

Exploring the role of exosomes in the pathogenesis and treatment of cardiomyopathies: A comprehensive literature review

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ABSTRACT

Exosomes, a subset of small extracellular vesicles that play a crucial role in intercellular communication, have garnered significant attention for their potential applications in the diagnosis and treatment of cardiomyopathies. Cardiomyopathies, which encompass a spectrum of heart muscle disorders, present complex challenges in diagnosis and management. Understanding the role of exosomes in the etiology of cardiomyopathies such as dilated cardiomyopathy (DCM), restrictive cardiomyopathy (RCM), arrhythmogenic cardiomyopathy (AC), and hypertrophic cardiomyopathy (HCM) may open new possibilities for therapeutic intervention and diagnosis. Exosomes have indeed demonstrated promise as diagnostic biomarkers, particularly in identifying cardiac conditions such as atrial fibrillation (AF) and in the timely classification of high-risk patients with different forms of cardiomyopathy. In DCM, exosomes have been implicated in mediating pathological responses in cardiomyocytes, potentially exacerbating disease progression. Moreover, in RCM, AC, and HCM, exosomes present significant potential as diagnostic biomarkers and therapeutic targets, offering insights into disease pathogenesis and potential avenues for intervention. Understanding the influence of exosomes on disease progression and identifying the specific molecular pathways involved in cardiomyopathy pathogenesis may significantly advance diagnostic and treatment strategies. While key findings highlight the multifaceted role of exosomes in cardiomyopathy, they also emphasize the need for further research to elucidate molecular mechanisms and translate findings into clinical practice. This review highlights the evolving landscape of exosome research in cardiomyopathies and underscores the importance of ongoing investigations to harness the full potential of exosomes in improving patient outcomes.

1. Introduction

Cardiomyopathies are a heterogeneous group of pathologies characterized by structural or functional abnormalities in the heart muscle, resulting in cardiac dysfunction [1]. Approximately 30 years ago Geisterfer-Lowrance and colleagues documented the first case of cardiomyopathy, identifying an upregulation of the MYH7 gene (p. Arg403Glu) as the causative factor in HCM [2]. This work pioneered a new genetic era in which different pathogenetic cardiac variants were identified and classified based on their morphological traits [3]. The four distinct cardiomyopathy phenotypes based on morphological alterations are categorized as hypertrophic cardiomyopathy (HCM),

dilated cardiomyopathy (DCM), arrhythmogenic cardiomyopathy (AC), and restrictive cardiomyopathy (RCM) [4,5]. HCM is characterized by left ventricular hypertrophy, whereas DCM is defined by ventricular enlargement and the absence of left ventricular hypertrophy [6]. AC is characterized by the replacement of ventricular myocardium with fibrofatty tissue and the existence of ventricular arrhythmias.

Additionally, RCM is characterized by abnormally rigid, non-dilated left or right ventricles with severe diastolic dysfunction [7]. Among cardiomyopathies, HCM and DCM are the most prevalent, with DCM affecting approximately 1 in 250 individuals and HCM approximately 1 in 500. In contrast, AC and RCM are less common [8].

Imaging modalities such as echocardiography (Echo) and cardiac

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magnetic resonance (CMR) are commonly employed for diagnosing and monitoring patients [3]. However, these differential diagnostic techniques may not enable early detection of the disease. Consequently, assessing cardiac biomarkers, such as exosomes, presents a promising approach for early disease diagnosis, prognosis, and treatment [9].

Exosomes are vesicles with an average diameter of 100 nm, originating from the extracellular membrane and are enclosed in the lipid bilayer [10]. They are released into biological fluids, including blood, saliva, lymph, pericardial fluid, and urine to facilitate intracellular communications [11]. Fig. 1 illustrates the biogenesis and release of exosomes. Upon physiological and pathological stimulation, exosomes are formed through the inward budding of a late endosome, also known as a multivesicular body (MVB). The intraluminal vesicles (ILVs) within the MVB bud inward into the endosomal lumen. When the MVB fuses with the cell membrane, these ILVs are released as exosomes [1]. These released nanoparticles carry genetic material and proteins from their cell of origin that may act as biomarkers. Exosomes facilitate the transfer of molecules between cells via membrane vesicle trafficking, thereby influencing the immune system by interacting with dendritic cells and B cells [12] and mediating adaptive immune responses to pathogens and tumors [13]. Exosomes have been studied in various fields, including cancer, cardiovascular diseases, and neurodegenerative diseases, showing potential as disease biomarkers and therapeutic targets [14]. Exosome-contained biological substances, such as mRNAs, miRNAs, signaling molecules, proteins, and lipids, initiate intracellular communication, which is crucial for diagnosing the early stages of cardiomyopathies and serve as potential biomarkers [13,14]. In this regard, the study conducted by Qin and his team observed that exosomes play a vital role in promptly identifying and classifying high-risk patients and treating them for underlying cardiomyopathic conditions [15].

Moreover, Huang and colleagues sought to unveil potential risks associated with disease progression and outcomes and provide insights into unexplored molecular mechanisms targeting the pathophysiology of these underlying disease conditions [7]. For instance, serum-derived exosomes from pediatric DCM patients upregulated the expression of

atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in primary cardiomyocytes, suggesting that exosome-mediated pathological hypertrophy in cardiomyocytes occurs independently of the renin-angiotensin-aldosterone system (RAAS) and the adrenergic system [8]. Additionally, endothelial-derived EVs from obese patients have been shown to increase the expression of critical intracellular proteins, such as cTnT and α -actin, which are involved in cardiomyocyte hypertrophy and fibrosis [16]. Furthermore, exosomes derived from immortalized cardio sphere-derived cells displayed high levels of β -catenin [17], a protein that plays a crucial role in the pathogenesis of ARVC. It contributes to fibrotic and lipid tissue deposition and mitigates intercalated disc remodeling, a unique characteristic of ARVC [18].

