It reduces myocardial cell apoptosis and inflammation. The miRNA promote cell survival and vascularization, which are crucial for heart recovery. Elevated levels of this miRNA in circulation can indicate ischemic stress and ongoing cardioprotection, making them potential biomarkers for early diagnosis of MI.[38]

These studies illustrate the diverse roles of exosomal biomarkers in diagnosing and managing CAD, reflecting their potential to enhance our understanding and treatment of this prevalent cardiovascular condition.

Cardiomyopathy

Cardiomyopathy is a disease that affects the heart muscle, causing it to become enlarged, thick, or rigid. This can make it harder for the heart to pump blood effectively, leading to heart failure and other complications. There are several types of cardiomyopathy, including Diabetic cardiomyopathy (DCM) , uremic cardiomyopathy (UCM), dilated, acute peripartum cardiomyopathy (PPCM)and septic. [39]

1. miR-1, miR-208, and miR-499: These are associated with myocardial injury and serve as potential diagnostic indicators for diabetic cardiomyopathy (DCM). They regulate cardiac muscle function and influence apoptosis and hypertrophy in cardiomyocytes.[39]

2. miR-19b-3p and miR-181b-5p: These influence cellular stress responses and apoptosis in cardiomyocytes, providing insights into cardiac health. They positively correlate with myocardial status, used for diagnosis and prognosis of DCM.[40]

3. miR-126 and miR-320: These enhances endothelial function and angiogenesis. Also inhibits angiogenesis and impacts cardiac function, reflecting changes in cardiac health and are increased in patients with DCM, serving as a diagnostic markers**.** [41]

4. miR-26a and miR-155: These are linked to muscle preservation and mediating cardiomyocyte pyroptosis in UCM. The miR-26a inhibits muscle wasting and improves insulin sensitivity. miR-155 regulates inflammatory responses and cell death in cardiomyocytes, influencing UCM progression.[41]

5. miR-122 and miR-29a: These are associated with mitochondrial function and cardiac parameters in obesity-related cardiomyopathy. miR-122 regulates mitochondrial protein function, impacting cardiac remodeling. miR-29a is involved in the regulation of extracellular matrix and fibrosis, combating obesity-mediated cardiac dysfunction.[42]

6. miR-30, miR-21, miR-133a, miR-29, and miR-26: These are being linked to Dialated cardiomyopathy and are associated with collagen production markers and factors that control fibrosis. They regulate the MMPs/TIMPs system involved in extracellular matrix metabolism and fibrosis.[43]

7. miR-146a: It is induced by the 16-kDa prolactin fragment (16K PRL) through NF-κB activation, leading to inhibition of angiogenesis. It promotes the release of miR-146a-enriched exosomes from endothelial cells (ECs), which can be taken up by cardiomyocytes, resulting in decreased levels of downstream factors like Notch1, Erbb4, and Irak1, thus affecting cellular mitogenesis and differentiation. Higher plasma levels of exosomal miR-146a are observed in PPCM patients. [44]

8.miR-223: Implicated in cardioprotection during sepsis, its levels are reduced in sepsis patients compared to healthy controls and in non-surviving versus surviving patients. It downregulates Sema3A and Stat3 through MSC-derived exosomes, leading to reduced macrophage inflammation and cardiomyocyte cell death.[45] Regulates pro-inflammatory markers such as IL-6, IL-1β, and ICAM-1. The decreased levels of miR-223 can result in increased expression of these inflammatory proteins, contributing to myocardial depression.[46]

Cardiac fibrosis

The pathological condition known as cardiac fibrosis is typified by an overabundance of extracellular matrix (ECM) components accumulating in the heart, mostly due to fibroblast activation. It is a medical condition that causes changes in the heart structure and compliance , which can eventually result in cardiac failure and death.[47]

