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Idiopathic Dilated Cardiomyopathy: Molecular Basis and Distilling Complexity to Advance

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Abstract

Cardiomyopathies are heterogeneous diseases of the myocardium associated with abnormal findings of chamber size, wall thickness, and/or functional contractility. In particular, dilated cardiomyopathy (DCM) is mainly characterized by ventricular chamber enlargement with systolic dysfunction and normal left ventricular (LV) wall thickness. Although DCM is thought to be induced mainly by genetic or environmental factors, in the majority of cases, the cause is unknown. With an estimated prevalence of 1:2500 and an incidence of 1:18,000 per year in adults, DCM is the most frequent indication for heart transplantation, which represents an enormous cost burden on healthcare systems. These figures warrant greater accuracy in patient diagnosis and prognosis and further insight into the underlying basis of DCM. Here, we discuss past and recent findings on the molecular mechanisms involved in DCM. Dilated cardiomyopathy has been linked to the overactivation of extracellular signal-regulated kinase (ERK1/2), which in turn is related to activation of low-density lipoprotein receptor-related protein-1 (LRP-1). Moreover, a redistribution of LRP-1 into cholesterol-enriched plasma membrane domains (lipid rafts) and alterations in cardiac DNA methylation have been reported in failing hearts. In conclusion, more comprehensive analyses of myocardial lipid rafts and epigenetic mechanisms may advance our understanding of DCM causes and progression. In turn, this understanding may promote the development of innovative treatments.

Keywords: dilated cardiomyopathy, heart failure, lipid rafts, LRP-1, molecular basis, cardiac muscle, vasculature

1. Introduction

Cardiomyopathies are heterogeneous diseases of the myocardium associated with abnormal findings of chamber size, wall thickness, and/or functional contractility [1]. Currently, cardiomyopathy is classified based on the dominant pathophysiology or by aetiological/pathogenetic factors. This system defines four distinct categories of cardiomyopathy: dilated, hypertrophic, restrictive, and arrhythmogenic (**Figure 1**) [2]. In particular, dilated cardiomyopathy (DCM) is mainly characterized by ventricular chamber enlargement or dilatation with systolic dysfunction and normal left ventricular (LV) wall thickness. In general, these abnormalities lead to progressive heart failure, a decline in LV contractile function, abnormalities in ventricular and supraventricular rhythm and conduction, thromboembolism, and finally, sudden or heart failure-related death [3]. Indeed, DCM is a common, largely irreversible cause of myocardial damage. It is thought to be induced by genetic or environmental factors that may manifest clinically over a wide range of ages (most commonly in the third or fourth decade, but also in young children). Dilated cardiomyopathy affects both sexes and all ethnic groups; it is typically identified when patients exhibit severe limiting symptoms and incapacity [4].