Given the rapid advancements in exosome research, our review provides an up-to-date synthesis of their roles in cardiomyopathies, highlighting emerging diagnostic and therapeutic avenues.

2. Search methodology

A comprehensive literature search was conducted using various online databases, including PubMed, Embase, Scopus, and ProQuest. To ensure the selection of relevant studies, the keywords "Exosomes OR Exosome [Title]" were used in combination with terms related to various cardiomyopathies, such as "Dilated Cardiomyopathy, Hypertrophic Cardiomyopathy, Restrictive Cardiomyopathy OR RCM, Arrhythmogenic Cardiomyopathy OR AC, Heart Muscle Diseases, Cardiomyopathy, Cardiomyopathies [Title/Abstract]." Articles were meticulously reviewed and selected based on inclusion criteria that focused on English-language studies published between 1990 and 2024. The search specifically targeted research articles and reviews that explore the involvement of exosomes in human cardiomyopathies. This approach was designed to compile a diverse range of studies relevant to the objectives of this narrative review.

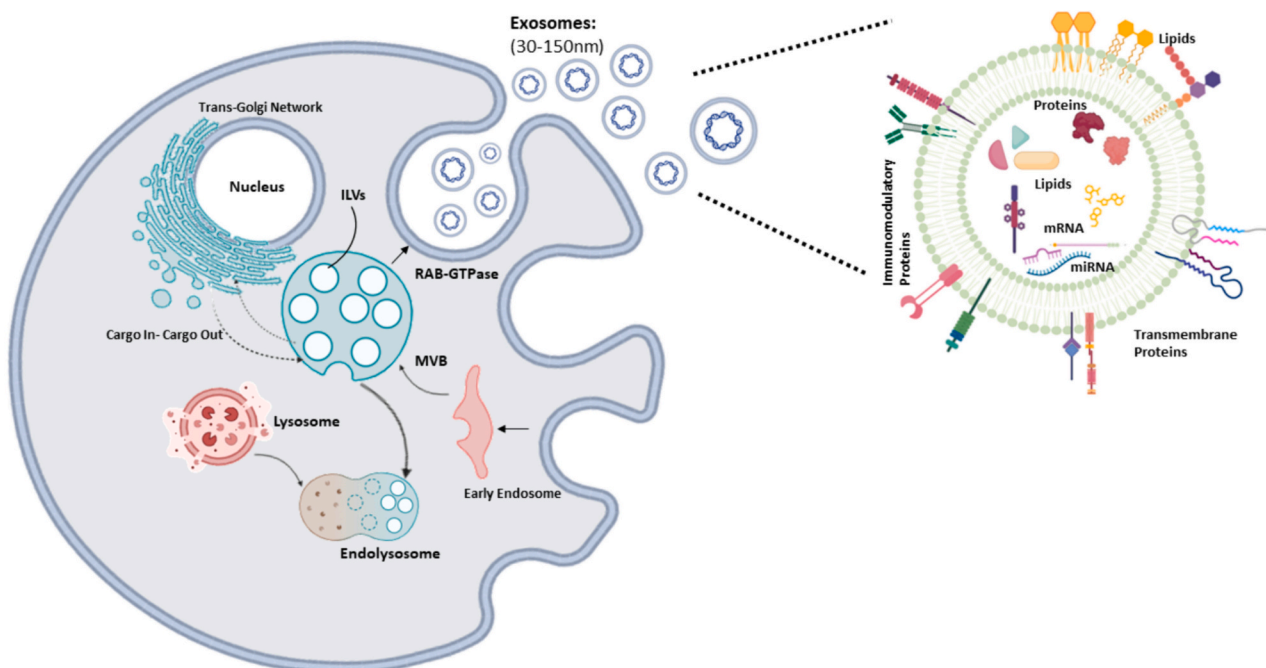


Fig. 1. Schematic representation of exosome biogenesis and release. Exosome biogenesis occurs within multivesicular bodies (MVBs), which are formed through the maturation of early endosomes following endocytosis. During this process, intraluminal vesicles (ILVs) are generated via inward budding of the endosomal membrane. MVBs can either fuse with lysosomes for degradation or be transported to the plasma membrane for exosome release. This secretion is regulated by RAB GTPases. Exosomes contain a variety of macromolecules, including transmembrane proteins, miRNAs, mRNAs, proteins, DNA, and lipids, which are released into the extracellular space upon fusion with the plasma membrane.

3. Cardiomyopathies

3.1. Dilated cardiomyopathy

Dilated cardiomyopathy (DCM) is a common complication of heart disease, characterized by an increase in myocardial mass and volume. It is one of the most prevalent types of cardiomyopathies, with an estimated prevalence of 36.5 cases per 100,000 individuals [19]. Contractile dysfunction and an enlarged left ventricle are typically linked to DCM. A significant proportion of DCM cases—approximately 35%—are attributed to idiopathic hereditary factors, while acquired causes include hormonal imbalances, infections, inflammation, and hypertension [20]. Given the limited literature on the role of exosomes in the pathogenesis of DCM, further research is needed to explore the interactions between various cell-derived exosomes and their impact on cardiomyocytes, as well as the subsequent effects on left ventricular remodeling in DCM [8].