1. **miR-155:** These are prevalent in exosomes generated from macrophages and, by encouraging aberrant fibroblast proliferation and differentiation, can exacerbate inflammation and cardiac fibrosis.[48]
2. **miRNA-425**: Circulating exosomal miRNA-425 was discovered by Wang et al.[49] as a new biomarker that can forecast the development of cardiac fibrosis and heart dysfunction in heart failure patients.
3. **miRNA-744**: Alongside miRNA-425, Wang et al.[49] also reported that miRNA-744 functions as a biomarker for cardiac fibrosis progression in heart failure patients.
4. **HSP70**: Yang et al.[50] discovered that HSP70 on the surface of serum exosomes is linked to cardiac fibrosis and negatively correlated with age, indicating that it may be a useful target for diagnosing myocardial fibrosis associated with aging.
5. **miR-208a:** Following myocardial infarction, it is increased in rat cardiomyocytes and transported to fibroblasts through exosomes, resulting in cardiac fibrosis and fibroblast proliferation. This pro-fibrotic effect could be reversed by a miR-208a antagonist.[50]
6. **miR-217:** Cardiomyocyte-derived miR-217 delivered by exosomes exacerbated pressure overload-induced myocardial fibrosis and cardiac dysfunction.[18]
7. **miR-92a-3p**: Liu et al.[51] discovered that miR-92a-3p in exosomes generated from endothelial cells is associated with atherogenesis, suggesting its involvement in the mechanisms of cardiovascular disease.
8. **miR-21**: Mice with myocardial infarcts showed enhanced cardiac function and less scarring when miR-21-rich exosomes were directly injected into the region. Nevertheless, the capacity of exosomes produced from heart failure patients' cardiac stromal cells to stimulate endothelial tube formation and cardiomyocyte proliferation was hampered by dysregulated miR-21-5p expression.[52,53]

9. **miR-142-3p:** By activating myofibroblasts via miR-142-3p-WNT signaling, exosomes from activated CD4+ T cells accelerated post-ischemic cardiac fibrosis.[54]
10. **HIF-1α**: Sun et al.[55] demonstrated that by encouraging the development of new blood vessels during myocardial infarction, overexpression of HIF-1α in exosomes derived from mesenchymal stem cells decreased heart fibrosis.
11. **miR-30d**: Li et al.[56] showed that negative cardiac remodeling and elevated expression of genes linked to inflammation and fibrosis are correlated with decreased expression of miR-30d in heart-derived exosomes.
12. **miR-30d:** It targets integrin α5 in fibroblasts through a paracrine mechanism, preventing fibroblast proliferation and fibrosis, and is increased in cardiomyocytes in ischemic heart failure models in rats and mice.[56]

These miRNAs have been identified as potential exosomal biomarkers that modulate fibroblast proliferation, differentiation, and cardiac fibrosis. Targeting these miRNAs in exosomes may help diminish fibrosis and ameliorate cardiac function.

Valvular heart disease (VHD)

Valvular heart disease (VHD) is associated with significant anatomical and functional abnormalities of cardiac valves, leading to conditions such as stenosis and regurgitation. Recent studies have identified specific microRNAs (miRNAs) that play crucial roles in the pathogenesis of VHD and may serve as biomarkers for diagnosis:

1. **miR-23**: Acts as a negative feedback regulator of hyaluronic acid synthase 2 (Has2), preventing excess deposition of hyaluronan in cardiac jelly, which is crucial for cardiac valve formation. Aberrant regulation of miR-23 can lead to cardiac valve defects.[57]
2. **miR-9**: Involved in the regulation of cardiomyocyte energetics and fibrosis, associated with the progression of myxomatous mitral valve disease and congestive heart failure.[58]
3. **miR-181c**: Similar to miR-9, it regulates cardiomyocyte energetics and is linked to valve disorder progression and heart failure.[58]
4. **miR-495**: Associated with mitochondrial function and involved in the regulation of fibrotic processes in valve diseases.[58]
6. **miR-599**: Also involved in cardiomyocyte energetics and linked to the pathophysiology of valvular heart disease and heart failure.[58]
7. **lncRNA HOTAIR**: Modulates calcification-related gene expression in valvular smooth muscle cells (VSMCs) during aortic valve disease, influencing the progression of calcification.[59]

