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Theranostic biomarkers in hypertrophic cardiomyopathy: insights in a long road ahead

Carmem L. Sartorio[1], Davide Lazzeroni[1,2], Gloria Bertoli[3], Paolo G. Camici[1]

[1]Department of Cardiovascular Diseases, Vita Salute University and San Raffaele Hospital, Milan, Italy, [2]Prevention and Rehabilitation Unit, Fondazione Don Gnocchi, Parma, Italy, [3]Institute of Molecular Bioimaging and Physiology (IBFM), National Research Council (CNR), Milan Italy

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1. ABSTRACT

The study of biomarkers and their related signalling pathways has allowed the development of new therapeutic strategies in a range of disorders. However, in hypertrophic cardiomyopathy (HCM), which is the most common hereditary cardiac disease, there are many potential biomarkers described, but their specificity and applicability for HCM remains an open field. The aim of the present review is to provide an overview of molecules that could give some insight into the pathophysiologic mechanisms underlying HCM, especially to those with "theranostic" - a combination of diagnostics and therapy - potential. The clinical and pre-clinical state of the art and theranostic perspectives of this topic is discussed in the present review. The better understanding of this subject would provide an algorithm, to optimize the integration of diagnosis, prognostics and therapeutics findings in HCM, leading to a tailored approach for this pathology.

2. INTRODUCTION

Hypertrophic cardiomyopathy (HCM) is a cardiomyopathy characterized by the presence of increased left ventricular (LV) wall thickness that is not solely explained by other cardiac or systemic diseases capable to induce LV hypertrophy (1, 2).

HCM is an inherited cardiomyopathy, being considered the most prevalent monogenic

cardiac disease (3). Few cardiac diseases are as heterogeneous as HCM. In fact, its timing of onset, phenotype and clinical course show extreme individual variability. Even within members of the same family, the reasons for such diversity are not completely understood and potentially range from genetic/epigenetic to environmental factors.

Although the majority of patients are asymptomatic, about 15–20% of HCM patients develop adverse remodelling and about 5–10% progress to an end-stage phase, characterized by severe functional deterioration of the LV (defined by a LV ejection fraction – LVEF <50%), extreme degrees of fibrosis, adverse remodelling, hemodynamic decompensation and poor outcome (4). The triggers of this process from stable HCM to this adverse phenotype remain unclear. The annual incidence for cardiovascular death in HCM is 1–2% as the consequence of heart failure, thromboembolism and sudden cardiac death (SCD), generally due to spontaneous ventricular fibrillation (5).

Traditionally, the focus of HCM literature has been polarized on the prevention of SCD, through the identification of factors associated with higher risk of ventricular arrhythmias (6). By comparison, limited attention has been devoted to the chronic evolution towards LV remodelling, dysfunction and heart failure (HF) (7–9).

For these reasons, one of the most important challenges in HCM management nowadays is the identification of theranostic biomarkers that could be useful for prematurely stratify patients with higher risk of evolution from a stable disease towards LV dysfunction and heart failure.

The purpose of the present review is to provide an overview of different biomarkers of HCM that could give some insights into the progression of the disease from the classical phenotype to overt dysfunction and failure.

3. CLINICAL PROGNOSTIC BIOMARKERS IN HCM

World Health Organization has defined a biomarker as “any substance, structure, or process that can be measured in the body or its products and influence or predict the incidence of outcome or disease” (10). Several biomarkers have been associated with adverse remodelling and outcome in HCM. These biomarkers could be classified in: clinical biomarkers, circulating biomarkers, structural and functional biomarkers. Functional and structural biomarkers are investigated using cardiac imaging techniques while circulating biomarkers are investigated with laboratory tests.

3.1. Structural, functional and circulating biomarkers

3.1.1. Structural biomarkers

The most important structural biomarkers in HCM are represented by indexes of LV hypertrophy, LV geometry, left atrial volume and myocardial fibrosis. The main theranostic implications of structural biomarkers consist in the identification of patients with high risk of progression toward end-stage disease, that must be benefited by therapies with specific effects on the prevention of heart failure or arrhythmias such as beta-blockers, ace-inhibitors or other anti-arrhythmic drugs (11).

In HCM, hypertrophy is generally asymmetrical (with wall thickness >15 mm) and affects predominantly the interventricular septum, although it may also be symmetrical or localized to the apex or other isolated LV segments. Echocardiography is very accurate in measuring LV wall thickness and HCM is defined by values of LV wall thickness ≥15 mm, not solely explained by abnormal loading conditions. The debate on whether the magnitude of LV hypertrophy influence the prognosis of HCM patients has produced conflicting results in the last 40 years. Although there is wide evidence that extreme LV wall thickness (≥30 mm) represents an independent predictor of SCD, nowadays the long-term prognostic role of LV hypertrophy in terms of mortality and evolution towards HF is still on debate (2). In fact, the degree of maximum LV wall thickness should be considered in the context of a multifactorial approach to better stratify the risk of HCM patients. Even *LV geometry* is altered in patients with HCM, ranging from concentric hypertrophy to spade-shaped cavity of apical HCM. Although generally LV volumes are normal or reduced in HCM, in a minority of patients the disease evolves towards severe remodelling of the LV with cavity enlargement and wall thinning. This adverse remodelling may evolve to overt systolic or diastolic dysfunction (or both) with the corresponding clinical presentation of HF with preserved or reduced ejection fraction (EF) (12).

Left atrial (LA) enlargement is common in HCM, both indexes of LA enlargement and function provide important prognostic information in HCM, including risk of atrial fibrillation and long-term adverse outcome (13, 14).

In addition to wall thickness and chamber size measurement, the characterization of myocardial tissue allowed by cardiac magnetic resonance (CMR) has a significant value in determining disease progression in HCM. Late-gadolinium enhancement (LGE) techniques identify patchy mid-wall replacement *fibrosis* in 50–80% of cases, correlating positively with regional wall thickness, and inversely with wall thickening and LV

systolic function (15). In patients who are asymptomatic or only mildly symptomatic, LGE represents an independent predictor of all-cause and cardiac mortality. When extensive (20% of the whole LV), LGE is an independent predictor of sudden cardiac death (16). The theranostic utility of the identification of myocardial fibrosis, the final histopathological evolution of many cardiac injuries, comprises the possibility to develop future specific therapies to contrast the intramyocardial fibrotic processes. Indeed, sustained progress in uncovering mechanisms of myocardial fibrosis has accelerated the clinical development of new several anti-fibrotic therapies (17).

3.1.2. Functional biomarkers

The most important functional biomarkers in HCM are represented by indexes of systolic and diastolic left ventricular function, LV outflow obstruction and coronary microvascular dysfunction (CMD). Measuring and following the evolution of functional biomarkers may help physician to better stratify patients that require drugs with positive effects on myocardial dysfunction, such as beta-blockers and ace-inhibitors (11).