Exosomes play a significant role in DCM by mediating pathological responses in cardiomyocytes and potentially propagating the disease process. For instance, exosomes derived from pediatric DCM patients have been shown to modulate cardiomyocytes, suggesting a role in the development and progression of heart failure in pediatric patients [21]. Exosomes have been found to contain a variety of biomolecules, including heat shock proteins, myosin heavy chain, cardiac troponins, proteins related to fibrosis, nucleic acids (DNA, mRNA, miRNAs), and metabolites [22]. DCM, which can lead to heart failure, is often linked to genetic inheritance. Within this context, the ability of exosomes to transfer molecular cargo, including miRNAs, between cells underscores their significance in the pathophysiology of DCM. Exosomes secreted from hypertrophically stimulated DCM cardiomyocytes have been shown to activate fibrosis in the heart, while exosomes from familial DCM cardiomyocytes promote fibrogenesis via a microRNA-mediated pathway [1]. Exosome research in the context of DCM is ongoing. While some molecules have been identified, a comprehensive profiling of exosomal content remains necessary. Exosomes associated with DCM have been found to contain heat shock proteins, myosin heavy chain, cardiac troponins, and fibrosis-related proteins, all of which are linked to cardiac remodeling, fibrosis, inflammation, and dysfunction. Additionally, exosomes carry nucleic acids, including DNA, mRNA, and miRNAs, which have been studied for their potential as biomarkers for cardiovascular disorders and their regulatory functions [23]. Due to their lipid-rich composition, exosomes may reflect intracellular signaling or metabolic pathways and may also carry metabolites from cellular metabolism. The potential of exosomes as diagnostic tools for cardiomyopathy is a promising area of research. Their unique characteristics suggest that they could serve as noninvasive and efficient biomarkers for disease detection and monitoring. Several miRNAs, such as miR-218-5p, miR-210, miR-1, miR-133a, miR-29b, and miR-455, have been identified as potential biomarkers for diagnosing and predicting outcomes in individuals with DCM [23]. Also, exosomal microRNAs have been implicated in heart failure, acting as communicators between cardiovascular cells [24,25]. These miRNAs can influence cardiomyocyte hypertrophy and other processes related to heart failure, highlighting the involvement of exosomes in the pathophysiology of DCM [14,26,27]. MicroRNAs play a significant role in the diagnosis of DCM by regulating mechanisms that contribute to the disease's progression. For instance, miRNA-208 has been associated with poor clinical outcomes and shows potential as a diagnostic marker [28]. The upregulation of specific miRNAs, such as miRNA-218-5p, in exosomes has been identified as a critical factor in promoting fibrogenesis in DCM. This finding sheds light on the molecular mechanisms through which exosomes influence disease progression [5,13].

Exosomes from DCM patients contribute to fibrogenesis through several mechanisms. Firstly, they can mediate pathological responses in cardiomyocytes and potentially propagate the disease process, as suggested by studies on serum exosomes from pediatric DCM patients [21].

Secondly, exosomes can promote inflammation and cardiac fibrosis, critical aspects of DCM pathogenesis [22]. Specifically, exosomes derived from DCM cardiomyocytes have been shown to upregulate specific miRNAs, such as miRNA-218-5p, contributing to fibrogenesis and impaired cardiac function [13]. Additionally, exosomes from systemic sclerosis (SSc) patients display alterations in their content of profibrotic and anti-fibrotic factors, indicating that exosomes from pathological conditions can have altered cargo that influences fibrogenesis [5]. This aspect was also highlighted by Posadino and colleagues, who reported that miRNAs potentially present in the serum exosomes of SSc patients can activate fibrosis and induce endothelial-to-mesenchymal transition in human pulmonary microvascular endothelial cells (HPMECs) [29].

Studies have demonstrated that exosomes derived from cardiomyocyte-derived cells can improve cardiac function and reduce myocardial fibrosis in animal models of DCM, indicating their therapeutic efficacy in mitigating the pathological processes associated with DCM [5] (Fig. 2). This suggests that exosome-derived microRNAs have the potential to enhance cardiac function and reduce fibrosis, which are critical aspects of DCM pathogenesis [30]. Additionally, exosomes from induced pluripotent stem cells can deliver cardioprotective miRNAs and prevent cardiomyocyte apoptosis, suggesting that exosomes can also have protective effects depending on their origin and cargo [22]. Exosomes have been implicated in promoting inflammation and cardiac fibrosis, critical aspects of DCM pathogenesis. Although miRNA-derived exosomes hold potential as therapeutic targets [27,31–33], their role in the treatment of DCM is not yet fully understood. Further research is required to explore their precise mechanisms of action and to optimize their therapeutic application [22].

3.2. Hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiovascular disease, characterized by abnormal thickening (hypertrophy) of the left ventricular myocardium that occurs independently of abnormal loading conditions. This condition is associated with significant histopathological features, including myocyte hypertrophy and disarray, as well as increased cardiac fibrosis [34]. These pathological features can lead to significant cardiac complications, including arrhythmias, impaired diastolic function, and left ventricular outflow tract obstruction [35]. In the general adult population, the frequency of HCM is approximately 1 in 500 individuals [36]. Sarcomere protein gene variations are the primary cause of HCM, which is primarily inherited as an autosomal dominant characteristic [37]. Nevertheless, nongenetic or environmental factors that influence the phenotype of HCM, though not yet fully understood, may contribute to its clinical variability and diverse phenotypic expression [38].