Heart failure

Heart failure (HF) is a is a complex disorder characterized by the heart's inability to pump enough blood to meet the body's demands, resulting in a variety of systemic consequences. Recent research has highlighted the potential of specific microRNAs (miRNAs) as biomarkers for diagnosing HF.[60] Here are some key miRNAs associated with heart failure, along with their functions and relevant studies:

1. **CD14, SerpinG1, SerpinF2:** These are systemic vascular markers associated with the development of heart failure (HF) in patients suspected of acute HF.[61]
2. **miR-146a:** Increased levels are associated with the attenuation of inflammatory targets and provide cardioprotective effects against oxidative stress in HF patients.[62]
3. **miR-486:** Similar to miR-146a, it plays a role in mitigating inflammation and protecting against oxidative stress in HF.[62]
4. **miR-155:** Activated in response to TGF-β treatment, associated with hypertrophic responses in cardiomyocytes.[63]
5. **miR-21:** Promotes cardiac hypertrophy through paracrine signaling; regulates key pathways linked to hypertrophy and fibrosis in cardiomyocytes.[64,65]
6. **miR-21-3p (miR-21):** Acts as a paracrine mediator of cardiomyocyte hypertrophy, targeting pathways that lead to cardiac enlargement and dysfunction.[64]
7. **miR-214, miR-135b, miR-125a, miR-145-5p**: Involved in angiogenesis by upregulating or downregulating target gene expression in endothelial cells (ECs).[66–71]

CONCLUSION

To conclude, while exosomes are key components that have been extensively studied in relation to cardiovascular diseases (CVDs), it is vital to also focus on the salivary assessment of these exosomes. Salivary exosomes provide a non-invasive method for monitoring and diagnosing CVDs, carrying a diverse array of biomarkers that reflect cardiovascular health. Their ability to transport specific indicators associated with conditions such as

coronary artery disease and heart failure positions them as valuable tools for early detection and personalized treatment approaches.

Among the various contents of exosomes, microRNAs (miRNAs) are increasingly recognized for their roles in CVD. These small RNA molecules are essential for regulating gene expression and are linked to numerous cardiovascular processes. Therefore, research should emphasize the evaluation of salivary miRNAs to fully harness their potential as biomarkers. By integrating salivary miRNA analysis into clinical practice, we could significantly enhance our understanding and management of cardiovascular diseases, underscoring the need for further exploration in this promising area.

DECLARATIONS

Conflicts of interest and financial disclosures

The author declares that he has no conflict percent and there was no external source of funding for the research in question.

Ethical approval

The study was approved by the Institutional Ethics Committee and was conducted in accordance with the Declaration of the World Medical Association.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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REFERENCES

1 Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990–2019. J Am Coll Cardiol2020;76:2982–3021. https://doi.org/10.1016/j.jacc.2020.11.010.

2 Kim SJ, Mesquita FCP, Hochman-Mendez C. New Biomarkers for Cardiovascular Disease. Texas Hear Inst J 2023;50. https://doi.org/10.14503/THIJ-23-8178.

3 Frąk W, Wojtasińska A, Lisińska W, Młynarska E, Franczyk B, Rysz J. Pathophysiology of Cardiovascular Diseases: New Insights into Molecular Mechanisms of Atherosclerosis, Arterial Hypertension, and Coronary Artery Disease. Biomedicines 2022;10:1938.https://doi.org/10.3390/biomedicines1008193 8.

4 Brouwers S, Sudano I, Kokubo Y, Sulaica EM. Arterial hypertension. Lancet 2021;398:249–61. https://doi.org/10.1016/S0140-6736(21)00221-X.

