Journal of Molecular and Cellular Cardiology Plus 4 (2023) 100037

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology Plus

journal homepage: https://www.journals.elsevier.com/journal-of-molecular-and-cellular-cardiology-plus

# MicroRNA profiling of the feline left heart identifies chamber-specific expression signatures in health and in advanced hypertrophic cardiomyopathy

Jessica Joshua [a,b], Jeff L. Caswell [a], Josep M. Monné Rodriguez [c], Anja Kipar [c], M. Lynne O'Sullivan [d], Geoffrey Wood [a], Sonja Fonfara [b,*]

[a] University of Guelph, Ontario Veterinary College, Department of Pathobiology, Guelph N1G 2W1, Ontario, Canada
[b] University of Guelph, Ontario Veterinary College, Department of Clinical Studies, Guelph N1G 2W1, Ontario, Canada
[c] University of Zurich, Vetsuisse Faculty, Institute of Veterinary Pathology, Winterthurerstrasse 268, CH-8057 Zurich, Switzerland
[d] University of Prince Edward Island, Department of Companion Animals, Charlottetown C1A 4P3, Prince Edward Island, Canada

ARTICLE INFO

*Keywords:*
Cat
Cardiac
Left atrium
Left ventricle
Small RNA sequencing
Bioinformatics

ABSTRACT

Hypertrophic cardiomyopathy (HCM) is a common heart disease in humans and cats, nonetheless, the disease pathogenesis is still poorly understood. MicroRNAs are suspected to be involved in the disease process but the myocardial microRNA expression pattern in cats has not been identified. We hypothesized that microRNA profiles differ between healthy cats and cats with HCM. Small RNA sequencing on left ventricle (LV) and left atria (LA) samples from healthy cats (8 LV, 8 LA) and cats with HCM (7 LV, 5 LA) was performed. We identified 1039 differentially expressed microRNAs (False Discovery Rate <0.01, fold change >2). Cats with HCM were found to have a distinct microRNA expression profile with apparent regional heterogeneity. Comparing the HCM and control hearts, we detected 80 differentially expressed microRNAs for the HCM LV, and 37 for the LA. These included LV and LA enriched miR-21, miR-146b, and reduced miR-122-5p, which were recently suggested as key microRNAs for the HCM pathogenesis, and miR-132, which might be of therapeutic interest. Several top enriched microRNAs: miR-3958, miR-382-5p, miR-487a-5p (HCM LV); miR-chrD4_30107-3p (HCM LA); miR-3548 (HCM LV and LA) have either not been reported in the heart or only little is known. We identified potentially relevant microRNAs and further investigations into their role are required. Genes known to be targeted by the differentially expressed microRNAs were associated with inflammation and growth pathways in the HCM LV and LA, cardioprotective pathways in the LV, and fibrosis and structural changes in the LA when compared to healthy hearts.

## 1. Introduction

Hypertrophic cardiomyopathy (HCM) has a prevalence of 15 % in the general pet cat population [1]. The disease is characterized by thickening of the heart muscle, particularly the left ventricle, and impaired diastolic function [2]. HCM in cats is considered to be analogous to HCM in humans [3], with a similar phenotype and clinical presentation [4]. In humans, HCM has a genetic cause with >1500 mutations [5]. In cats, the disease is largely considered idiopathic [6] although 4 mutations have been identified to date, all of which affect genes that are also found in humans with HCM [7–10]. These genetic mutations were shown to have incomplete penetrance and cats that carry these genotypes do not necessarily show an HCM phenotype [11–15]. These observations suggest that other factors, such as microRNAs, are involved that influence the phenotype and progression of HCM.