In this regard, exosomes produced by brown adipocytes with iron overload have been shown to significantly affect the regulation of tropomyosin 1 alpha (Tpm1), a gene associated with both the HCM and the dilated DCM [39,40]. The internalization of exosomes by HL-1 cardiomyocytes, particularly those generated from iron overload brown adipocytes, indicates their potential impact on cardiomyocyte function. This observation suggests that exosomes are crucial in mediating the communication between different cell types, mainly brown adipocytes, and cardiomyocytes [41]. The study by Zhang and colleagues demonstrated that exosomes derived from iron overload brown adipocytes have a further pronounced effect on the regulation of Tpm1 in comparison to free iron alone, suggesting a more substantial impact of iron overload brown adipocytes on cardiomyocytes [42]. These findings indicate that exosomes play a crucial role in mediating the effects of iron overload-brown adipocytes on cardiomyocytes, potentially contributing to the onset of iron overload cardiomyopathy [43].

Moreover, the study by Song et al. (2019) revealed that GLA-null cardiomyocytes secrete more exosomes than control cardiomyocytes, indicating a potential link between autophagic dysfunction and

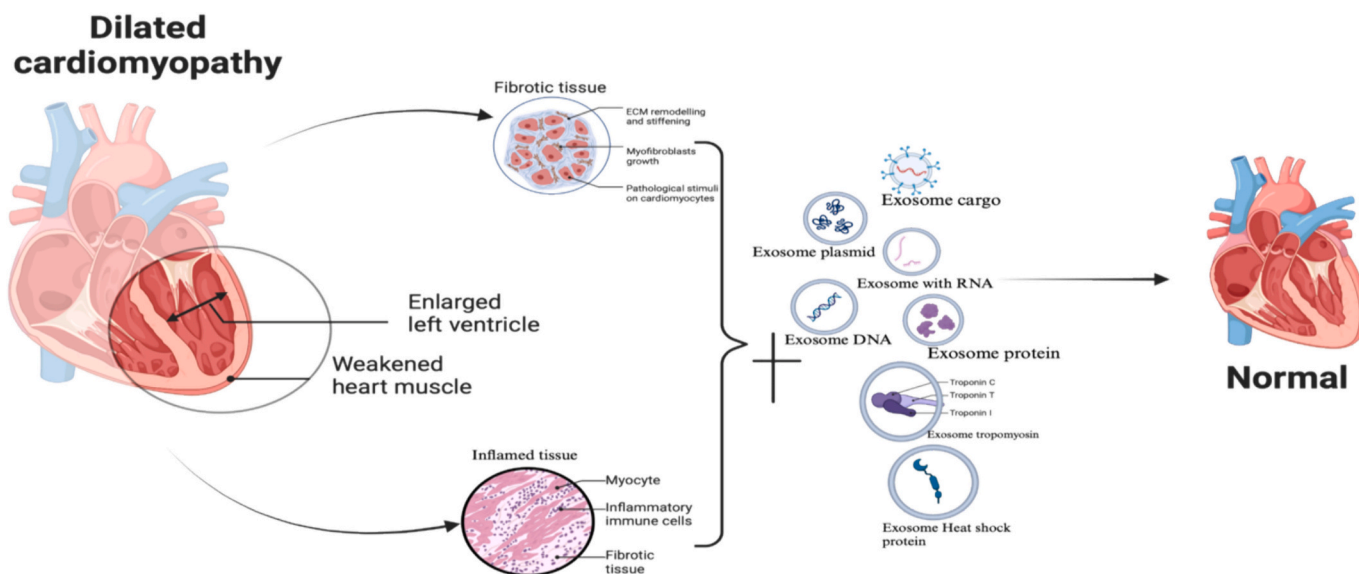


Fig. 2. The figure illustrates how the different exosomes can be used to treat dilated cardiomyopathy.

increased exosome secretion in Fabry disease-associated HCM [44]. This finding suggests that exosome biogenesis and secretion may be affected in GLA-null cardiomyocytes, highlighting the role of exosomes in the pathophysiology of Fabry disease-associated HCM [45]. This observation also underscores the potential involvement of exosomes in the pathogenesis of Fabry disease-associated HCM and the need for further investigation into the mechanisms underlying the altered exosome biogenesis and secretion in GLA-null cardiomyocytes [44].

The findings from these studies provide valuable insights into the role of exosomes in HCM and highlight potential mechanisms through which exosomes derived from GLA-null cardiomyocytes and iron-overloaded brown adipocytes may contribute to the pathophysiology of HCM, as summarized in Table 1 and Fig. 3 [42,44]. These results not only open avenues for further research into the cellular and molecular mechanisms underlying the effects of exosomes on cardiomyocyte function, but also provide a strong foundation for the development of potential therapeutic strategies targeting exosome-mediated pathways in HCM.