5 Geraghty L, Figtree GA, Schutte AE, Patel S, Woodward M, Arnott C. Cardiovascular Disease in Women: From Pathophysiology to Novel and Emerging Risk Factors. Hear Lung Circ2021;30:9–17. https://doi.org/10.1016/j.hlc.2020.05.108.

6 Bahbah EI, Noehammer C, Pulverer W, Jung M, Weinhaeusel A. Salivary biomarkers in cardiovascular disease: An insight into the current evidence. FEBS J 2021;288:6392–405. https://doi.org/10.1111/febs.15689.

7 Miller CS, Foley JD, Bailey AL, Campell CL, Humphries RL, Christodoulides N, et al. Current Developments in Salivary Diagnostics. Biomark Med 2010;4:171–89. https://doi.org/10.2217/bmm.09.68.

8 Foley JD, Sneed JD, Steinhubl SR, Kolasa J, Ebersole JL, Lin Y, et al. Oral fluids that detect cardiovascular disease biomarkers. Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114:207–14. https://doi.org/10.1016/j.oooo.2012.03.003.

9 Deddens JC, Vrijsen KR, Colijn JM, Oerlemans MI, Metz CHG, van der Vlist EJ, et al. Circulating Extracellular Vesicles Contain miRNAs and are Released as Early Biomarkers for Cardiac Injury. J Cardiovasc Transl Res 2016;9:291–301.

https://doi.org/10.1007/s12265-016-9705-1.

10 Neves KB, Rios FJ, Sevilla-Montero J, Montezano AC, Touyz RM. Exosomes and the cardiovascular system: role in cardiovascular health and disease. J Physiol 2023;601:4923–36. https://doi.org/10.1113/JP282054.

11 Jadli AS, Parasor A, Gomes KP, Shandilya R, Patel VB. Exosomes in Cardiovascular Diseases: Pathological Potential of Nano-Messenger. Front Cardiovasc Med 2021;8:767488. https://doi.org/10.3389/fcvm.2021.767488.

12 Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987;262:9412–20. https://doi.org/10.1016/S0021-9258(18)48095-7.

13 Pan B-T, Johnstone RM. Fate of the transferrin receptor during maturation of sheep reticulocytes in vitro: Selective externalization of the receptor. Cell 1983;33:967–78. https://doi.org/10.1016/0092-8674(83)90040-5.

14 Kalluri R, LeBleu VS. The biology , function , and biomedical applications of exosomes. Science(80-)2020;367. https://doi.org/10.1126/science.aau6977.

15 Liu Q, Piao H, Wang Y, Zheng D, Wang W. Circulating exosomes in cardiovascular disease: Novel carriers of biological information. Biomed Pharmacother 2021;135:111148. https://doi.org/10.1016/j.biopha.2020.111148.

16 Iwai K, Minamisawa T, Suga K, Yajima Y, Shiba K. Isolation of human salivary extracellular vesicles by iodixanol density gradient ultracentrifugation and their characterizations. J Extracell Vesicles 2016;5. https://doi.org/10.3402/jev.v5.30829.

17 Cui M, Han Y, Yang J, Li G, Yang C. A narrative review of the research status of exosomes in cardiovascular disease. Ann Palliat Med 2022;11:363–77. https://doi.org/10.21037/apm-21-3364.

18 Nie X, Fan J, Li H, Yin Z, Zhao Y, Dai B, et al. miR-217 Promotes Cardiac Hypertrophy and Dysfunction by Targeting PTEN. Mol Ther - Nucleic Acids 2018;12:254–66. https://doi.org/10.1016/j.omtn.2018.05.013.

19 Ribeiro-Rodrigues TM, Laundos TL, Pereira-Carvalho R, Batista-Almeida D, Pereira R, Coelho-Santos V, et al. Exosomes secreted by cardiomyocytes subjected to ischaemia promote cardiac angiogenesis. Cardiovasc Res 2017;113:1338–50. https://doi.org/10.1093/cvr/cvx118.