MicroRNAs are short non-coding RNAs that negatively regulate gene expression post transcription [16]. They silence or degrade target messenger RNA sequences based on imperfect or perfect base-pairing of the complimentary seed sequence to the 3′ untranslated region [17]. MicroRNAs are important for maintaining health and the aberrant expression of microRNAs are involved in the pathogenesis of many diseases including HCM in humans and rodent models [18–23]. Cardiac-enriched microRNAs

*Abbreviations:* cAMP, 3′-5′-cyclic adenosine monophosphate; COX2, cyclooxygenase 2; EFNA4, ephrin A4; EFNA5, ephrin A5; ERK/MAPK, extracellular signal regulated kinase/mitogen activated protein kinase 1; HCM, hypertrophic cardiomyopathy; IL1, interleukin-1; IL6, interleukin-6; IPA, ingenuity pathway analysis; LA, left atrium; LV, left ventricle; MRTF, a transcription factor involved in cardiac structure and fibrosis; MyD88, myeloid differentiation primary response 88; PCA, principal component analysis; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B; RhoGDI, Rho GDP-dissociation inhibitor; SAV1, salvador homolog 1; STAT3, signal transducer and activator of transcription 3; STK3, serine/threonine-protein kinase 3; TNFA, tumor necrosis factor-alpha.

* Corresponding author.
*E-mail address:* sfonfara@uoguelph.ca (S. Fonfara).

http://dx.doi.org/10.1016/j.jmccpl.2023.100037
Received 29 December 2022; Received in revised form 31 May 2023; Accepted 1 June 2023
2772-9761/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

contributing to cardiac hypertrophy, fibrosis and/or associated with specific disease presentations have been reported for humans [21,24–26]. In human patients with HCM, myocardial microRNA profiles differed significantly from those of healthy individuals [20,27].

There are over 38,000 microRNAs annotated in miRbase v22.1 for 271 species, with around 2600 mature sequences experimentally validated in humans [28]. While much research on microRNAs has been done in human HCM, the role of microRNAs in feline HCM is largely unknown. So far, microRNA expressions have been profiled from various normal feline tissues, but not from the heart [29]. One study that looked at microRNA expression in serum samples from cats with HCM using microarrays identified significant differences in the expression of 11 microRNAs in cats with HCM compared to healthy cats [30]. Based on microRNA studies in human HCM and rodent models of HCM [20,31,32], we hypothesized that microRNA profiles would also be altered in feline HCM. Furthermore, regional differences in microRNA and long-noncoding RNA expression were observed in the human heart [33–35]. Previously, we found regional differences in gene activation, cytokine and remodeling enzyme gene expression in the healthy and HCM cat heart [36–39]. Therefore, we suspected regional differences in microRNAs between the left ventricle (LV) and the left atrium (LA). The objective of the study was to characterize the LV and the LA microRNA profiles in healthy cats and cats with HCM. Small RNA sequencing was used to determine the global microRNA expression profiles of the left heart, and bioinformatic analysis was performed to identify the regulated genes, signaling pathways and networks associated with HCM processes.

## 2. Material and methods

This prospective observational postmortem study was reviewed and approved by the Animal Care Committee of the University of Guelph. Clinical assessment, handling of cats, restrain, echocardiography, and euthanasia were performed in all cats as determined by the Animal Welfare Act of the Federal Food Safety and Veterinary Office (Switzerland), Veterinarians Act of Ontario (Canada), and the Professional Practice Standard for Veterinary Euthanasia from the College of Veterinarians of Ontario (Canada) and the Canadian Council on Animal Care as part of recognized veterinary practice. The cats with HCM were clinical patients of a cardiology referral service and were assessed under the Animal Welfare Act of the Federal Food Safety and Veterinary Office (Switzerland) and the Veterinarians Act of Ontario (Canada). HCM was diagnosed by board-certified specialists in veterinary cardiology. A diastolic LV wall thickness >6 mm in the absence of other diseases that could cause LV wall thickening was considered diagnostic for HCM [2]. Most cats had been long-term patients of the cardiology service and progression of their disease was observed over time. All HCM samples were obtained from pet cats that had been euthanized for medical reasons. The owners of these cats provided informed consent that the hearts could be collected postmortem and used for research purposes. Hearts from control cats were donated to the study by a commercial company that carries out animal safety studies for Food and Drug Administration as well as European Medicines Agency regulatory submissions in support of veterinary drug development, and is certified by the Canadian Council on Animal Care (https://kingfisherint.com; https://ccac.ca/en/about-the-ccac/). These cats were from a research cat population and were healthy controls in a study. All cats underwent gross and histopathologic examinations conducted and/or supervised by a board-certified veterinary pathologist as the gold standard to confirm the presence of HCM in the cats with HCM, the absence of cardiac diseases in the healthy cats and the absence of systemic diseases in all cats. Inclusion criteria were the presence of HCM in the cats with HCM and the absence of cardiac and systemic diseases in the control cats. Cats with cardiac diseases other than HCM or systemic diseases were excluded from the study.