Although LV *ejection fraction* (EF) is usually preserved or supernormal in classic HCM (with values > 70%), indexes of longitudinal LV systolic function are generally impaired (18). The most common LV dysfunction in HCM is represented by diastolic dysfunction, generally due to hypertrophy, elevated wall stiffness, elevated filling pressure and outflow obstruction (12). In particular, HCM is characterized by variable degrees of *diastolic dysfunction* that may be severe enough to cause symptoms of HF despite a normal EF. However, assessing diastolic function in cardiomyopathies is challenging. The best estimation originates from a combined measurement of mitral inflow velocities, tissue Doppler imaging, pulmonary vein flow velocities, pulmonary artery systolic pressure and LA size progression of diastolic dysfunction, as part of adverse remodelling over time. Diastolic function is strongly related to adverse outcome, particularly when a restrictive LV filling pattern becomes evident (4).

Dynamic *LV outflow obstruction* at rest is found in > 25% of HCM patients while in a proportion close to 40% a gradient ≥50 mmHg can be elicited with exercise or during provocative manoeuvres such as Valsalva (19). LV outflow obstruction is a major determinant of symptoms and it is associated with increased risk of heart failure-related complications and death (20).

In HCM, both severe structural remodelling of intramural coronary arterioles and increased perivascular fibrosis has been described. These structural changes, in addition to the increased extravascular compression, cause severe CMD (21). Within the septal myocardium, the small intramural coronary arteries showed variable degrees of medial hypertrophy-dysplasia and intimal hyperplasia and were closely related to fibrotic scars (22).

In most HCM patients, *microvascular function* and coronary reserve, assessed by both PET imaging or stress echocardiography, are blunted diffusely (12). The degree of coronary microvascular dysfunction is a powerful long-term predictor of adverse LV remodelling and systolic dysfunction, (23) as well as an independent predictor of death and unfavourable outcome in HCM (24–26). The detection of CMD provides unique theranostic information for the stratification of HCM, thereby suggesting the utility to treat patients with new drugs that improve myocardial blood flow and coronary flow reserve (27). Moreover, in patients with HCM, a diffuse down-regulation of myocardial beta-adrenoceptors and an increased concentration of catecholamine in the synaptic cleft have been found using PET imaging. Of note, these abnormalities correlate with the degree of LV dysfunction and may play a role in the development of end-stage of the disease (28). Further studies are warranted to understand whether this mechanism may represent a theranostic target in HCM.

3.1.3. Circulating Biomarkers in HCM

The most common circulating biomarkers used in the clinical practice to stratify the risk of progression from dysfunction to failure both in HF with preserved or reduced EF are natriuretic peptides and troponin levels. In HCM several studies have investigated the theranostic usefulness of these two biomarkers to identify the subgroup of patients with worst clinical decline and prognosis thereby providing novel monitoring parameters to evaluate the progression towards heart failure and the effects of specific drugs on the clinical course of the disease.

3.1.3.1. Natriuretic peptides: marker of myocyte stress in HCM

Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are stable peptides that are synthesized predominantly in the atria and left ventricle in response to elevated wall tension. Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) are biologically active peptides synthesized and released primarily from cardiac myocytes as a response to neurohormonal activation, myocardial stretch, and wall tension (29). Circulating plasma BNP levels are elevated in numerous cardiac pathologies in the presence of hemodynamic overload and increased cardiac fibrosis (30, 31). The use of natriuretic peptides is a well-established tool in the diagnosis, prognosis, and management of patients with HF and systolic dysfunction (32).

In HCM, plasma levels of ANP and BNP correlate positively with cardiac filling pressures, making them excellent markers for the presence of LV dysfunction and abnormal LV wall stress (33).

Various studies reported higher levels of ANP and BNP in HCM compared with normal subjects. Moreover plasma BNP levels correlated positively with symptoms of heart failure, severity of hypertrophy, Doppler echocardiographic signs of LV diastolic dysfunction, peak oxygen consumption and functional impairment (34–37).

BNP in obstructive and non-obstructive HCM is 85-fold and 23 fold elevated, respectively, compared with controls (38, 39).

Both BNP and ANP are significantly higher in the subgroup that shows evidence of obstruction, and both correlate positively with left intraventricular pressure gradient (38, 39).

Elevated NT-pro-BNP levels are associated with incipient LV remodelling and fibrosis assessed by cardiac magnetic resonance, and could be used to diagnose insidious unfavourable LV remodelling and higher risk of sudden death in HCM (40).

As prognostic factors, plasma levels of NT-pro-BNP and ANP are independent predictors of cardiovascular events in patients with HCM (41, 42). Geske *et al*. measuring BNP levels in 772 HCM patients, found that BNP is an independent predictor of morbidity and mortality (43).

3.1.3.2. Troponin: a marker of myocyte injury in HCM

Cardiac troponins, including cardiac troponin T and troponin I, are recognized as sensitive and specific markers of myocardial injury, being well-established diagnostic and prognostic markers in acute coronary syndrome. These troponins have been reported to predict adverse outcome in patients with HF even in the absence of coronary artery stenosis (44–46).

The mechanisms of myocyte injury in HCM are not completely understood. Abnormal elevation in troponin circulating levels may be caused by relative myocardial ischemia due to the imbalance between a hypertrophic heart and the insufficient coronary blood supply due to abnormal vessels (47). Moreover, myocyte abnormalities determined by gene mutation may directly cause myocyte injury (48). Troponins could be useful in determining the patients with higher risk to develop dilated phase of HCM and the effects of drug treatment on the evolution of HCM, but further studies are necessary.

Sato *et al*. comparing HCM patients with normal and altered troponin T levels found that higher levels of this molecule were associated with a significantly lower fractional shortening and thicker interventricular septum (49). Moreover, in the same study, 4 of 12 patients with increased troponin T experienced end stage dilated HCM, suggesting that an increase in troponin T concentration in HCM may be an indicator of subclinical myocyte injury and/or progression to dilated HCM.

Kubo and cols., following 183 HCM patients, during a mean follow-up of 4 years, found that an abnormal serum concentration of high-sensitivity cardiac troponin T is an independent predictor of adverse outcome with a linear correlation between high-sensitivity cardiac troponin T values and risk of cardiovascular events (50). The same group hypothesized, in a subsequent study, that both serum troponin T and plasma BNP might supplement each other to predict prognosis in HCM patients, describing these biomarkers as useful parameters to identify patients at risk for clinical deterioration (51).

3.1.3.3. Others circulating biomarkers

Higher levels of interleukin-6 were shown in HCM patients, compared with controls, even in the absence of other inflammatory conditions. A vicious circle has been proposed in HCM pathogenesis, by which LV hypertrophy promotes interleukin-6 release, thus promoting further hypertrophy (52). Moreover, patients with HCM exhibits increased levels of pro-apoptotic molecules such as tumour necrosis factor-α (TNF-α) and soluble Fas ligand, thereby suggesting the hypothesis that cardiomyocyte apoptosis might play a decisive role in promoting fibrosis and disease progression in HCM patients (52). In fact, marked increases in TNF-α have been reported in the end-stage phase of HCM (53). Finally, several metalloproteinases (MMPs), are increased in the serum of HCM patients, such as MMP-9 and MMP-3, respectively associated with LGE and increased arrhythmic risk (54).