20 Hu J, Wang S, Xiong Z, Cheng Z, Yang Z, Lin J, et al. Exosomal Mst1 transfer from cardiac microvascular endothelial cells to cardiomyocytes deteriorates diabetic cardiomyopathy. Biochim Biophys Acta - Mol Basis Dis 2018;1864:3639–49. https://doi.org/10.1016/j.bbadis.2018.08.026.

21 Davidson SM, Riquelme JA, Zheng Y, Vicencio JM, Lavandero S, Yellon DM. Endothelial cells release cardioprotective exosomes that may contribute to ischaemic preconditioning. Sci Rep 2018;8:15885. https://doi.org/10.1038/s41598-018-34357-z.

22 Li H, Liao Y, Gao L, Zhuang T, Huang Z, Zhu H, et al. Coronary Serum Exosomes Derived from Patients with Myocardial Ischemia Regulate Angiogenesis through the miR-939-mediated Nitric Oxide Signaling Pathway. Theranostics 2018;8:2079–93. https://doi.org/10.7150/thno.21895.

23 Han Y, Jia L, Zheng Y, Li W. Salivary Exosomes: Emerging Roles in Systemic Disease. Int J Biol Sci 2018;14:633–43. https://doi.org/10.7150/ijbs.25018.

24 Schneider A, Simons M. Exosomes: vesicular carriers for intercellular communication in neurodegenerative disorders. Cell Tissue Res 2013;352:33–47. https://doi.org/10.1007/s00441-012-1428-2.

25 Liang C, Zhang L, Lian X, Zhu T, Zhang Y, Gu N. Circulating Exosomal SOCS2-AS1 Acts as a Novel Biomarker in Predicting the Diagnosis of Coronary Artery Disease. Biomed Res Int 2020;2020:9182091. https://doi.org/10.1155/2020/9182091.

26 Ling H, Guo Z, Shi Y, Zhang L, Song C. Serum Exosomal MicroRNA-21, MicroRNA-126, and PTEN Are Novel Biomarkers for Diagnosis of Acute Coronary Syndrome. Front Physiol 2020;11:654. https://doi.org/10.3389/fphys.2020.00654.

27 Jensen RV, Hjortbak MV, Bøtker HE. Ischemic Heart Disease: An Update. Semin Nucl Med.2020;50:195–207. https://doi.org/10.1053/j.semnuclmed.2020.02.007.

28 Chen L, Yang W, Guo Y, Chen W, Zheng P, Zeng J TW. Exosomal lncRNA GAS5 regulates the apoptosis of macrophages and vascular endothelial cells in atherosclerosis. PLoS One 2017;12.

29 Bi S, Wang C, Jin Y, Lv Z, Xing X, Lu Q. Correlation between serum exosome derived miR-208a and acute coronary syndrome. Int J Clin Exp Med 2015;8:4275–80.

30 Li L-J, Gu Y-J, Wang L-Q, Wan W, Wang H-W, Yang X-N, et al. Serum exosomal microRNA-146a as a novel diagnostic biomarker for acute coronary syndrome. J Thorac Dis 2021;13:3105–14. https://doi.org/10.21037/jtd-21-609.

31 Pan J, Alimujiang M, Chen Q, Shi H, Luo X. Exosomes derived from miR-146a-modified adipose-derived stem cells attenuate acute myocardial infarction−induced myocardial damage via downregulation of early growth response factor 1. J Cell Biochem 2019;120:4433–43. https://doi.org/10.1002/jcb.27731.

32 Santoso MR, Ikeda G, Tada Y, Jung J, Vaskova E, Sierra RG, et al. Exosomes From Induced Pluripotent Stem Cell–Derived Cardiomyocytes Promote Autophagy for Myocardial Repair. J Am Heart Assoc 2020;9. https://doi.org/10.1161/JAHA.119.014345.