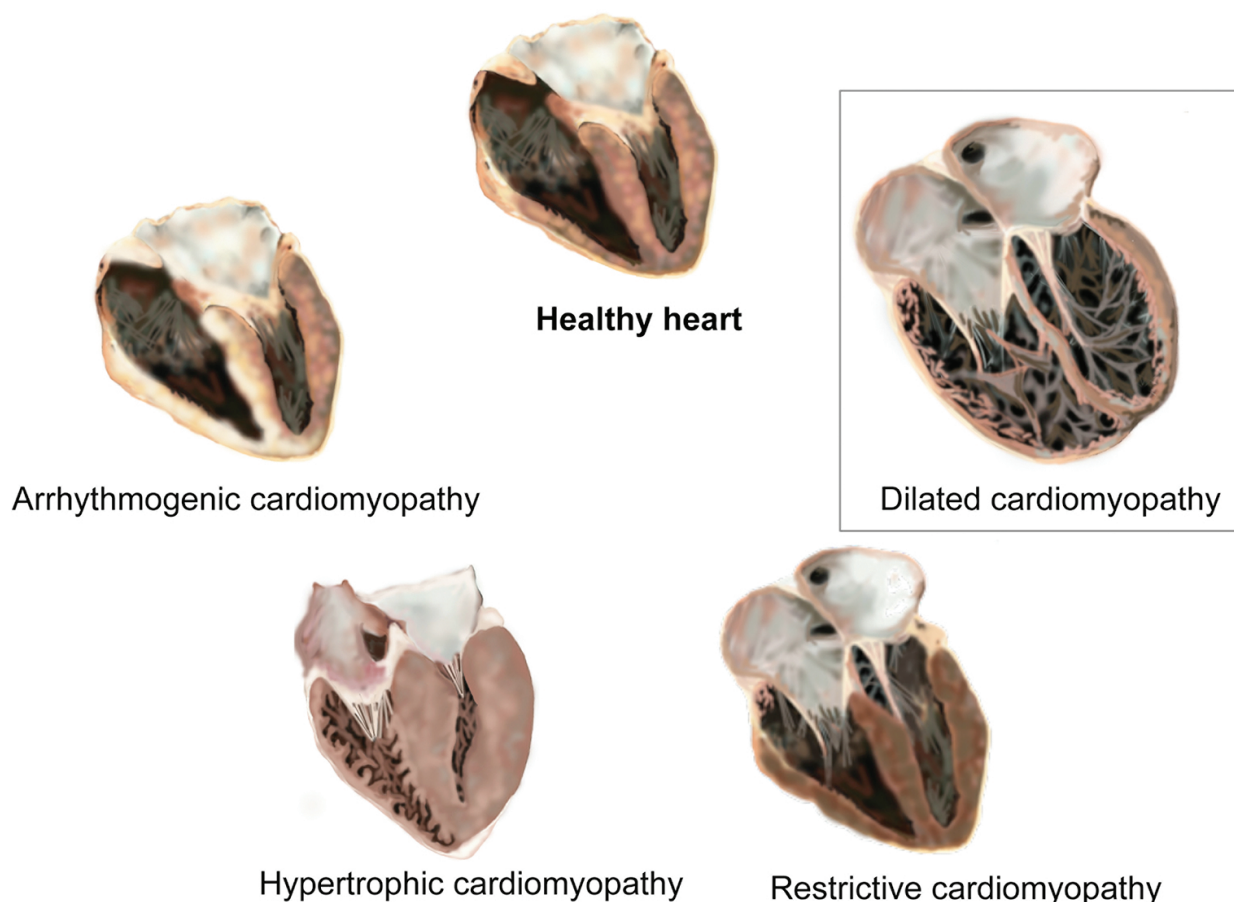


Figure 1. Heterogeneous diseases of the myocardium. The different types of cardiomyopathies fall into four principal categories, based on the muscle disorder involved. This chapter is confined to dilated cardiomyopathy.

This chapter summarizes the most important traits (aetiology, diagnosis, treatment, and pathophysiology) that characterize DCM. We focus on studies that provided novel insights into the underlying molecular basis of this extremely complex human disorder. In a sense, we will present new pieces of an intriguing puzzle, with the aim of bringing some order into the chaos of the molecular reality.

2. Dilated cardiomyopathy: characteristic features

Dilated cardiomyopathy has an estimated prevalence of 1:2500, an incidence of 1:15,000–18,000 per year in adults, and an estimated prevalence of 2:3 among children with unknown diseases [4, 5]. The clinical course of DCM can be progressive; one study reported that about 35% of individuals died within 5 years after diagnosis [1]. The origin of death is divided evenly between sudden death and pump failure. To date, prolonged survival has been achieved with neurohormone blockers (angiotensin-converting enzyme inhibitors and β -adrenoceptor antagonists) and devices (cardiac resynchronization and implanted defibrillators). Nevertheless, the only definitive treatment is heart transplantation, which is hampered in many instances by the limited number of heart donors and by graft rejection over time. Therefore, DCM is the most frequent indication for heart transplantation, which results in an enormous cost burden for healthcare systems throughout the world. Diagnosis is typically based on patient history and the presence of LV dilatation and impaired ejection fraction, with or without regurgitation; these signs are detected with echocardiography, cardiac magnetic resonance imaging, or both.