Moreover, many of the recent studies are dedicated to identify circulating molecules in the biofluids that could depict genetic/epigenetic regulation of HCM pathological progression and onset. One example are the circulating microRNAs, which will be discussed in the following *epigenetic* section.

4. GENETICS OF HCM

4.1. HCM main mutations

HCM is a monogenic cardiac disease with an autosomal dominant pattern of heritability and different penetrance, with a prevalence in the general population of 1/500 (3). It is the most common genetic

Table 1. Main mutations described in HCM

Location	Gene Symbol	Encoded Protein	Pathogenicity	Incidence
Thick filament genes	MYH7	Beta-myosin heavy chain	++	25–35%
	MYH6	Alpha-myosin heavy chain	+	R
	MYL2	Regulatory myosin light chain 2	++	<5%
	MYL3	Essential myosin light chain 3	++	R
	TTN	Titin	+	R
Thin filament genes	TNNT2	Troponin T (myocardial)	++	3–5%
	TNNI3	Troponin I (myocardial)	++	<5%
	TNNC1	Troponin C (myocardial)	++	R
	TPM1	Tropomyosin 1-alpha	++	<5%
	ACTC	Alpha-cardiac actin 1	++	R
Intermediate filament gene	MYBPC3	Myosin-binding protein C (cardiac)	++	20–30%
Z-Disc genes	ACTN2	Alpha-actin 2	++	R
	MYOZ2	Myozenin 2	++	R
	LBD3	LIM domain-binding protein 3	+	R
	CSRP3	Muscular LIM protein	+	R
	TCAP	Telethonin	+	R
	VLC	Vinculin/Metavinculin	+	R
Calcium handling genes	JPH2	Junctophilin-2	+	R
	PLN	Phospholamban	++	R
	CASQ2	Calsequestrin	+	R

$^{++}$ greater or $^{+}$ minor evidence of pathogenicity; R: rare (<1%)

heart disease, generally caused by mutations in cardiac sarcomere genes that can be identified in over 50% of patients (55). There are many described familiar mutations causing cardiac hypertrophy. Worse cardiac function and prognosis are generally related to the mutated genes codifying proteins constituents of the sarcomere, and for this reason HCM has been described as a “sarcomeric disease” (56, 57).

Corroborating this concept, trials using high throughput sequencing assays reported the prevalence of sarcomeric genes variants in HCM (58) and related mutations account for about 60% of HCM incidence (59). Moreover, these mutations in sarcomeric proteins have been associated with HCM severity in different continental populations (60–62), highlighting the overall importance of its identification in genetic screening of HCM individuals.

The sarcomeric genes altered in HCM could be classified according the proteins codified by them in those comprised in the thick, thin or intermediate filaments of the sarcomere. However, beyond the sarcomere, there are a variety of genes that encode other proteins as those related to the Z-disc (63, 64) and intracellular calcium modulators (65–67) exerting a role in HCM. The Table 1 summarizes the main described genes and respective encoded proteins in HCM, as well as their pathogenicity and reported prevalence (55, 62, 68–70). Among them, two are worth of notice – the MYH7 (encoding the beta-myosin heavy chain protein) and the MBPC3 (encoding the cardiac myosin binding protein C) – found in approximately 75% of all HCM individuals genotyped (71). Interestingly, HCM patients with sarcomere mutations are also characterized by greater impairment of coronary flow reserve and prevalence of LGE, compared with genotype-negative patients, thereby suggesting a genetic regulation of arteriolar remodelling, what could decisively play a role in the adverse progression of this disease. (48) Moreover, genetic mutations enrolling the thin-filament in HCM patients were recently suggested as a possible cause for the evolution from left ventricular hypertrophy to failure. The individuals carrying these mutations presented a greater tendency to develop systolic dysfunction, enlargement of volumes and HF symptoms rather than concentric geometry with severe (restrictive) diastolic dysfunction (72). Also, those individuals carrying multiple mutations are significantly younger at diagnosis, at higher risk of SCD and development of greater LV hypertrophy, compared with single-mutation carriers (73).

However, these causal mutations described above represent one feature among others, as the final phenotype seems to be the consequence of

interactions between the causal genes, genetic background (modifier genes) and maybe environmental aspects (74).

4.2. Modifiers in HCM

HCM is a disease with a heterogeneous clinical manifestation, suggesting that there are a variety of aspects modifying the disease outcome. The genome-wide methods available nowadays have allowed to integrate factors that already are known to affect the phenotype, which enhances the ability to estimate the location and effect of additional modifiers. The "modifiers", as common polymorphisms or founder mutations, raised a great interest in HCM, especially because they could affect its phenotypic expression and severity, such as the extent of hypertrophy and the risk of SCD.

One of the most important polymorphisms reported are these related to renin-angiotensin-aldosterone system (RAAS), which can alter the severity and phenotypical expression of HCM. In this context, former studies demonstrated that the combination of RAAS polymorphisms and MYBPC3 genotype resulted in an increased pro-left ventricular hypertrophy effect in HCM patients (75, 76). Gender related polymorphisms may also play a role in modifying HCM severity, which could explain the differences observed in HCM phenotype between men and woman (77). To illustrate this, Lind and cols. described the relationship between genetic variation in sex hormone receptors and LV hypertrophy in Australian HCM individuals (78). In this study both the reduced CAG-nucleotide repeats within the androgen receptor and male carriers of the A allele (SNP rs6915267) located in the promoter region of estrogen receptor 1 gene were significantly associated with enhanced LV hypertrophy.

The theranostic impact of the genetic modifiers on manifested phenotype would be useful to stablish risk stratification and clinical management of HCM patients.

4.3. Implications of genetic testing in HCM

With the growing importance of genetic screening in the clinical practice and patient management, the expansion of this technique is progressively occurring. The limitations could be attributed to its high cost, the complex analysis of the upcoming results and the poor knowledge regarding the related phenotype association to the genetic data obtained. However, nowadays the importance of genetic diagnosis in HCM families is undeniable and, the recent development of new technologies are leading to reduce the costs and facilitating data interpretation. The expansion of genetic testing contributes effectively to expand the comprehension of genetic mechanisms involved in HCM phenotype and would enable early identification, treatment and surveillance of relatives at risk (79). Moreover it would provide tools to refine the criteria of patient stratification, genetic counselling, future development of personalized therapies. In this regard, the identification of more specific therapeutic approaches directed to the underlying mechanisms, leading to molecular disturbances seen in HCM, would promote a revolutionary progress in its treatment and prevention of adverse outcomes.