3.3. Arrhythmogenic cardiomyopathy and atrial fibrillation

Arrhythmogenic Cardiomyopathy (AC) is a rare, inheritable genetic heart disease characterized by palpitation, syncope, and cardiac arrest secondary to ventricular tachycardia (VT) or fibrillation [46]. The prevalence of AC is estimated to range between 1 in 1000 and 1 in 5000 individuals. Initially termed arrhythmogenic right ventricular cardiomyopathy (ARVC), the condition was first associated with developmental abnormalities of the right ventricle. ARVC has been identified as a leading cause of ventricular dysfunction leading to sudden cardiac death (SCD), with a prevalence of approximately 1 in 2500 as of 2023 [10]. Approximately 60 % of AC cases carry a genetic pathogenic variant caused by heterogenous mutations in genes that encode proteins in the desmosomal complex such as desmoglein-2 (DSG2), Plakophilin 2 (PKP2), Desmoplakin (DSP), Desmocollin-2 (DSC-2) [47]. These

mutations in the desmosomal complex lead to dystrophy of the ventricular myocardium, with progressive replacement by fibro-fatty tissue, particularly in the free wall of the right ventricle (RV) [15]. This pathological remodeling exacerbates electric instability and impairs ventricular mechanical function, leading to arrhythmias and progressive heart failure. Further investigation has revealed that left ventricular dysfunction is also included within the classification of AC [48]. Consequently, AC encompasses classical right, left ventricular, and biventricular phenotypes [15]. In this context, atrial fibrillation (AF) is a common manifestation in AC, resulting from structural changes in the ventricular walls, and is a characteristic clinical feature in patients with ARVC. Therefore, the detection of AF can be directly associated with ARVC [49].

The use of exosomes for diagnosing and treating ARVC caused by desmosomal complex mutations is advancing rapidly. Exosome-based diagnosis of AC offers several advantages, as the number of exosomes produced, their origin, and the cargo they carry are specific to each pathological condition. Exosomes are also enriched with microRNAs (miRNAs), highlighting their potential as biomarkers for AC, surpassing the diagnostic capabilities of the traditional gold-standard 12-lead electrocardiography (ECG) [7]. In a study by Mazruk et al., the first AC model indicating miRNAs as a potential cause of desmosomal dysfunction was investigated. The study showed an upregulation in the miR-130a in transgenic mouse models, which resulted in increased ventricular dysfunction at 8 weeks, followed by fibrofatty tissue deposition, lipid accumulation, and apoptosis at 10 weeks. This finding suggests a direct link between miR-130a and AC, as miR-130a downregulates DSC2, leading to desmosomal dysfunction, fibrosis, and lipid accumulation. However, the presence of miR-130a in humans has not yet been confirmed, limiting the reproducibility and translational impact of these findings [50].

In the meantime, Sommariva and colleagues conducted the first miRNA profiling study for AC among human patients, investigating the correlation between two specific miRNAs (miR-21-5p and miR-135b)

Table 1
Effects of Exosomes on Cardiomyocyte Function in HCM.

Source of Exosomes	Target Cell Type	Impact on Target Cell	Potential Role in HCM Pathophysiology	References
Iron Overload-Brown Adipocytes	HL-1 Cardiomyocytes	Altered cardiomyocyte function; increased regulation of Tpm1	May contribute to the development of iron overload cardiomyopathy (IOC) and influence HCM progression	[42,43]
GLA-null Cardiomyocytes	Cardiomyocytes	Increased exosome secretion; autophagic dysfunction	Linked to Fabry disease-associated hypertrophic cardiomyopathy; suggests altered exosome biogenesis and secretion	[44,45]

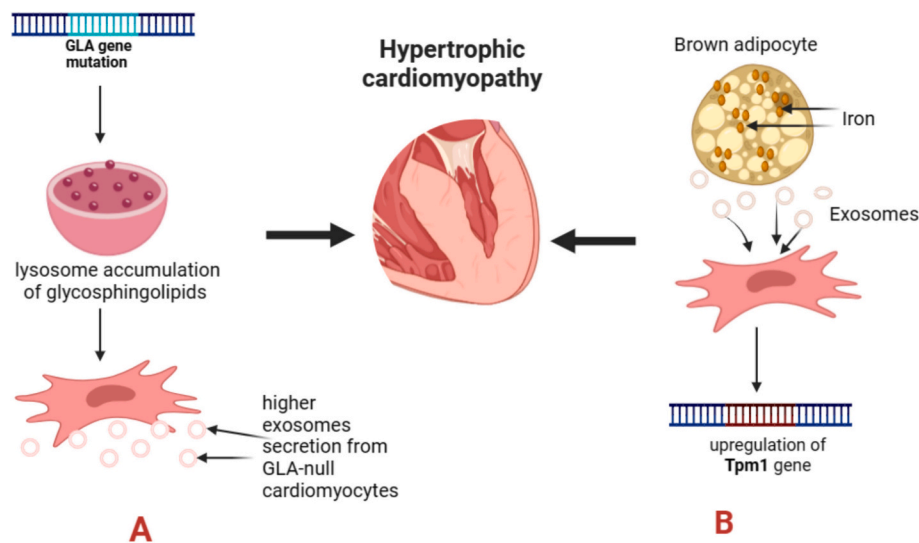


Fig. 3. The figure illustrates two mechanisms contributing to hypertrophic cardiomyopathy (HCM). Panel A depicts cellular dysfunction in GLA-null cardiomyocytes, characteristic of Fabry disease-associated HCM. An increase in exosome secretion from these cardiomyocytes, potentially linked to autophagic dysfunction, suggests that exosomes may play a role in the pathophysiology of Fabry disease-associated HCM. Panel B highlights the impact of iron overload in brown adipose tissue, which triggers the release of exosomes derived from brown adipocytes. These exosomes influence the regulation of Tpm1, a gene implicated in the development of HCM.

and two key signaling pathways, Wnt and Hippo, which are implicated in AC pathogenesis. Their analysis identified two targets: bone morphogenetic protein receptor type 2 (*BMPR2*), which is involved in adipogenesis, and Transforming Growth Factor Beta Receptor 2 (*TGFBR2*), associated with extracellular matrix (ECM) production and fibrosis. Hence, the upregulation of miR-21-5p and miR-135b suggests disruption of these signaling pathways, leading to cardiomyocyte fibrosis. While this study supports the potential of miRNAs as diagnostic biomarkers for AC, the selection of miR-21-5p and miR-135b has been met with some controversy, as other miRNAs with greater differential expression (DE) were not considered [51]. Despite this, both studies have paved the way for diagnosing AC through non-invasive techniques.