At the histological level, the main pathological derangements observed in explanted diseased hearts are patchy interstitial fibrosis surrounding myocardial filaments, marked lipid deposition, cardiac muscle atrophy, and lipid accumulation [6–8]. Moreover, further investigations of both patient samples and animal disease models have shown that DCM hearts exhibit marked vascular alterations [9, 10]. In most cases, the causal mechanism of disease is poorly understood. This reality has induced some authors to argue that heart disease in DCM arises from an obscure origin, and this viewpoint has given rise to the term ‘idiopathic’ DCM. Recently, relevant advances have been made in our understanding of the causes of this disease. The main causes include familial and genetic disorders, infectious and toxicity-related processes, autoimmunity, and inflammation [4].

Frequently, DCM has been defined as the result of an extremely complex genetic architecture that involves disruptions in a variety of myocardial proteins, which are provoked by rare variants in some genes; moreover, many of these genes are also involved in other cardiomyopathies, such as muscular dystrophy or syndromic diseases [11]. In brief, numerous DCM-associated genes have been identified. This information has provided a better understanding of disease pathogenesis, and it has promoted advances in mutation analytical techniques to facilitate the recognition of subjects and progeny that carry these mutations [12]. Alterations in more than 50 loci and genes have been identified, which mostly encode either cardiac myocyte-specific proteins or structural, nuclear membrane, and calcium metabolism proteins.

However, it is estimated that genetic disorders account for only 20–35% of DCM cases [12, 13]. Some researchers have predicted that genetic associations may have been missed due to the limited nature of previous studies; accordingly, they point to a need for more comprehensive studies in much larger cohorts of families that are rigorously phenotyped [11]. In addition, some cases of DCM are believed to be related to autoimmune and inflammatory processes [14–16]; metabolic, nutritional, and endocrine deficiencies; or heart muscle damage following exposure to viruses, exogenous drugs, or toxins (e.g., chronic alcohol consumption) [2]. Peripartum cardiomyopathy also represents a subset of LV systolic dysfunction. In the latter cases, initial symptoms of heart failure occur during the late stages of pregnancy [17]. Although there may be a variety of causes for DCM, the clinical presentation of this disease seems to be uniform, both in humans and in animal models that have been used to dissect DCM development, progression, and treatment [10].

Standard treatment of DCM involves neurohormonal inhibition of the renin-angiotensin-aldosterone system (i.e., angiotensin-converting enzyme inhibitors, angiotensin II receptor type 1 blockers, or mineralocorticoid receptor blockers) or blocking the sympathetic nervous system with β -blockers. In patients, it is crucial to focus on improving cardiac function and reducing mechanical stress. Although progress has been made in arrhythmia therapy and in sudden death prevention, many impediments for improving patient outcomes remain unresolved [4]. Innovative therapies, such as stem cell-based applications, are also being investigated [18].

3. Dilated cardiomyopathy affects both cardiac muscle and the vasculature

Dilated cardiomyopathy is associated with pronounced remodelling of one or both ventricles, which results in large changes in the shapes of ventricles and in the architecture of myocardial fibres. As mentioned previously, the main microscopic hallmarks of failing hearts are marked collagen deposition, patchy interstitial fibrosis, degenerated cardiac muscle cells, and sparse blood vessels. At the ultrastructural level, remodelling comprises mitochondrial abnormalities, T-tubular dilatation, and intracellular lipid droplet accumulation [10]. Because the mitochondrion is the main site of ATP production and cardiac myocytes are particularly sensitive to the supply of energy, deficits in mitochondrial function have been linked to DCM [19]. Additionally, altered levels of connexin-43 and modulation of its phosphorylation state can induce electromechanical uncoupling between neighbouring cardiac muscle cells [20].

A number of studies have indicated that programmed cell death or apoptosis contributes actively to human end-stage heart failure. Indeed, cell death occurs in myocardial ischemia-reperfusion [21], ischemia-reperfusion injury [22], and fatal myocarditis [23]. However, the role of apoptosis in the DCM myocardium remains controversial, due to some limitations in the techniques that have been used to measure apoptosis [24–26]. A positive Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) signal seems to require fragmentation of only 10% of all DNA; as a result, the level of apoptosis may be highly overestimated [24]. Furthermore, cells that are apoptotic, necrotic, undergoing DNA repair, or living can emit