Myocardial samples from healthy male cats (8 left ventricle (LV) and 8 left atria (LA), age: 1.5 years old) and male cats with HCM (7 LV and 5 LA, age: 3–15 years old) (Table 1) were collected immediately after euthanasia and stored in RNAlater at −80 °C until RNA isolation.

### 2.1. RNA isolation

For extraction of total RNA, 700 μL of QIAzol Lysis Reagent (Qiagen) and a 2 mm stainless steel bead were added to myocardial tissue samples and lysed using a Tissue Lyser II (Qiagen) for 8 min at 30/s frequency for high throughput sample disruption and homogenization. Total RNA was isolated from the samples using the miRNeasy Mini Kit (Qiagen) according

**Table 1**
Demographic information and statistics summary of sRNA-seq data. Left ventricle (LV) and left atrium (LA) samples were collected from healthy cats and cats with hypertrophic cardiomyopathy (HCM). Breeds in the study include Domestic Shorthair (DSH), Domestic Longhair (DLH), Chartreux, European Shorthair (ESH) and Ragdoll.

| Sample | Group | Age (years) | Breed | Sex | Reads post trimming, # | Reads for analysis, % | Mature reads, % | Ribosomal reads, % | Unassigned, % |
|---|---|---|---|---|---|---|---|---|---|
| LV1 | Healthy LV | 1.5 | DSH | Male | 7,949,223 | 94 | 68 | 2 | 7 |
| LV2 | Healthy LV | 1.5 | DSH | Male | 8,585,895 | 95 | 69 | 2 | 7 |
| LV3 | Healthy LV | 1.5 | DSH | Male | 8,084,147 | 96 | 71 | 2 | 8 |
| LV4 | Healthy LV | 1.5 | DSH | Male | 7,334,672 | 94 | 73 | 2 | 6 |
| LV5 | Healthy LV | 1.5 | DSH | Male | 8,204,467 | 96 | 78 | 2 | 5 |
| LV6 | Healthy LV | 1.5 | DSH | Male | 7,328,982 | 91 | 50 | 17 | 10 |
| LV7 | Healthy LV | 1.5 | DSH | Male | 7,804,861 | 90 | 56 | 11 | 11 |
| LV8 | Healthy LV | 1.5 | DSH | Male | 8,141,622 | 77 | 53 | 13 | 10 |
| LV9 | HCM LV | 3 | DSH | Male | 7,635,908 | 95 | 75 | 2 | 7 |
| LV10 | HCM LV | 9 | DSH | Male | 7,288,369 | 97 | 78 | 2 | 6 |
| LV11 | HCM LV | 8 | DLH | Male | 8,463,889 | 94 | 58 | 17 | 9 |
| LV12 | HCM LV | 11.9 | DLH | Male | 6,578,612 | 93 | 54 | 15 | 13 |
| LV13 | HCM LV | 14 | Chartreux | Male | 8,205,172 | 97 | 70 | 1 | 9 |
| LV14 | HCM LV | 8 | ESH | Male | 8,214,560 | 94 | 48 | 2 | 29 |
| LV15 | HCM LV | 11 | ESH | Female | 9,349,153 | 96 | 60 | 2 | 18 |
| LA1 | Healthy LA | 1.5 | DSH | Male | 7,292,626 | 97 | 80 | 1 | 5 |
| LA2 | Healthy LA | 1.5 | DSH | Male | 8,266,977 | 94 | 65 | 2 | 11 |
| LA3 | Healthy LA | 1.5 | DSH | Male | 8,163,264 | 95 | 69 | 2 | 10 |
| LA4 | Healthy LA | 1.5 | DSH | Male | 7,290,056 | 95 | 67 | 3 | 13 |
| LA5 | Healthy LA | 1.5 | DSH | Male | 7,675,167 | 95 | 74 | 2 | 6 |
| LA6 | Healthy LA | 1.5 | DSH | Male | 7,753,055 | 93 | 57 | 14 | 10 |
| LA7 | Healthy LA | 1.5 | DSH | Male | 9,509,335 | 91 | 61 | 12 | 9 |
| LA8 | Healthy LA | 1.5 | DSH | Male | 7,017,891 | 91 | 55 | 1 | 11 |
| LA9 | HCM LA | 9 | DSH | Male | 7,918,819 | 96 | 67 | 2 | 8 |
| LA10 | HCM LA | 3 | Ragdoll | Male | 7,739,632 | 96 | 68 | 2 | 8 |
| LA11 | HCM LA | 10 | DSH | Male | 5,848,821 | 94 | 44 | 19 | 15 |
| LA12 | HCM LA | 8 | ESH | Male | 8,625,530 | 96 | 69 | 1 | 10 |
| LA13 | HCM LA | 11 | ESH | Female | 9,109,097 | 94 | 56 | 1 | 20 |