The rising availability of genetic screening of HCM patients and relatives permitted also the identification of a new subset of subjects carrying a mutation for HCM but with normal phenotype. They were so called: ''genotype positive-phenotype negative'' HCM (80). There is a lack of information in this group regarding the risk of sudden cardiac death, the consequence of physical activities and the risk factors for progression to clinical manifested disease. Nevertheless, this is a challenging topic, since this subgroup of HCM individuals is "at risk" of developing the clinical phenotype and adequate surveillance, counselling, preventive measures and therapeutic interventions should be early considered. Previous reports reported abnormal modifications in the myocardium of this group of individuals, but whether these changes are enough to result in clinically evident adverse effects remains unclear. It was already described that the development HCM phenotype habitually arises during the second decade of life, but it remains uncertain how it progresses in individuals carrying HCM-associated mutations (4). Therefore this group could represent an open field for further studies in order to identify targets to future genetic and molecular therapies, especially directed to those with potential trigger mechanisms to bring HCM from genotype to phenotype.

In addition, one of the aims of genetic screening of patients with HCM is to differentiate the individuals with HCM derived from sarcomeric mutations and those considered "HCM phenocopies", which comprise individuals with several cardiac or systemic diseases that can mimic HCM features. These can include a variety of metabolic and mitochondrial diseases, in addition to syndromes and rare diseases. The correct differential diagnosis of those patients allow the adequate prognosis, follow up, clinical supervision and specific therapies of such different pathological panoramas (68).

Moreover, considering the epidemiologic aspect of all clinical and genetic studies available in the actuality, the prevalence for HCM, initially established in 1:500 in the CARDIA study (3), could be revised including those individuals with clinical manifested HCM and those "at risk" – carrying HCM genes but without developing the clinical phenotype. It would allow to increase the awareness in cardiology

practice, leading to enhancement of diagnosis and early therapeutic intervention – thus reducing HCM related mortality (81).

In addition, one of the great challenging coming from genetic studies is to develop novel genetic therapies in HCM, targeting gene mutations or particular genotypes, as discussed further.

4.4. Gene therapy in HCM

Regarding gene therapy in HCM, there is a long way ahead before finding a feasible approach. Nowadays there are no genetic therapies able to reverse or prevent HCM in humans. Most of clinical trials for gene therapy do not comprise patients with HCM. One of the reasons is that gene therapy has a more pertinent application for recessive inherited pathologies associated to mutations and reduced or lacking of enzyme function (55). Furthermore, according the north American and European register of clinical trials (82, 83) the majority of the current genetic studies in HCM are observational, looking towards to delimit the prevalence of some mutations and to define which of these are associated to disease outcome or using genetic data to select patients for drug tests. This is the case of the DELIGHT trial (DiltiazEm Long-term In Genotype-positive Hypertrophic cardiomyopathy as preclinical treatment), which demonstrated that pre-clinical administration of diltiazem (an calcium channel blocker) may prevent the development of hypertrophy in HCM patients with positive genotype (84).

Moreover, using the findings coming from genetic studies in humans, transgenic animal models hosting diverse mutations – mimicking the human genotype/phenotype - were developed to investigate the underlying mechanisms of HCM (85–87). It provides an important tool to investigate the effects of pharmacological treatments on specific mutations related to HCM. As an example, the treatment of mouse model of human HCM (cardiac troponin T - cTnT-Q^{92}) with the angiotensin II blocker losartan prevented the development of cardiac interstitial fibrosis.(88) Using the same mouse model, Tsybouleva *et al*. reported the central role of aldosterone in the transition from sarcomeric mutations to cardiac phenotype in HCM, describing new molecular mechanisms enrolled in hypertrophy and fibrosis as well as the efficacy of mineralocorticoid receptor blockade to revert HCM cardiac manifestation. (89) Other studies demonstrated that, in a transgenic rabbit model of human HCM, both simvastatin and atorvastatin, 3-hydroxyl-3-methylglutaryl coenzyme A reductase inhibitors, were able to reduce septal and posterior wall width, collagen accumulation, LV mass and ameliorated LV filling pressure (90, 91). However, a clinical test, treating HCM patients with statins during 9 months, was not able to confirm these findings (92).

The studies in animal models provide data that could be used as base for future clinical trials in order to confirm the use of personalized pharmacological treatments depending on the genetic profile in HCM patients.

On the other hand, data coming from genetic studies lead to the development of promising *in vitro* studies, which could provide some insights about future possibilities for HCM gene therapy. Patient-specific induced pluripotent stem cell cardiomyocytes (iPSC-CMs) were developed to elucidate the mechanisms underlying HCM development. Lan *and cols.* Described iPSC differentiated cardiomyocytes derived from HCM patients carrying one specific mutation (missense mutation - Arg663His) in MYH7 gene. This study reached interesting results regarding pharmacological modulation of calcium homeostasis leading to a protective effect on hypertrophic and electrophysiological abnormalities. (93, 94). But the main outcome could be considered the creation of HCM – IPSC cardiomyocytes itself, that provides an important tool for the identification basal mechanisms of and new therapeutic approaches for future studies in HCM. Following this idea, another study using IPSc cardiomyocytes from HCM patients carrying another mutation, in Arginine442Glycine - in MYH7 gene, was related to structural and electrophysiological alterations, sensitive to pharmacological treatments. (95). Despite these iPSCs cardiomyocytes derived from HCM patients present some limitations, such as immaturity, it raised a great interest as an experimental approach. In this regard, there are companies and institutions committed to develop Bio-banks dedicated to human iPSC cardiomyocytes derived from HCM patients. (96, 97) This biotechnological approach provides an important pre-clinical model for translational research in HCM and identification of new therapies and drug screening.

Moreover, there are some first insights in gene therapy designed for HCM *in vivo*. One example is the study performed in homozygous *Mybpc3*-targeted knock-in (KI) mice, mimicking severe neonatal HCM. In this study, Mearini and colleagues demonstrated that gene therapy, using adeno-associated virus (AAV9)-Mybpc3, could prevent both neonatal cardiomyopathy as well as long-term disease manifestation. (98) Moreover, MYK-461 – a novel small-molecule inhibitor of sarcomere power – was recently described as being protective in a mice model of HCM. Early administration of MYK-461 in mice expressing alpha-cardiac myosin heavy chain missense mutations (R403Q, R719W, or R453C) inhibited hypertrophic and pro-fibrotic gene programs. Moreover this treatment attenuated the development and progression typical histomolecular changes of HCM.(99) The same substance is now being tested in a clinical trial (phase 1).(100) Taking

together, these studies would provide an interplay between genetic observations and therapeutic interventions, finding new targets to prevent the adverse progression of HCM.

Although the results coming from pre-clinical models give significant insights in the basal mechanisms of HMC as well as new interventional approaches, their applications remain inconclusive and further studies are necessary to construct the translational bridge from bench to bedside. In brief, the research of genetics in HCM has reached many relevant therapeutic direct and indirect approaches in HCM. Nevertheless, the genetic profile is not sufficient to justify HCM development and phenotype/transcriptional profile. Epigenetic modifications and protein post.-translational modifications may play a crucial role in triggering HCM phenotype, adverse outcomes and progression to heart failure. In the next section will be dedicated to analyse how epigenetics is involved in HCM disease.

5. EPIGENETICS OF HCM

One of the most striking features of the HCM is the heterogeneity of the symptoms, clinical course and cardiac phenotype of patients carrying the same mutations. (101, 102). The clinical outcome of HCM seems to be the result of the influence of genetic mutations, detrimental effect of age in protein-quality control process as well as environmental factors (lifestyle, diet, reduced physical practice) and clinical parameters such as blood pressure.(102).