33 Li W, Li Y, Zhi W, Liu C, Fan W, Miao Q, et al. Diagnostic value of using exosome-derived cysteine-rich protein 61 as biomarkers for acute coronary syndrome. Exp Ther Med 2021;22:1437. https://doi.org/10.3892/etm.2021.10872.

34 Zhang P, Liang T, Chen Y, Wang X, Wu T, Xie Z, et al. Circulating Exosomal miRNAs as Novel Biomarkers for Stable Coronary Artery Disease. Biomed Res Int 2020;2020:3593962. https://doi.org/10.1155/2020/3593962.

35 Gao F, Kataoka M, Liu N, Liang T, Huang Z-P, Gu F, et al. Therapeutic role of miR-19a/19b in cardiac regeneration and protection from myocardial infarction. Nat Commun 2019;10:1802. https://doi.org/10.1038/s41467-019-09530-1.

36 Huang X, Zuo J. Emerging roles of miR-210 and other non-coding RNAs in the hypoxic response. Acta Biochim Biophys Sin (Shanghai).2014;46:220–32. https://doi.org/10.1093/abbs/gmt141.

37 Chen Y, Zhao Y, Chen W, Xie L, Zhao Z-A, Yang J, et al. MicroRNA-133 overexpression promotes the therapeutic efficacy of mesenchymal stem cells on acute myocardial infarction. Stem Cell Res Ther 2017;8:268. https://doi.org/10.1186/s13287-017-0722-z.

38 Peng Y, Zhao J-L, Peng Z-Y, Xu W-F, Yu G-L. Exosomal miR-25-3p from mesenchymal stem cells alleviates myocardial infarction by targeting pro-apoptotic proteins and EZH2. Cell Death Dis 2020;11:317. https://doi.org/10.1038/s41419-020-2545-6.

39 Xu Y, Wan W, Zeng H, Xiang Z, Li M, Yao Y, et al. Exosomes and their derivatives as biomarkers and therapeutic delivery agents for cardiovascular diseases: Situations and challenges. J Transl Intern Med 2023;11:341–54. https://doi.org/10.2478/jtim-2023-0124.

40 Copier CU, León L, Fernández M, Contador D, Calligaris SD. Circulating miR-19b and miR-181b are potential biomarkers for diabetic cardiomyopathy. Sci Rep 2017;7:13514. https://doi.org/10.1038/s41598-017-13875-2.

41 Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, et al. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol 2014;74:139–50. https://doi.org/10.1016/j.yjmcc.2014.05.001.

42 Li F, Zhang K, Xu T, Du W, Yu B, Liu Y, et al. Exosomal microRNA-29a mediates cardiac dysfunction and mitochondrial inactivity in obesity-related cardiomyopathy. Endocrine 2019;63:480–8. https://doi.org/10.1007/s12020-018-1753-7.

43 Rubiś P, Totoń-Żurańska J, Wiśniowska-Śmiałek S, Holcman K, Kołton-Wróż M, Wołkow P, et al. Relations between circulating microRNAs (miR-21, miR-26, miR-29, miR-30 and miR-133a), extracellular matrix fibrosis and serum markers of fibrosis in dilated cardiomyopathy. Int J Cardiol 2017;231:201–6. https://doi.org/10.1016/j.ijcard.2016.11.279.

44 Halkein J, Tabruyn SP, Ricke-Hoch M, Haghikia A, Nguyen N-Q-N, Scherr M, et al. MicroRNA-146a is a therapeutic target and biomarker for peripartum cardiomyopathy. J Clin Invest 2013;123:2143–54. https://doi.org/10.1172/JCI64365.

45 Wang X, Gu H, Qin D, Yang L, Huang W, Essandoh K, et al. Exosomal miR-223 Contributes to Mesenchymal Stem Cell-Elicited Cardioprotection in Polymicrobial Sepsis. Sci Rep 2015;5:13721. https://doi.org/10.1038/srep13721.