equivalent signals in the TUNEL assay [27], which renders the use of this method even more questionable. Consequently, although some authors have reported putative increases in apoptotic markers with different methodologies, including caspase-3 activity, DNA fragmentation (TUNEL), and electron microscopy [28], others have failed to detect changes in apoptosis. For instance, Bott-Flügel *et al.* did not detect any correlations between caspase-3 activity, the induction of DNA fragmentation, and haemodynamic or echocardiographic variables in patients with end-stage heart failure, including DCM. Moreover, they did not find significant differences in caspase-3 activation between DCM and control myocardium [26]. **Figure 2** shows that only slight amounts of caspase-3 mRNA and protein were detected in LV samples from patients (unpublished results). These findings suggest that cardiac muscle cells might trigger apoptotic self-destruction, without completing the process. Hence, DCM is characterized by marked abnormalities in the function and integrity of cardiac muscle.

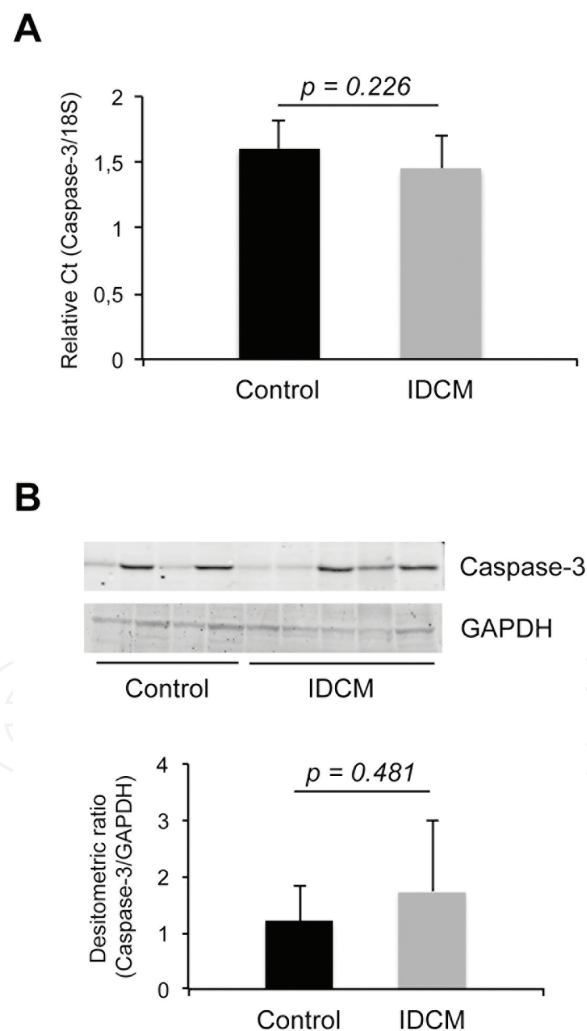


Figure 2. Comparative analysis of apoptosis in left ventricle samples collected from human explanted DCM hearts and control hearts from non-cardiac decedents. Representative caspase-3 gene (A) and protein (B) expression levels by quantitative RT-PCR and Western blotting, respectively.

Cardiac endothelial dysfunction was also associated with disease progression and a poor prognosis in patients with DCM. In the late 1940s, preliminary observations showed a correlation between heart weight and the total cross-sectional size of the main coronary vessels [29]. Since then, a number of studies have recognized that cardiac vasculature is a key regulator of the integrity and function of the myocardium. In an attempt to take this a step further, Brutsaert *et al.* studied the mechanical properties of the mammalian ventricular myocardium before and after damaging the endocardial surface [30]. Those authors speculated that the endocardium could affect myocardial performance by either forming an electrochemical barrier, releasing a chemical substance or messenger, or both. They subsequently demonstrated that nitric oxide synthase activity regulated the contractile responsiveness of ventricular myocytes [31]. Perhaps more significantly, additional studies in Langendorff-perfused and post-infarcted rat hearts confirmed that endothelial damage led to progressive myocardial dysfunction and that, conversely, protecting the associated vasculature preserved global myocardial homeostasis [32, 33].