In the following year, Yamada and colleagues reported a direct correlation between the upregulation of miR-494 and elevated levels of caspase -3, contributing to progressive myocardial atrophy in ventricular arrhythmia [52]. Additionally, Puzzi et al. (2019) investigated the effects of plakophilin-2 (PKP2) deficiency, which leads to impaired cell-cell adhesion and increased fibrosis. Through gene expression analysis, they observed an upregulation of miR-200b. To validate the role of miR-200b in AC, they applied anti-miR-200b, which resulted in the complete restoration of cell-collagen adhesion, confirming an association between miR-200b upregulation, PKP2 deficiency and AC [53]. Succeeding this, Marinas, et al., 2020 identified six upregulated miRNAs (miR-122-5p, miR-133a-3p, miR-133b, miR-142-3p, miR-182-5p, and miR-183-5p) that target 16 genes related to desmosomal complex deficiencies or transcription factors, including JUP, DSG2, DSP/PKP2, PKP2/JUP, and TCF7L2, as well as the Wnt, Hippo, and TGF- β signaling pathways. This six-miRNA panel demonstrated strong diagnostic potential as a non-invasive tool for AC. However, as this was a validation study, the cohort size was limited to 86 subjects, with only 17 AC cases, which restricts the generalizability of the findings [54].

Recently, Khudiakov and colleagues investigated the relationship between five upregulated miRNAs (hsa-miR-1-3p, hsa-miR-21-5p, hsa-miR-206, and hsa-miR-122-5p) that are directly associated with ARVC. These miRNAs regulate the expression of the metalloproteinase-2 gene and genes coding for collagen isoforms. Additionally, they modulate vimentin expression in mesenchymal and cardiac fibroblasts, suggesting a role in the pathogenesis of ARVC [55]. However, the study had several limitations, particularly the small number of differentially expressed genes examined, which reduced the robustness of the data validation.

Exosomes also play a crucial role in the diagnosis of atrial fibrillation

(AF). For instance, Shaihov-Teper et al. (2021) found that epicardial fat (eFat) promotes AF, as the vesicles it secretes exhibit proinflammatory and proarrhythmic properties, facilitating AF development [56]. Similarly, Mun et al. (2019) demonstrated that exosomes secreted by myofibroblasts can influence cardiomyocytes by downregulating the L-type calcium channel Cav1.2, thereby contributing to the progression of AF [57]. Moreover, Wang et al. (2019) compared circulating exosomes in the plasma of AF patients and control groups, identifying three differentially expressed miRNAs—miR-483-5p, miR-142-5p, and miR-223-5p—associated with AF. Of these, miR-483-5p was independently linked to AF, underscoring the diagnostic potential of exosomes in AF detection [58]. Fig. 4 illustrates the role of miRNAs as diagnostic biomarkers for ARVC. The application of exosomes in diagnosing and treating ARVC is advancing rapidly due to their feasibility and specificity in detecting miRNAs through non-invasive bodily fluid sampling techniques [7].

Exosomes, a promising diagnostic tool for AC, are currently undergoing clinical trials for use in point-of-care testing. For example, Khan et al. (2015) demonstrated that exosomal miRNA-294 derived from embryonic stem cells could inhibit myocardial fibrosis by promoting angiogenesis and the proliferation of myocardial progenitor cells, thus improving cardiac function following myocardial infarction [59]. In a more recent study, Lin et al. (2021) isolated exosomes from immortalized cardiosphere-derived cells engineered to express high levels of β -catenin, a protein implicated in the pathogenesis of AC. These exosomes, enriched with NF- κ B, were administered intravenously for one month in DSG2^{mt/mt} mice, which exhibited features of left and right ventricular dysfunction and fibrous tissue deposition [17]. Repeated administration of exosomes enhanced the immune response, mitigated intercalated disc remodeling, reduced fibrous infiltration, improved cardiac function, and suppressed arrhythmias. However, the study has several limitations. Although DSG2^{mt/mt} mice model some aspects of AC, they do not fully replicate key human features, such as arrhythmogenicity and fibrosis, and their hearts lack the fatty infiltrations typically seen in human AC [17]. Additionally, these mice exhibit an early and aggressive phenotype (e.g., severe cardiomyopathy and QTc prolongation), which does not represent the broad spectrum of disease presentation in humans [17].