Moreover, epigenetic processes would account for the modifications contributing in the development of HCM. They include all the inherited processes that regulate gene expression without altering the genomic sequence. Major epigenetic mechanisms described are DNA methylation, phosphorylation, modifications of histone proteins and transcription regulation by small noncoding RNAs (microRNAs or miRNAs). In the current review, selected epigenetic processes will be highlighted focusing on their role in the context of HCM.

5.1. Histone modification

The manipulation of chromatin structure, via nuclear DNA packaging, is a key process to regulate the access to genetic information. Histone proteins are able to interact with DNA, maintaining the chromatin in a condensed/silenced or relaxed/active state. Histone modifications are responsible of the chromatin accessibility to transcription. Some of the molecules involved in the process of histone modification were found altered in HCM. In this context, two different opposite effects have been described for histone deacetylases (HDACs) members of class II. The HDAC2 activation by phosphorylation in response to hypertrophic stimulus has shown a pro-hypertrophic effect (103). Conversely, HDAC5, belonging to Class IIa HDACs, negatively regulates HCM by deacytilating HDAC2 (104, 105). Other factors as p300 and p300/CBP-associated factor (pCAF) were associated to cardiac hypertrophy and transcriptional activation of heart specific genes. (106) However, their role in HCM remains unclear.

Moreover, Brg1, a chromatin-remodeling protein, was also described as potentially enrolled in HCM outcome. In patients presenting enhanced levels of Brg1, it positively correlated with the severity of HCM symptoms and myosin heavy chain alterations. It was also reported that Brg1 interacts with HDACs and poly (ADP-ribose) polymerase-1, and, considering its activation in HCM patients, Brg1/Brm-associated-factor could be a target for the treatment of cardiac hypertrophy.(107)

5.2. DNA Methylation /demethylation

DNA methylation is another epigenetic mechanism that, altering chromatin structure, regulates gene expression. DNA methylation has been described in HCM on cardiac troponin C DNA sequence, where a cluster mutation in exon 8 and 9 involved C->T transition within CpG dinucleotide and lead to the creation of a genomic instable region.(108) High methylation level of CpG islands has been described also in an important causal gene of HCM, the myosin binding protein C3 (MYBPC3), supporting a mutagenic mechanism via deamination of the methylated CpG, which results in the development of a transition mutation from a cytosine to a thymine. (109). There are other examples illustrating the role of methylation in pre-clinic models of HCM. Changes in expression of DNA methylation genes DNMTs and Tet methylcytosine dioxygenase (Tets), induced by a cAMP analogue, lead to a global DNA methylation increase in adult mouse atrium cardiomyocytes and results in the downregulation of several HCM genes (*Myh7*, *Gata4*, *Mef2c*, *Nfatc1*, *Myh7b*, *Tnni3* and *Bnp*)(110).

Also demethylation coming from JMJD2A, a histone trimethyl demethylase, was found enhanced in cardiac tissue from HCM patients underwent myectomy. In the same study, JMJD2A was described to promote hypertrophic response to stress and to downregulate H3K9 trimethylation, leading to activation of pro-hypertrophic genes in mice with aortic constriction–induced pressure overload. Thus JMJD2A could be speculated as a drug target for transcriptional therapy against cardiac hypertrophy and heart failure. (111)

Regarding phosphorylation, when this process occurs in serine 10 (H3S10) of Histone H3, it is usually a mark associated to mitosis and chromosome condensation.(112) H3S10 phosphorylation occurs

transiently after stress by the activity of calcium/calmodulin-dependent protein kinase II δ (CaMKIIδ), and does not involve all H3 histones.(113) CaMKII signaling was largely described as contributor to cardiac hypertrophy and having a role in the progression of heart disease.(114) CaMKIIδ is the principal isoform in the heart, regulating the phosphorylation of histone H3 at serine-10 in hypertrophy. This last was shown to reactivate a program of fetal cardiac genes, suggesting an epigenetic mechanism by which CaMKIIδ controls cardiac hypertrophy and with prognostic interest to development of heart failure. Further studies are needed to ascertain the role of these mechanisms in HCM, despite it has been previously demonstrated in mice with pressure overload and humans with advanced stage of heart failure.(115, 116) In this context, the effect of CaMKII-dependent signaling was reported in isolated cardiomyocytes from HCM patients. Increased CaMKII activity and phosphorylation of targets lead to alteration of ion channels function, culminating in prolonged action potential as well as increased cellular arrhythmias. (117) It supports the speculation that CaMKII, due its multifaceted effects - promoting epigenetic alterations, beyond to be enrolled in sarcomeric mutations, participating also in mechanisms underlying electrophysiological abnormalities found in HCM – could represent a novel theranostic target in HCM.

5.3. microRNAs

5.3.1. MiRNAs in HCM

In the last ten years, microRNAs (miRNAs), small noncoding endogenous RNA, have emerged as important regulators of the heart physiopathology. MiRNAs are small (20–22 nucleotides in length) non-coding RNA transcribed by RNA polymerase II and III from different genomic regions, generally in intronic ones. Their post-translational regulation of protein expression is the result of miRNA-mediated gene silencing – due degradation or translational repression. MiRNAs have been increasingly recognized as an important class of regulatory small noncoding RNAs, both in physiological and pathological conditions in the heart.(118, 119) Their function as negative regulators of gene expression acting in a complex functional network in which each single miRNA is able to control thousands of direct target genes, and each single mRNA coding sequence could be controlled by many different miRNAs.(102) Due to the diverse underlying mechanisms of HCM outcome, beyond clinical and genetic analysis, transcriptome and miRNome profiles seem to be important tools in the molecular analysis to unveil the triggers or modulators of HCM. However, the morphological heterogeneity and incomplete penetrance of this pathology render difficult the precise description of the progression by miRNA profiling and the establishment of an analogy between mutations and miRNA expression. Nevertheless, several attempts have been made to propose single or groups of miRNAs having a role as regulators of cardiomyocytes hypertrophy or fibrosis observed in HCM.(120, 121) These profiles expressed the alteration of miRNAs observed in tissues of both animal models and patients with HCM. There are a variety of miRNAs described as modulators of the hypertrophic pattern in the heart. However, even sharing some common features between diverse cardiac maladies presenting cardiac hypertrophy, the focus of the current section will be directed to miRNAs reported in HCM – in both human or animal models.

In this regard, Bagnall and cols. performed an elegant study in which they observed the miRNA profile during the development of hypertrophy and failure in a murine double mutant model of severe HCM (crossbreeding mice with the HCM-causing mutations Gly203Ser in cardiac TnI-203 and Arg403Gln in alpha-myosin heavy chain - MHC-403).(120) The phases were divided in asymptomatic, mildly asymptomatic and overt HCM. Interestingly, mir-1 was downregulated whereas mir-21 was upregulated in all stages of HCM development in this mouse model. Moreover, both miRNAs were reported in a similar pattern also in human heart HCM (122, 123). In addition, significantly increased levels of mir-21 was found in the plasma of HCM patients (124), but lacking significant correlation with hypertrophy.