46 Wang H, Zhang P, Chen W, Feng D, Jia Y, Xie L. Serum MicroRNA Signatures Identified by Solexa Sequencing Predict Sepsis Patients' Mortality: A Prospective Observational Study. PLoS One 2012;7:e38885.

https://doi.org/10.1371/journal.pone.0038885.

47 Fan J, Ren M, He Y. Diagnostic and Therapeutic Properties of Exosomes in Cardiac Fibrosis. Front Cell Dev Biol 2022;10. https://doi.org/10.3389/fcell.2022.931082.

48 Wang C, Zhang C, Liu L, A X, Chen B, Li Y, et al. Macrophage-Derived mir-155-Containing Exosomes Suppress Fibroblast Proliferation and Promote Fibroblast Inflammation during Cardiac Injury. Mol Ther 2017;25:192–204. https://doi.org/10.1016/j.ymthe.2016.09.001.

49 Wang L, Liu J, Xu B, Liu Y, Liu Z. Reduced exosome miR-425 and miR-744 in the plasma represents the progression of fibrosis and heart failure. Kaohsiung J Med Sci 2018;34:626–33. https://doi.org/10.1016/j.kjms.2018.05.008.

50 Yang J, Yu X, Xue F, Li Y, Liu W ZS. Exosomes derived from cardiomyocytes promote cardiac fibrosis via myocyte-fibroblast cross-talk. Am J Transl Res 2918;10. https://doi.org/30662677.

51 Liu Y, Li Q, Hosen MR, Zietzer A, Flender A, Levermann P, et al. Atherosclerotic Conditions Promote the Packaging of Functional MicroRNA-92a-3p Into Endothelial Microvesicles. Circ Res 2019;124:575–87. https://doi.org/10.1161/CIRCRESAHA.118.314010.

52 Qiao L, Hu S, Liu S, Zhang H, Ma H, Huang K, et al. microRNA-21-5p dysregulation in exosomes derived from heart failure patients impairs regenerative potential. J Clin Invest 2019;129:2237–50. https://doi.org/10.1172/JCI123135.

53 Song Y, Zhang C, Zhang J, Jiao Z, Dong N, Wang G, et al. Localized injection of miRNA-21-enriched extracellular vesicles effectively restores cardiac function after myocardial infarction. Theranostics 2019;9:2346–60. https://doi.org/10.7150/thno.29945.

54 Cai L, Chao G, Li W, Zhu J, Li F, Qi B, et al. Activated CD4+ T cells-derived exosomal miR-142-3p boosts post-ischemic ventricular remodeling by activating myofibroblast. Aging (Albany NY) 2020;12:7380–96. https://doi.org/10.18632/aging.103084.

55 Sun J, Shen H, Shao L, Teng X, Chen Y, Liu X, et al. HIF-1α overexpression in mesenchymal stem cell-derived exosomes mediates cardioprotection in myocardial infarction by enhanced angiogenesis. Stem Cell Res Ther 2020;11:373. https://doi.org/10.1186/s13287-020-01881-7.

56 Li J, Salvador AM, Li G, Valkov N, Ziegler O, Yeri A, et al. Mir-30d Regulates Cardiac Remodeling by Intracellular and Paracrine Signaling. Circ Res 2021;128. https://doi.org/10.1161/CIRCRESAHA.120.317244.

57 Lagendijk AK, Goumans MJ, Burkhard SB, Bakkers J. MicroRNA-23 Restricts Cardiac Valve Formation by Inhibiting Has2 and Extracellular Hyaluronic Acid Production. Circ Res.2011;109:649–57. https://doi.org/10.1161/CIRCRESAHA.111.247635.

58 Yang VK, Loughran KA, Meola DM, Juhr CM, Thane KE, Davis AM, et al. Circulating exosome microRNA associated with heart failure secondary to myxomatous mitral valve disease in a naturally occurring canine model. J Extracell Vesicles.2017;6. https://doi.org/10.1080/20013078.2017.1350088.