Khan et al. (2015) found that exosomal miRNA-294 derived from embryonic stem cells could inhibit myocardial fibrosis by promoting angiogenesis and the proliferation of myocardial progenitor cells, improving cardiac function post-myocardial infarction [59]. Similarly,

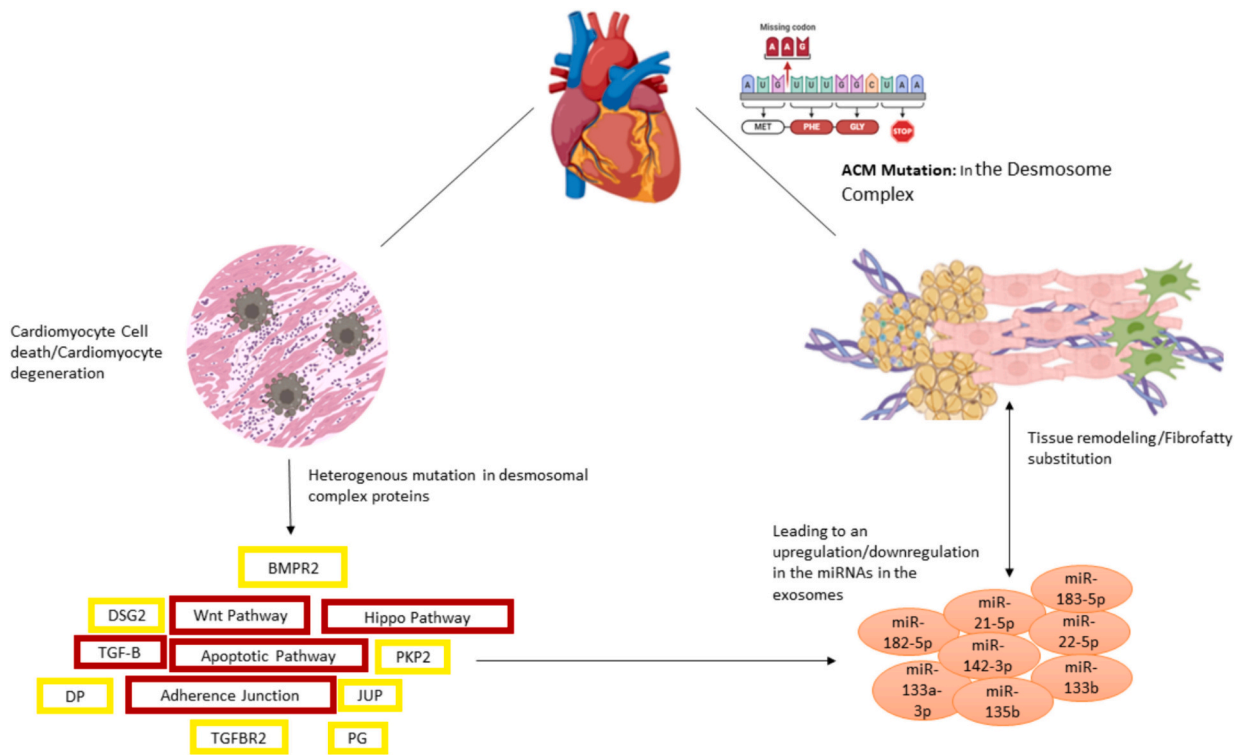


Fig. 4. AC mutations trigger signaling cascades such as the Wnt and TGF- β signaling pathways, and mutations in the adherence junctions induce fibrosis in cardiomyocytes. Upon fatty fibro tissue formation, exosomes containing miRNAs are released. Hence, they could be used as a noninvasive diagnostic tool via blood samples or pericardial fluids.

Liu et al. (2019) demonstrated that miRNA-320d inhibits cardiomyocytes by targeting signal transducers, showing potential as a targeted therapeutic strategy for atrial fibrillation [60]. Despite the promising results from these studies, further preclinical and clinical research is required before these findings can be applied to human cases of this heterogeneous disease [17]. To date, the use of exosomes for treating AC remains limited to animal models, primarily due to unresolved safety concerns, such as off-target effects [5]. Moreover, although the body of evidence supporting the role of exosomes as non-invasive diagnostic and therapeutic tools in AC is growing, their clinical application remains significantly constrained by technological challenges [47].

3.4. Restrictive cardiomyopathy

Restrictive cardiomyopathy (RCM) is frequently regarded as the rarest and most difficult form of heart muscle disease to define and categorize [61]. This complexity arises from the broad spectrum of underlying disorders associated with RCM, which pose significant challenges to both its classification and diagnosis [61]. Moreover, RCM encompasses the most diverse range of etiologies and histopathological features among cardiomyopathies, often requiring invasive diagnostic procedures such as cardiac catheterization or endomyocardial biopsy for accurate identification [61]. Additionally, the genetic basis of RCM is increasingly recognized as overlapping with that of other cardiomyopathies, as many of the implicated genes are shared across different forms of heart muscle disease [61].

RCM is characterized by increased stiffness of the heart muscle, resulting in impaired diastolic relaxation while the ventricles remain non-dilated [62]. Different forms of RCM exhibit variations in their causes, clinical manifestations, diagnostic procedures, and treatment options. The development of RCM can be attributed to several factors, broadly categorized into infiltrative diseases, storage diseases, and systemic conditions [62]. (1) Infiltrative diseases involve the accumulation