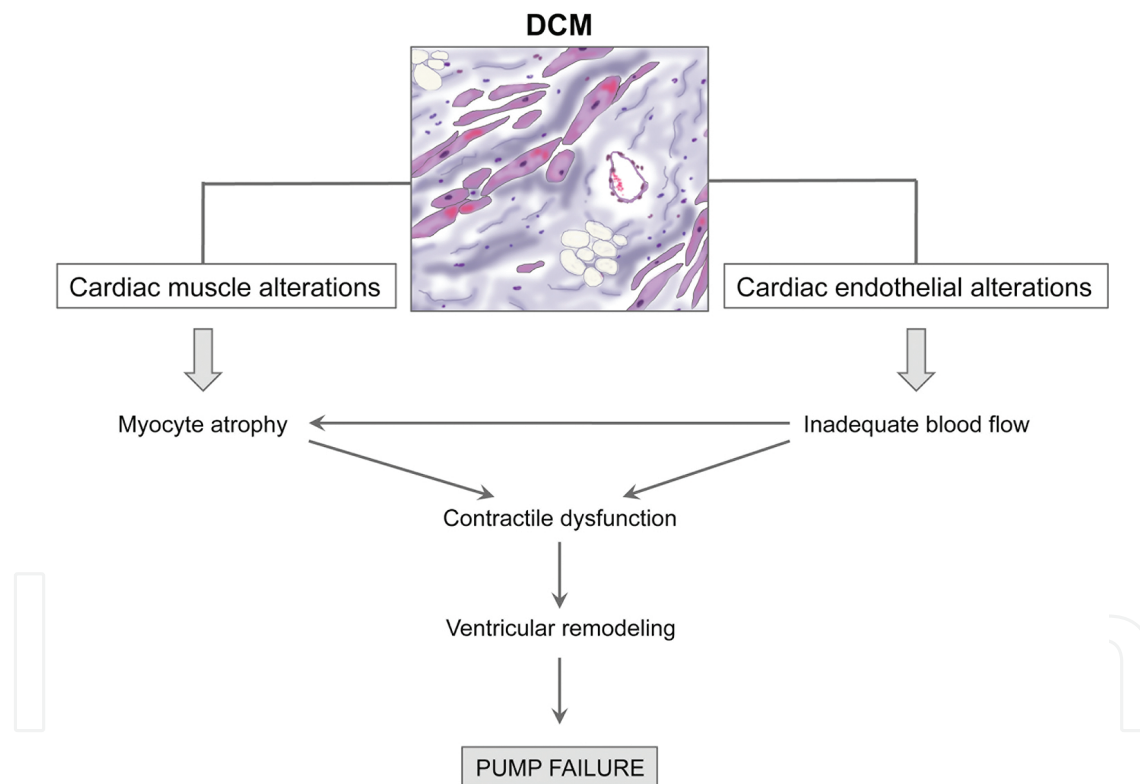


Figure 3. Foundation of DCM as a two-hit heart disease. The integrity and function of both cardiac muscle and vasculature are adversely compromised in DCM, which leads to LV remodelling and pump failure.

Advances in medical imaging techniques have become crucial for performing more comprehensive analyses of vascular derangements in DCM. Angiography revealed that a mismatch between artery size and LV mass in patients with DCM contributed to myocardial hypoperfusion [34–37]. Computed tomography measurements of DCM cardiac vasculature on a multislice scanner have also clearly shown side branch paucity and shortened, thinned

epicardial arteries [9]. Therefore, the epicardial coronary arteries in patients with DCM are not adequately sized for the enlarged LV mass. Notably, a variety of studies described significantly reduced, sparse microvasculature in diseased myocardium samples [9, 38, 39]. In this context, numerous studies in patients with DCM have reported that the circulating levels of distinct bone marrow-derived cell populations are peripherally increased after vascular damage [40]. Although there is a correlation between the circulating levels of these progenitor cells and the progression and clinical outcomes of DCM, the clinical usefulness of this overrepresentation awaits further validation.

Collectively, these findings led to a re-examination of the pathophysiology of DCM. In 2009, Roura and Bayes-Genis [10] reviewed the extensive data from animal models and patients and concluded that DCM is a two-hit disease, where both cardiac muscle and endothelial alterations contribute equally to contractile deficiency and pump failure (**Figure 3**).