It is worth of notice that miR-1 and miR-133, belonging to the same transcriptional unit, have been found downregulated in mice and human profiles in HCM.(120, 123) Furthermore, a former study described the central role of miR-133 and mir-1 as controllers of cardiomyocyte hypertrophy, both in murine model and in cardiac tissue from HCM patients. In the same study, *in vitro* and *in vivo* assays demonstrated that overexpression of both miR-133 or miR-1 inhibited cardiac hypertrophy and conversely reduced levels of miR-133 stimulated an important cardiac hypertrophic pattern.(125). However, increased levels of miR-1 and miR-133 were detected in the circulation of HCM patients.(124, 126) A possible explanation for this inverse pattern would be attributed to cardiomyocyte death due ischemic episodes (127) that are possible to occur during the whole HCM patient's lives.(128) This fact remarks the importance of larger studies and accurately interpretation of data, because circulating microRNA levels not always reflect tissue levels.

Song and collaborators have reported miR-451 as negative modulator of cardiac hypertrophy in HCM. (122) Another study demonstrated the role of miR-195 and miR-451 in HCM development, by targeting MO25 in the LKB1/ adenosine monophosphate-activated kinase signaling. Moreover, in transgenic mice, the solely cardiac-specific overexpression of miR-195 was sufficient to induce cardiomyopathy.(129) This

regulatory pathway could become a therapeutic target in HCM.

For miR-221, a specific role in HCM has been demonstrated. It was found upregulated in left ventricular tissue from HCM patients, and, in the same study, it was shown to increase myocyte cell size in rat cardiomyocytes under pressure overload hypertrophic stimulus, probably downregulating p27, and inducing the re-expression of fetal genes, leading the authors to suggest miR-221 as a target for treatment of cardiac hypertrophy.(130)

Several other miRNAs have been profiled in HCM carrying MYBPC3 mutation compared with hearts of non-failing donors and revealed the downregulation of miR-34*, miR-96, miR-181a-2*, miR-184, miR-204, miR-222*, miR-371, miR-383, miR-497, miR-708, and the upregulation of miR-10b/b*, miR-10a*. (131) In the same study, the analyses on the predicted targets of these miRNAs revealed that the beta-adrenergic or cardiac hypertrophy signalling pathways could be involved in the HCM phenotype. This result suggested that altered miRNA expression could contribute to desensitization of the beta-adrenergic response which was consistent with the observed reduction in phosphorylation/activation of proteins that are target of beta-adrenergic receptor (i.e. cMyBP-C and cardiac troponin I). (132, 133)

Despite the differences and limitations observed when comparing animal models and human trials, there are some miRNAs similarly regulated in both profiles. This is the case of miR-21, miR-122, miR-132, that showed the same upregulation and conversely the downregulated miR-1, -30b, -133b and -150. (118).

Cardiac contractility depends on the expression of two myosin heavy chain (MHC) genes *alpha* and *beta, often described as altered in HCM.* In this regard, some miRNAs called “MyomiRs”, which are encoded and co-expressed by myosin genes, were described as enrolled in the control of myosin content, myofibril identity and muscle performance. (134) One of these is miR-208a, that was reported as a cardiac-specific microRNA, encoded by an intronic region of the alpha-MHC gene (fast, Myh6). This miRNA is essential to electric conduction (135), development of cardiac hypertrophy, fibrosis and beta-MHC (Myh7) expression in reaction to stress and hypothyroidism in mice.(129) Moreover, the inhibition of miR-208a in Dahl Salt rats suppressed pathological myosin switching and cardiac remodeling, improving survival and cardiac function.(136) In the same study, plasmatic miR-423–5p, which has been described as a circulating biomarker for human heart failure (137), was reduced in animals treated with antimiR-208a. Moreover, miR-208a plays a central role for the expression of Myh7 and also of its isoform - Myh7b - in the mouse heart. This last, Myh7b, encodes miR-499 and also slow myosin, both in cardiac and skeletal muscle. (134) It was demonstrated that miR-499 is increased in human and murine cardiac hypertrophy and cardiomyopathy, and its solely overexpression is sufficient to cause murine heart failure, exacerbating maladaptation due to pressure overload.(138) There is a fine interplay between miR-208a and miR-499, as miR-208a is required for expression of Myh7b/miR-499 and miR-499 can replace the cardiac functions of miR-208a in case of its absence. (134) This crossregulation was also reported in miR-208a–mutant animals, in which the combined reduction in miR-208a and miR-499 was considered a mark of antimiR-208a efficacy.(136) Another MyomiR is miR-208b, which is encoded by an intronic region of cardiac Mhy7 (slow, beta-MHC). It was reported that miR-208b presumably would increase during cardiac hypertrophy, because miR-208a and miR-208b share very similar sequences - which differs by only 2 base pairs in the targeted region- and can suppress same target genes.(135, 136) Despite the importance of MyomiRs in cardiac hypertrophy, there is a lack of information about their role in HCM.

5.3.2. Circulating miRNAs in HCM

Human tissue samples, derived from cardiac myectomies or explanted hearts, have been used to identify changes in miRNA profile. As myocardium is composed by several cell types other than cardiomyocytes, we cannot exclude the possibility that changes in cell composition (e.g. increase number of fibroblasts) between HCM and donor group might influence the identified miRNA signature. To select “pure myocardium samples” and perform differential analysis, modern techniques, as laser capture microscopy, should be used. These limitations reinforce the use of circulating miRNAs as new biomarkers for HCM diagnosis. Indeed, circulating miRNAs are potentially suitable for use as clinical biomarkers, as some miRNAs are secreted and can be found conserved in circulating biofluids (especially serum and plasma). This feature allows to access relevant biological information coming from circulating miRNAs through minimal-invasive procedures (blood withdraw) and rapid, specific and sensitive detection of these molecules, as they present a long half-life within the sample.(139) Actually, degradation of small RNA sequences is circumvented by packaging miRNAs in microparticles (exosomes, microvesicles, and apoptotic bodies) or by binding to proteins (i.e., Ago2) or high-density lipoproteins (HDLs). (140) The stability of miRNAs in the circulation raises also the intriguing possibility that they could be taken up by surrounding cells to regulate their gene expression. It is speculated that cardiomyocytes and fibroblasts under stress may send signals to other cell types and induce the release of miRNAs from other cells into circulation.