to manufacturer's instructions and suspended in RNase free water. RNA was quantified (Nanodrop 2000; ThermoFisher Scientific) and its integrity and purity measured by Agilent Bioanalyzer (RNA6000 Nano assay). Samples with a 260/280 ratio of >1.9 and RNA integrity number > 5 were used for sequencing. Extracted RNA samples were stored at −80 °C until used for sequencing.

### 2.2. Small RNA sequencing

Sequencing libraries were generated for all samples using the NEBNext small RNA Library Prep Kit for Illumina (New England Biolabs) as per manufacturer's instructions. Briefly, 1 μg of total RNA from each sample was ligated to 3′ and 5′ adaptors, and poly(A) tails were added using poly(A) polymerase. RNA was reverse transcribed and amplified by PCR with primers specific to the adaptor sequences. MicroRNA enrichment was completed by extracting the 15–30 nucleotide fraction of the polyacrylamide gel. Library quality was assessed using the Bioanalyzer 2100 system (Agilent Technologies). Sequencing was performed on the Hiseq 2500 SR50 bp (Illumina) platform, with 150 M reads total.

### 2.3. Bioinformatic analysis

Processing: Quality of sequencing data was assessed using FastQC v.0.11.2 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). For each sample, raw reads were processed for adapter and quality trimming using trim_galore v.0.4.1 (http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/).

The following parameters for low-quality reads were applied: stringency = 5 for adaptor trimming, minimum phred score = 20. Quality was reassessed using FastQC and any remaining index adaptors were removed during sRNA bench analysis (https://arn.ugr.es/srnatoolbox/srnabench/), with parameter minReadLength = 15.

Quantification: No feline microRNAs are included in miRbase 22.1. MicroRNAs detected by Lagana et al. (2017) were considered as known microRNA [29]. RNACentral (http://rnacentral.org/) was the reference for other RNA types such as rRNAs and tRNAs. Using genome mapping mode (https://arn.ugr.es/srnatoolbox/static/sRNAtoolbox_manual.pdf), reads were mapped to the Felis_catus genome (ensemble_rel96) and mature microRNA expression was quantified.