4. Molecular basis of dilated cardiomyopathy: past and new actors

Molecularly, in humans, DCM has been related to intramyocardial accumulation of α -2 macroglobulin (α -2M) [41] and increased activation of extracellular signal-regulated kinase (ERK1/2) [42]. In a mouse model of DCM, the disease was generated by a mutation in the lamin A/C gene [43, 44]. In these mice, chemically suppressing ERK1/2 activation prevented LV dilatation and greatly restored the cardiac ejection fraction.

The α -2M protein is a highly abundant plasma protease inhibitor, which has been shown to activate various tyrosine kinases and mitogen-activated protein kinases [45]. Moreover, α -2M was one of the first molecules to be described as a ligand of low-density lipoprotein (LDL) receptor-related protein 1 (LRP-1) [46]. A recent review described LRP-1 as a multifunctional receptor of the LDL-receptor family that mediates the clearance of a variety of structurally diverse extracellular molecules [47]. Moreover, LRP-1 plays key roles in various biological processes by interacting with multiple intracellular signalling pathways [48, 49]. As a result, LRP-1 tyrosine phosphorylation can be activated in response to diverse extracellular molecules, such as platelet-derived growth factor [50–52]. In particular, ERK1/2 activation is recognized as one of the main LRP-1 molecular relays [53–55]. In the following pages, we present and discuss novel data demonstrating that, together with the pivotal role of LRP-1 in the vascular wall and in the aetiology of atherosclerosis [56], LRP-1 is redistributed and overactivated within some specialized plasma membrane domains, termed lipid rafts, in DCM myocardium.

The decreased capillary density found in patients with DCM was shown to arise from impairments in myocardial endothelial cell survival and insufficient revascularization. These processes involve several intracellular signalling pathways, including those mediated by vascular endothelial (VE)-cadherin/ β -catenin, angiopoietins, and vascular endothelial growth factors (VEGFs). In particular, VE-cadherin, β -catenin, angiopoietin-2, VEGF-A, VEGF-B, and VEGF receptor-1 (VEGFR-1) expression levels were downregulated in DCM [9, 57, 58]. Roura

et al. [9] further demonstrated that reduced expression of β -catenin, an important angiogenic regulator [59], occurred exclusively in myocardial vascular cells in failing hearts.

Remarkably, different DNA methylation patterns have been detected in genes that regulate pathways related to heart disease and in genes with unknown functions in DCM. For example, variations in DNA methylation were observed in lymphocyte antigen 75, tyrosine kinase-type cell surface receptor HER3, homeobox B13, and adenosine receptor A2A [60]. These validated targets are most likely involved in modifying DCM rather than independently causing disease. Furthermore, other authors found differentially methylated gene promoters and a depletion of mitochondrial DNA that resulted in a thymidine kinase deficiency in DCM hearts [61, 62]. Indeed, investigating epigenetic mechanisms represents an attractive approach for finding novel mechanisms of disease.

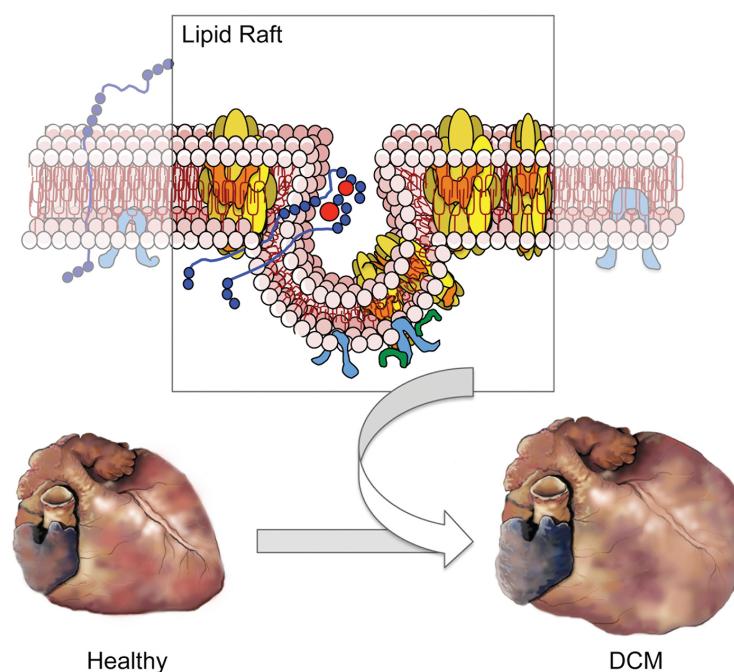


Figure 4. Potential impact of lipid raft-associated signalling on DCM. Schematic illustration of a lipid raft domain in a portion of the cell surface. Proteins potentially involved in disease progression are either packed into or excluded from these specialized plasma membrane areas.