59 Carrion K, Dyo J, Patel V, Sasik R, Mohamed SA, Hardiman G, et al. The Long Non-Coding HOTAIR Is Modulated by Cyclic Stretch and WNT/β-CATENIN in Human Aortic Valve Cells and Is a Novel Repressor of Calcification Genes. PLoS One 2014;9:e96577. https://doi.org/10.1371/journal.pone.0096577.

60 Jadli AS, Parasor A, Gomes KP, Shandilya R, Patel VB. Exosomes in Cardiovascular Diseases: Pathological Potential of Nano-Messenger. Front Cardiovasc Med 2021;8. https://doi.org/10.3389/fcvm.2021.767488.

61 Zhang Y-N, Vernooij F, Ibrahim I, Ooi S, Gijsberts CM, Schoneveld AH, et al. Extracellular Vesicle Proteins Associated with Systemic Vascular Events Correlate with Heart Failure: An Observational Study in a Dyspnoea Cohort. PLoS One 2016;11:e0148073. https://doi.org/10.1371/journal.pone.0148073.

62 Beg F, Wang R, Saeed Z, Devaraj S, Masoor K, Nakshatri H. Inflammation-associated microRNA changes in circulating exosomes of heart failure patients. BMC Res Notes 2017;10:751. https://doi.org/10.1186/s13104-017-3090-y.

63 Basma H, Johanson AN, Dhar K, Anderson D, Qiu F, Rennard S, et al. TGF-β induces a heart failure phenotype via fibroblasts exosome signaling. Heliyon 2019;5:e02633. https://doi.org/10.1016/j.heliyon.2019.e02633

64 Bang C, Batkai S, Dangwal S, Gupta SK,

Foinquinos A, Holzmann A, et al. Cardiac fibroblast–derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. J Clin Invest 2014;124:2136–46. https://doi.org/10.1172/JCI70577.

65 Fredj S, Bescond J, Louault C, Potreau D. Interactions between cardiac cells enhance cardiomyocyte hypertrophy and increase fibroblast proliferation. J Cell Physiol 2005;202:891–9. https://doi.org/10.1002/jcp.20197.

66 Liu Y, Luo F, Wang B, Li H, Xu Y, Liu X, et al. STAT3-regulated exosomal miR-21 promotes angiogenesis and is involved in neoplastic processes of transformed human bronchial epithelial cells. Cancer Lett 2016;370:125–35. https://doi.org/10.1016/j.canlet.2015.10.011.

67 Umezu T, Tadokoro H, Azuma K, Yoshizawa S, Ohyashiki K, Ohyashiki JH. Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. Blood 2014;124:3748–57. https://doi.org/10.1182/blood-2014-05-576116.

68 Thuringer D, Jego G, Berthenet K, Hammann A, Solary E, Garrido C. Gap junction-mediated transfer of miR-145-5p from microvascular endothelial cells to colon cancer cells inhibits angiogenesis. Oncotarget 2016;7:28160–8. https://doi.org/10.18632/oncotarget.8583.

69 van Balkom BWM, de Jong OG, Smits M, Brummelman J, den Ouden K, de Bree PM, et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. Blood 2013;121:3997–4006. https://doi.org/10.1182/blood-2013-02-478925.

70 Liang X, Zhang L, Wang S, Han Q, Zhao RC. Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a. J Cell Sci 2016;129:2182–9. https://doi.org/10.1242/jcs.170373.

71 Wang K, Jiang Z, Webster KA, Chen J, Hu H, Zhou Y, et al. Enhanced Cardioprotection by Human Endometrium Mesenchymal Stem Cells Driven by Exosomal MicroRNA-21. Stem Cells Transl Med 2017;6:209–22. https://doi.org/10.5966/sctm.2015-0386.