Differential microRNA expression: Once the mature microRNA read counts were compiled, principal component analysis (PCA) was performed to visualize the similarity of expression between samples. DESeq2 (http://bioconductor.org/packages/release/bioc/html/DESeq2.html) was used to detect differentially expressed microRNAs between various groups. The following comparisons were examined: Healthy LV vs. Healthy LA, HCM LV vs. HCM LA, HCM LV vs. Healthy LV, and HCM LA vs. Healthy LA.

### 2.4. Pathway and network analysis

Ingenuity Pathway Analysis software (IPA, Qiagen) was used for in silico target prediction and to determine which canonical pathways and networks are associated with differentially expressed microRNAs. Only mRNA targets with high confidence or those that were experimentally validated were used for analysis. The Benjamini-Hochberg correction was used for adjusted *P*-value, with significance threshold set to 1.3 (equivalent to $P < 0.05$).

### 2.5. Validation by reverse transcription quantitative polymerase chain reaction (RT-qPCR)

In order to validate the microRNA sequencing data, we selected 4 microRNAs based on their high differential expression in HCM hearts compared to controls. 5 LV and 5 LA samples were collected from male healthy cats (1.5 years of age) and cats with HCM (3–15 years of age) ($n = 20$ total). Total RNA was extracted from the samples as per the RNA isolation protocol above and converted to cDNA by reverse transcription using 1st strand cDNA synthesis kit (Agilent Technologies), according to manufacturer's instructions. RT-qPCR was performed using miRNA QPCR Master Mix, universal reverse primer (Agilent Technologies) according to manufacturer's instructions and the Lightcycler 480 (Roche Diagnostics). PCR primer sequences were selected based on published literature for housekeeping genes (RNA U6) and from our data for target microRNAs (miR-185-5p, miR-208-3p, miR-21-5p, miR-132-3p). Forward primer sequences (5′-3′) were as follows: miR-185-5p: GCTGGAGAGAAAGGCAGTTCCTGA, miR-208-3p: GCTATAAGACGAACAAAAGGTTTG, miR-21-5p: GCTAGCTTATCAGACTGATGTTGAC, miR-132-3p: GCAACGTAACAGTCTACAGCC.

Conditions for polyadenylation and RT respectively were 37 °C for 30 min, 95 °C for 5 min for the first program and 55 °C for 5 min, 25 °C for 15 min, 42 °C for 30 min, 95 °C for 5 min for the second program. The cycling conditions for qPCR were 95 °C for 10 min, 45 cycles of 95 °C for 10 s, 60 °C for 15 s and 72 °C for 20 s, followed by dissociation curve analysis. Relative quantification was calculated by $2^{-\Delta\Delta Ct}$ method. All RT-qPCR statistical analysis was performed using GraphPad Prism 9.0. *P*-values <0.05 were considered as statistically significant.

## 3. Results

To characterize and define the feline miRNome (all expressed mature microRNAs) in the myocardium of healthy cats and those with HCM, 29 small-RNA libraries were generated from 8 male healthy cats (paired LV and LA samples from all cats) and 9 male cats with HCM (paired LV and LA samples from 3 cats, individual LV samples from 4 cats and LA samples from 2 cats). Our analysis revealed 1039 microRNAs after removal of repetitive sequences and those with poor expression. The total number of reads per sample averaged 7.3 million reads, which was 91 % of the total reads acquired from sequencing. The remaining were discarded due to adaptor trimming and repetitive sequences. The genome reads were 7.1 million reads per sample on average, indicating excellent alignment to the reference. Of these reads, 54 % were mature microRNAs. Only 1 % of the reads were hairpins and the remaining consisted of other reads, such as tRNA or rRNA. Unassigned reads were 15 % of the overall reads, which could include novel microRNAs.

PCA was conducted to assess the similarities and differences in microRNA expression levels of HCM and healthy samples both in the LV and the LA (Fig. 1). The low intra-group variability indicates that within each group: Healthy LV, Healthy LA, HCM LV and HCM LA, the samples have similar microRNA profiles. The high inter-group differences with separation between HCM and healthy samples as well as between LV and LA samples indicate that the gene expression is driven by disease associated factors, along principal component 1 and by regional differences, along principal component 2. The higher dispersion of HCM samples shows a more heterogenous expression relative to the tightly clustered healthy samples, revealing an inherent variability in HCM hearts than in healthy hearts.