For more than 40 years, the Singer and Nicolson model of the cell membrane, where proteins are viewed as icebergs floating in a sea of lipids, has provided a solid foundation for studying cell membrane properties [63]. This enduring model was subsequently reinterpreted with the discovery of localized, highly cholesterol- and glycosphingolipid-enriched plasma membrane areas, referred to as 'lipid rafts' [64]. Many proteins, particularly those involved in cell signalling and cytokine presentation, were found to be densely packed together in these specialized surface domains [65]. Accordingly, lipid rafts facilitate interactions between protein receptors and mediators by maintaining them tightly packed together in one location. Interestingly, LRP-1 was reported to be associated with lipid rafts [66, 67].

As previously mentioned, recent research has given us new molecular aspects of the disease. Particularly, in patients with DCM, LRP-1 was seen redistributed and further activated, through tyrosine phosphorylation, within lipid rafts enriched in caveolin-3 and flotillin-1 [68]. Of note, these observations suggested that movement of LRP-1 within these specialized membrane domains contribute to the overactivation of ERK1/2-mediated signalling described in DCM. However, further confirmatory exploration is warranted to determine whether this overactive signalling leads to the characteristic promotion of extracellular matrix metalloproteinase activity and subsequent LV remodelling (**Figure 4**) [69, 70].

To investigate this novel regulatory impact of lipid rafts on DCM, Roura *et al.* extracted lipid rafts from failing myocardium and detected elevated amounts of the mobilizing cytokine, stromal cell-derived factor (SDF)-1 [71]. In that same study, the authors showed that deficiencies in ILK and ERK1/2 signalling impeded SDF-1-mediated migration of circulating progenitor cells. As a result, impaired cell migration compromised endothelial maintenance and recovery, which contributed to the marked vascular derangements observed in diseased myocardium [72].

Taken together, these observations support the growing body of data that led to the recognition of myocardial lipid rafts and their associated proteins as modulators of cardiac performance and as novel therapeutic targets [73–75].

5. Conclusions

Heart failure has become an increasingly common disorder worldwide, and it is associated with substantial morbidity and mortality. Many causes of heart failure are easily identified in clinical practice, including abnormal heart valves, inherited cardiomyopathies, severe coronary artery disease, or hypertensive heart disease. However, the precise mechanisms that govern the progression of heart failure and ventricular remodelling in DCM remain obscure. Some authors have appropriately pointed out that, for both clinicians and researchers, attempting to discover the underlying genetic and environmental causes linked to complex human diseases, such as DCM, is like facing a drawer filled with thousands of puzzle pieces mixed together from an unknown number of jigsaw puzzles [76, 77]. The question remains, how can they begin to solve it?

There is a growing body of data that describes the multifaceted genetic diversity involved in DCM and the alterations in both cardiac muscle and vasculature that contribute to the disease. However, several crucial issues remain to be addressed. For example, it is not clear whether the marked vascular deficiencies observed in patients with DCM develop secondary to heart remodelling, or whether they directly contribute to myocardial alterations and to the temporal evolution of LV dilatation. Accordingly, researchers are providing novel mechanistic insights that might bring some order to this ‘disordered’ collection of data. For example, they have shown that lipid rafts participate in the mechanism underlying the spatial regulation of LRP-1-mediated ERK1/2 activity. Undoubtedly, further work is needed to increase our comprehension of the causes underlying this ‘obscure’ disease. To that end, the current state of knowledge

summarized in the present review provides a starting point for addressing the remaining questions in the pathophysiology of this disease. Moreover, we highlight new avenues for discovering potentially effective treatments.

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