Table 2. Circulating miRNAs in HCM

Circulating miRNA	Suggested clinical usefulness	Reference
miR-27a miR-199a-5p miR-29a	Diagnosis of hypertrophy Diagnosis of hypertrophy/fibrosis (myocardial remodelling)	(124)
miR-10b-5p miR-146a-5p miR-15a-5p miR-17–5p miR-18a-5p miR-19b-3p miR-21–5p miR-29a-3p miR-30d-5p miR-133a-3p miR-193–5p miR-192–5p miR-200a-3p miR-296–5p	Diagnosis of fibrosis	(142)
miR-29a and miR-155 miR-29a miR-155	Differential diagnosis of HNCM vs. senile cardiac amyloidosis Diagnosis of HOCM/cardiac hypertrophy (higher levels in individuals carrying MYH7 mutation) Diagnosis of HCM (lower levels in individuals carrying MYBPC3 mutation)	(143)

HCM, hypertrophic cardiomyopathy; HOCM, hypertrophic obstructive cardiomyopathy; HNCM, hypertrophic non-obstructive cardiomyopathy

In addition, miRNAs play a role in the intercellular communication processes either through directly action as paracrine signals or by modulating downstream intercellular signalling mediators. (141)

Analysing circulating miRNAs in HCM, Roncarati and co-workers identified 12 miRNAs (miR-21, miR-26a, miR-27a, miR-29a, miR-30a, miR-126–3p, miR-133a, miR-143, miR-145, miR-155, miR-199a-5p, and miR-199a-3p) increased in plasma from HCM patients. However, only 3 miRNAs (miR-27a, miR-29a, and miR-199a-5p) correlated with hypertrophy. Among these, only miR-29a was significantly associated with both hypertrophy and fibrosis, being considered as a potential biomarker for myocardial remodelling assessment in HCM (124).

In a recent study analysing human circulating miRNA profiling in a cohort of 56 HCM patients reported that 14 miRNAs (miR-18a-5p, miR-146a-5p, miR-30d-5p, miR-17–5p, miR-200a-3p, miR-19b-3p, miR-21–5p, miR-193–5p, miR-10b-5p, miR-15a-5p, miR-192–5p, miR-296–5p, miR-29a-3p and miR-133a-3p) were correlated with T1 from cardiac magnetic resonance, a parameter associated with cardiac diffuse fibrosis. (142) These miRNAs were upregulated in HCM patients with T_1 < 470 ms, in contrast to circulating markers of collagen turnover that were not able to predict diffuse myocardial fibrosis. This suggests that miRNAs are molecules suitable to be used as circulating biomarkers for cardiac fibrosis in HCM.

Derda and colleagues studied 8 miRNAs with previously reported cardiovascular implications (miR-1, miR-21, miR-29a, miR-29b, miR-29c, miR-133a, miR-155 and miR-499) in serum of HCM patients. MiR-29c was upregulated in aortic stenosis, while miR-29a and miR-155 expression levels are able to discriminate hypertrophic non-obstructive patients from those with senile cardiac amyloidosis. The expression of miR-29a was found increased in hypertrophic obstructive cardiomyopathy patients with a mutation in MYH7, correlating with markers of cardiac hypertrophy. Conversely, miR-155 was decreased in HCM patients carrying a MYBPC3 mutation. (143)

Those differences between miRNAs, depending on patient clinical features or carried mutations, raise the possibility of being used as differential biomarkers in HCM. The Table 2 recapitulates the some miRNAs reported in the blood stream of HCM patients and suggested clinical applications. Particularly miR-29a (in bold) is present in all studies comprising different geographic populations and methodologies of detection, highlighting the importance of this miRNA as clinical biomarker in HCM.

The findings on circulating miRNA profiles are essential to HCM research, even if additional studies are required to expand the feasibility of this method for biomarker's screening and to identify specific molecules to differentiate the early stages of HCM, allowing the implementation of therapeutic and preventive measures to mitigate its progression.

5.3.4. Potential miRNA based therapies in HCM

Besides being potentially informative for increased insight in the mechanisms underlying and sustaining HCM disease, miRNAs represent prospective targets for therapy. The described features of miRNAs make them suitable targets for silencing

Table 3. Main approaches of miRNA-based therapies in models of cardiac hypertrophy

miRNA	Approach	Model	Results	Reference
miR-1	Adenoviral construct to increase expression	male Sprague-Dawley rats underwent ascending aortic banding	Reduction of cardiac hypertrophic phenotype	(148)
miR-21*	Antago-miR	Angiotensin II-induced cardiac hypertrophy in mice	Reduction of heart hypertrophic phenotype	(149)
miR-21	Antago-miR	Heart failure in mice (TAC)	Reduction of interstitial fibrosis and improvement of cardiac function	(145)
miR-132	Antago-miR	Heart failure in mice (TAC)	Rescue of heart hypertrophic phenotype	(144)
miR-133a-2 precursor sequence	Adenoviral construct leading overexpression	AKT induced heart hypertrophy in mice	Decrease of cardiac hypertrophic phenotype	(125)
miR-133	Antago-miR	C57BL/6 mice	Repression of HCM phenotype	(125)
miR-208	Plasmids and Transfection Assays	Hypertrophy in KO Mice (TAC)	blockade of β*MHC* expression, reduced cardiac hypertrophy and fibrosis	(152)
miR-208a	Locked-Nucleic Acid (LNA) Antago-miR	Diastolic heart failure in Dahl-salt rats	Reduction of cardiac remodelling	(136)
miR-378	locked nucleic acid (LNA)-antimiR-378	Hypertrophy in Mice (TAC)	Reduction of cardiac hypertrophic phenotype	(150)
miR-451	Mimic (overexpression)	HeLa cells and neonatal cardiomyocytes (rats)	Suppresses to the development of HCM	(122)
miR-652	locked nucleic acid (LNA)-antimiR-652	Hypertrophy in Mice (TAC)	Reduction of cardiac hypertrophic phenotype	(151)

TAC, Transverse *aortic* constriction.* miRNA passenger strands

by antisense oligonucleotides or by restoring their function - through the use of synthetic double stranded miRNAs or viral vector based overexpression.

The reported HCM therapeutics based on miRNAs involves the modulation of expression of these noncoding RNAs experimental assays. In cells, it is carried out by inactivating the function of pro-hypertrophic miRNAs using synthetic miRNA inhibitors or by using oligonucleotides that mimic miRNA sequence to synthetically induce anti-hypertrophic miRNAs.

The possibility of the use of miRNAs based therapy in treatment of HCM is highlighted by several *in vivo* studies (125, 144) that were able to inhibit cardiomyocyte hypertrophy and fibrosis (Table 3). Moreover, other models of cardiac hypertrophy are also described, highlighting some mechanisms that could be shared and of interest for HCM management.

With the purpose to study the role of miR-133 in the HCM, Carè and co-workers (125) successfully used a miR-133 RNA-mimic sequence within an adenoviral vector construct and an antagomiR in the hearts of a mouse model of AKT induced HCM and in a normal C57BL/6 mouse line, respectively. Similarly, Thum and collaborators (145) and Ucar *et al*. (144) were able to inhibit cardiomyocyte hypertrophy using an antagomiR for miR-21 and for miR-132, respectively, in mice models of heart failure induced by pressure overload. An antimiR for miR-208a was successfully used to reduce miR-208a expression in a rat model of diastolic heart failure.(136)

Despite the promising use of miRNA-based therapies, modulation of the function of miRNAs raises several concerns. The uptake of miRNA-mimics can result not only in the restoration of miRNA function in affected cells but also in the overexpression of the miRNA in other cells. In the same way, the inhibition of miRNA activity by antimiRs can be done on off-target locations. Therefore, targeting would be important in order to upgrade miRNA therapy, which could be accomplished by nanotherapy approaches.(146) Furthermore, another pitfall to be considered for the miRNA-based therapies is the creation of unwanted side effects due to the distribution of miRNA in tissues, other than the cardiac tissue. This problem could be partially overcome by the use of lenti-, adeno-, or adeno-associated viruses.(147) As an illustrative example, adenoviral-based therapies with miR-1 resulted in in rat model decreased cardiac fibrosis, apoptosis and improved cardiac function.(148) Indeed, due to the suppressing effects of miR-1 overexpression on pro-hypertrophic IGF-1 signalling, the restoration of miR-1 expression levels in HCM patients would be helpful in reverting HCM phenotype and preserving heart from remodelling.