### 3.1. MicroRNA expression pattern are region-specific in the healthy and the HCM heart

To investigate regional differences in the healthy and HCM hearts, microRNAs of the healthy LV were compared to the healthy LA, followed by the comparison of the LV and the LA of the HCM heart. Heat maps and volcano plots were generated to visualize microRNA expression differences between groups as well as to show statistical significance in the case of large fold changes in microRNAs. In the healthy heart, 9 microRNAs were significantly more abundant and 28 were significantly less abundant in the LV compared to the LA (Fig. 2A, B; Table 2A). In the HCM heart, the differential expression was more marked, with 52 microRNAs significantly more abundant and 25 significantly less abundant in the LV compared to the LA (Fig. 2C, D; Table 2B). Several overlapping microRNAs were observed in healthy and HCM hearts: there was a significantly higher abundance of 6 microRNAs (microRNA(miR)-499-5p, miR-652-3p, miR-652-5p, miR-140-5p, miR-190a-5p and miR-365-3p) and a significantly lower abundance of 11 microRNAs (miR-148a-5p, miR-153-3p, miR-885-3p,

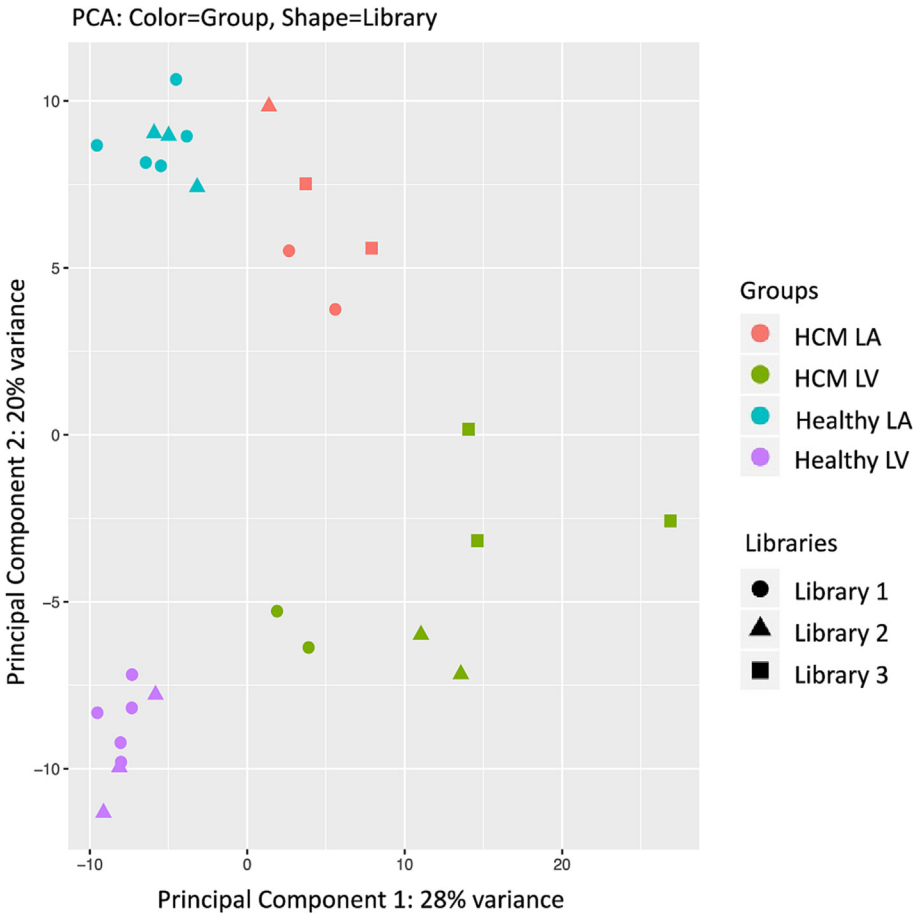


**Fig. 1.** Principal component analysis of all reads shows clustering of samples based on region and condition (healthy vs. HCM).
Left ventricle (LV) and left atrial (LA) samples have distinct microRNA profiles in healthy cats and cats with HCM. The clustering of expression profiles within the feline heart distinguishes HCM from healthy samples along principal component 1 (PC1), as well as LV from LA samples along principal component 2 (PC2). Colors indicate the observational groups: HCM LA ($n$ = 5), HCM LV ($n$ = 7), Healthy LA ($n$ = 8) and Healthy LV ($n$ = 8). Sequencing library construction was conducted in 3 batches with no batch effects observed.

miR-885-5p, miR-200a-3p, miR-200a-5p, miR-200b-3p, miR-200b-5p, miR-218-5p, miR-429-3p, and mir-2387-5p) in the LV when compared to the LA (Table 2).

### *3.2. Predicted pathways and networks are region specific in the healthy and the HCM heart*

To explore the biological functions of the differentially expressed microRNAs that were observed in the regional comparison, IPA was used to identify activated or inhibited canonical pathways, and their interactions. With IPA, we identified a lower expression of most major pathways, regulators and processes in the