of abnormal substances in the heart muscle, leading to its stiffening. Examples include amyloidosis (AL, ATTRm, ATTRwt, ApoA-I), sarcoidosis, hereditary hemochromatosis, and primary hyperoxaluria [62]. (2) Storage diseases are rare congenital metabolic disorders that result in abnormal intracellular storage of substances. These include Anderson-Fabry disease, Gaucher disease, glycogen storage diseases, mucopolysaccharidosis type II (Hurler syndrome), Niemann-Pick disease, Danon disease, and Friedreich's ataxia [62]. (3) Systemic diseases contributing to RCM include diabetes, scleroderma, myofibrillar myopathies, pseudothorax elasticum, Werner syndrome, sarcomeric protein disorders, carcinoid syndrome, idiopathic fibrosis, hypereosinophilic syndrome, chronic eosinophilic leukemia fibrosis, endocardial fibroelastosis, and metastatic malignancies [62]. Genetic factors also play a significant role in RCM, though the molecular mechanisms underlying its genetic forms are not yet fully understood [63]. More than 19 gene mutations have been identified as being associated with primary RCM, but the genetic overlap between RCM and other cardiomyopathies complicates classification. Key RCM-related genes include those encoding sarcomere proteins, such as cardiac troponin-I. However, increasing evidence points to the involvement of non-sarcomeric gene mutations, such as DES and FLNC, in the pathogenesis of RCM [62,63]. In addition to these factors, idiopathic causes such as radiation therapy and certain medications have been implicated in the onset of RCM [64].

The role of exosomes in disease is becoming increasingly well understood as more research explores their potential applications in diagnosing and treating various health conditions, either alone or in combination with other therapeutic strategies [65–68]. However, investigation in this direction is challenging because our understanding of the molecular mechanisms underlying primary cardiomyopathies, particularly in the case of RCM, remains limited and lacks comprehensive insight.

Our current understanding of the molecular mechanisms underlying RCM suggests a cascade of events triggered by specific mutations, which alter the structure or function of proteins involved in the heart's

sarcomere or associated structures [69]. These mutations initially cause structural changes or dysregulation in protein expression compared to their wild-type counterparts [69]. Once integrated into the sarcomere or associated structures, the mutated proteins disrupt normal protein-protein interactions, impairing sarcomeric dynamics and protein functionality [69]. This disruption leads to several pathophysiological consequences, including diastolic dysfunction, compromised structural integrity, impaired intercellular communication, and increased myocardial stiffness [69]. In addition to these mechanical effects, the mutation also activates specific signaling pathways via associated proteins, further influencing cellular processes [69]. One notable consequence is the altered secretion of exosomes, which play a key role in cellular communication and may contribute to the progression of the disease [69].

Although genetically linked RCM is a rare disorder, it is associated with a high mortality rate [69]. Therefore, advancing our understanding of the molecular pathways involved, including the role of exosomes, is critical for the development of more targeted diagnostic and therapeutic strategies to manage this life-threatening condition.

4. Future perspectives

Future research on exosomes in cardiomyopathies should focus on several critical areas. Detailed profiling of exosomal contents in different cardiomyopathy subtypes is crucial for identifying specific biomarkers that can facilitate early diagnosis and disease monitoring. Employing cutting-edge technologies like next-generation sequencing and proteomics will be key to achieving this objective. Elucidating the mechanisms of exosome biogenesis, secretion, and uptake in cardiomyocytes will enhance our understanding of their role in disease progression. This knowledge will be instrumental in developing targeted therapies that can modulate exosome functions to achieve therapeutic outcomes. Exosomes' therapeutic potential can be further explored by engineering them to deliver specific therapeutic agents, such as miRNAs or proteins, to treat cardiomyopathies. Although comprehensive preclinical and clinical trials are essential to validate the safety and effectiveness of these exosome-based therapies. Additionally, integrating exosome research with traditional diagnostic approaches, such as imaging and genetic testing, will provide a more comprehensive understanding of cardiomyopathies improving diagnostic precision and enabling the development of personalized treatments.

5. Conclusion

Exosomes have emerged as pivotal players in the pathogenesis and potential treatment of cardiomyopathies, offering promising avenues for diagnosis and therapy. These extracellular vesicles are increasingly recognized for their role in mediating intercellular communication, particularly in conditions such as DCM, HCM, AC, and RCM. Indeed, exosomes transport diverse molecular cargoes, including proteins, lipids, and RNAs, which can modulate pathological processes and disease progression.

For instance, in DCM, exosomes are implicated in promoting pathological cardiomyocyte responses, including fibrosis and inflammation. HCM research indicates that exosomes from iron-overloaded brown adipocytes significantly impact cardiomyocyte function, while studies on AC and RCM have revealed that exosomal miRNAs play crucial roles in modulating pro-fibrotic pathways and cardiac functions. While these findings highlight the diversified role of exosomes in cardiomyopathies, further research is needed to elucidate their precise molecular mechanisms and translate these insights into clinical practice. This will pave the way for the use of exosomes as both diagnostic biomarkers and therapeutic tools.

In conclusion, although exosome research in cardiomyopathies is still in its early stages, its potential to transform diagnostic and therapeutic practices is substantial. Continued research efforts and

translational initiatives are necessary to harness the full clinical potential of exosomes in managing cardiomyopathies.

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CRedit authorship contribution statement

Shadiya Fawzul Ameer: Writing – original draft, Visualization. **Manar Elsaka:** Writing – original draft, Visualization. **Summaiya Kahtoon:** Writing – original draft, Visualization. **Rabia-Illhem Kerzabi:** Writing – original draft, Visualization. **Gavino Casu:** Supervision, Methodology. **Roberta Giordo:** Writing – review & editing, Writing – original draft. **Hatem Zayed:** Supervision, Methodology. **Gianfranco Pintus:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the study's design, data collection, analysis, interpretation, or manuscript writing.

Data availability

No data was used for the research described in the article.

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