Considering the important aspect of fibrosis development in HCM, the efforts to modulate

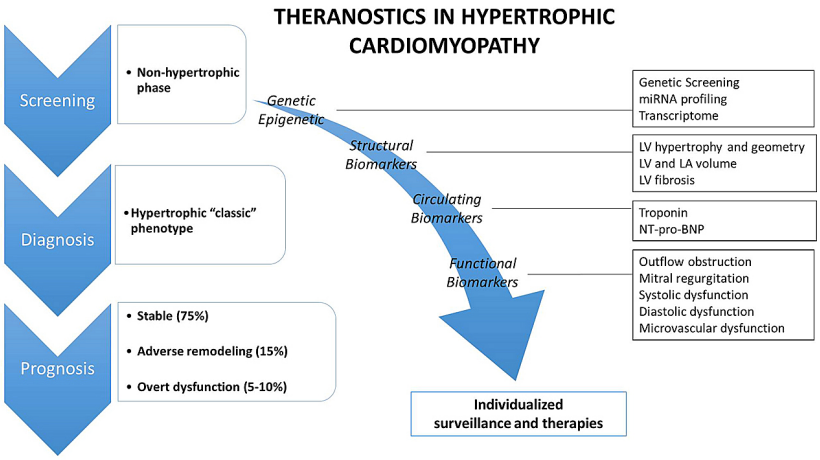

Figure 1. Proposed theranostic approach for tailored treatments and surveillance of HCM patients from pre-clinical to end-stage phase of the disease.

miRNAs regulating this process could be a novel therapeutic approach. Another possible tactic is to inhibit the microRNAs that are actively secreted by fibroblast in the HCM septum tissue. In fact it has been proposed that also miRNA passenger strand, usually indicated with miRNA-xxx*, could have a role in the paracrine communication between fibroblasts and cardiomyocytes. In this context, miR-21*, which is usually degraded, has been identified in HCM fibroblast-derived exosomes, and pharmacological inhibition of miR-21* in a mouse model of Angitensin II-induced cardiac hypertrophy attenuated the pathology. (149)

To increase the binding affinity of miRNA sequence and the stability of the sequence, locked nucleic acid-modified (LNA)-antimiR has been used. *In vivo* approaches revealed that LNA-antimiR-378 is able to reduce development of cardiomyocytes hypertrophy and fibrosis in mouse hearts. Mechanistically, miR-378 depletion was found to induce the expression of transforming growth factor beta 1 (TGF-β1) in mouse hearts and in cultured cardiomyocytes. Among various secreted cytokines in the conditioned-media of miR-378-depleted cardiomyocytes, only TGF-β1 levels were found to be increased, and this effect was prevented by miR-378 expression. Interestingly, treatment of cardiac fibroblasts with the conditioned media of miR-378-depleted myocytes activated pSMAD2/3 and induced fibrotic gene expression.(150)

In another example, administration of a locked nucleic acid (LNA)-antimiR-652 (miR-652 inhibitor) in HCM model of mice subjected to transverse aortic constriction (TAC) improved heart function associated with reduced cardiac fibrosis, apoptosis and B-type natriuretic peptide gene expression concomitant with preserved angiogenesis.(151) MiRNA-based therapeutics have not yet reached clinical trials for cardiovascular disorders, but the promising results in numerous animal models of hypertrophy suggest that it could be considered soon.

In cardiac tissue from HCM patients, miR-451 was reported as the most downregulated miRNA. Overexpression of miR-451 in HeLa cells and neonatal cardiomyocytes was able to regulate cardiac hypertrophy and cardiac autophagy (autophagosome formation), a feature observed in HCM development. This effect is explained because miR-451 targets TSC1 (a positive regulator of autophagy) and therefore could be speculated as a therapeutic target for this disease.(122)

In brief, the study of miRNAs expression profiles would allow the development of future approaches for diagnosis, prognosis and therapeutics in HCM patients. As described above, single miRNAs have been proposed as diagnostic biomarkers of HCM and used for the development of miRNA-based therapies on HCM models. Accumulated evidence suggests that microRNAs are essential regulators of cardiac remodeling. However, further studies are required to confirm its applicability and relevance in humans beings.

In the Figure 1 a scheme is presented as a suggested workflow to address HCM approaches in the clinical practice. Noteworthy is that genetic/ epigenetic studies present also the possibility of comparison of circulating molecules in the blood stream (serum/plasma) with those findings in cardiac tissue. This would provide a qualitative improvement in the interpretation of biological data. For this purpose, efforts should be directed to arrange all ethical aspects to create biobanks of both body fluids and heart tissue making feasible the construction of larger cohorts of patients in consistent studies of mechanisms involved in the onset and progression of HCM.

6. CONCLUSIONS AND PERSPECTIVES

Traditionally, the focus of HCM literature has been polarized on the prevention of SCD, through the identification of factors associated with higher risk of ventricular arrhythmias. By comparison, limited attention has been devoted to the life-long process of LV remodelling and progressive dysfunction that occurs in a substantial proportion of HCM patients and culminates in the end-stage or burned-out phase. For these reasons, nowadays one of the most important challenges in HCM management is the identification of theranostic biomarkers that could be useful for early stratification of patients with higher risk of evolution from a stable disease towards LV dysfunction and heart failure. In this context, genetics and epigenetics approaches available nowadays could offer the key to open the black box of HCM underlying mechanisms and provide important novel tools useful for the early diagnosis and development of tailored therapeutic interventions.

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Abbreviations: Hypertrophic cardiomyopathy (HCM), left ventricular/left ventricle (LV), ejection fraction (EF), sudden cardiac death (SCD), late-gadolinium

enhancement (LGE), coronary microvascular dysfunction (CMD), positron emission tomography (PET), single-photon emission computed tomography (SPECT), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), metalloproteinases (MMPs), renin-angiotensin-aldosterone system (RAAS), microRNAs (miRNAs), myosin heavy chain (MHC), beta-MHC (MYH7), alpha- MHC (MYH6)

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Send correspondence to: Davide Lazzeroni, Prevention and Rehabilitation Unit, Fondazione Don Gnocchi, Parma, Italy, Tel: 3905212054, Fax:+390521205441, E-mail: davide.lazzeroni@gmail.com