healthy LV compared to the healthy LA (Fig. 3A, 4A). Among the top pathways, the cardiac hypertrophy, RhoGDI (Rho GDP-dissociation inhibitor) signaling, Dopamine-DARPP32 feedback in cAMP signaling and dilated cardiomyopathy pathways were found to be more abundantly expressed in the healthy LV than the healthy LA (Fig. 3A). The network analysis identified STK3 (serine/threonine-protein kinase 3) and SAV1 (salvador homolog 1), which are part of the HIPPO pathway, EFNA4 (ephrin A4) and EFNA5 (ephrin A5), which are members of the family of tyrosine kinase receptors, and NCSTN (nicastrin), involved in NOTCH1 signaling, as more active in the healthy LV than the healthy LA (Fig. 4A).

In the HCM heart, the regional comparison identified a higher number of activated pathways in the LV than in the healthy heart, which included growth, proliferative and calcium signaling pathways (Fig. 3B). For the network analysis most major pathways, regulators and processes were less activated in the LV than the HCM LA. However, the PPARA (peroxisome proliferator activated receptor alpha), PPARGC1A (PPAR gamma coactivator 1 alpha), DGAT1 (diacylglycerol O-acyltransferase 1), ghrelin, KLF15 (Krüppel-like factor 15) and contraction of the heart were more active in the HCM LV than in the HCM LA (Fig. 4B). This indicates a higher baseline activity within the LA in health and an activation of pathways within the LV in HCM.

### *3.3. The microRNA signature of the feline HCM heart differs from the healthy heart*

To identify microRNA profiles that are associated with HCM, we compared the microRNAs of the HCM LV and LA with the microRNAs from the healthy LV and LA, respectively. Heatmaps and volcano plots were constructed to visualize the differences.

Clear differences were found in the microRNA expression patterns of healthy cats and cats with HCM (Fig. 5). In the HCM LV, 66 microRNAs were enriched and 14 microRNAs were reduced when compared to the healthy LV (Table 3A). In the HCM LA, 22 microRNAs were enriched and 15 microRNAs were reduced when compared to the healthy LA (Table 3B). In the HCM LV and LA, 11 microRNAs were similarly upregulated (miR-21-3p, miR-21-5p, miR-132-3p, miR-132-5p, miR-146b-5p, miR-96-5p, miR-182, miR-183-5p, miR-185-5p, miR-409-5p, miR-3548-5p) and 4 were similarly downregulated (miR-122-5p, miR-885-5p, miR-378-5p, miR-139-5p) when compared with the healthy counterparts.

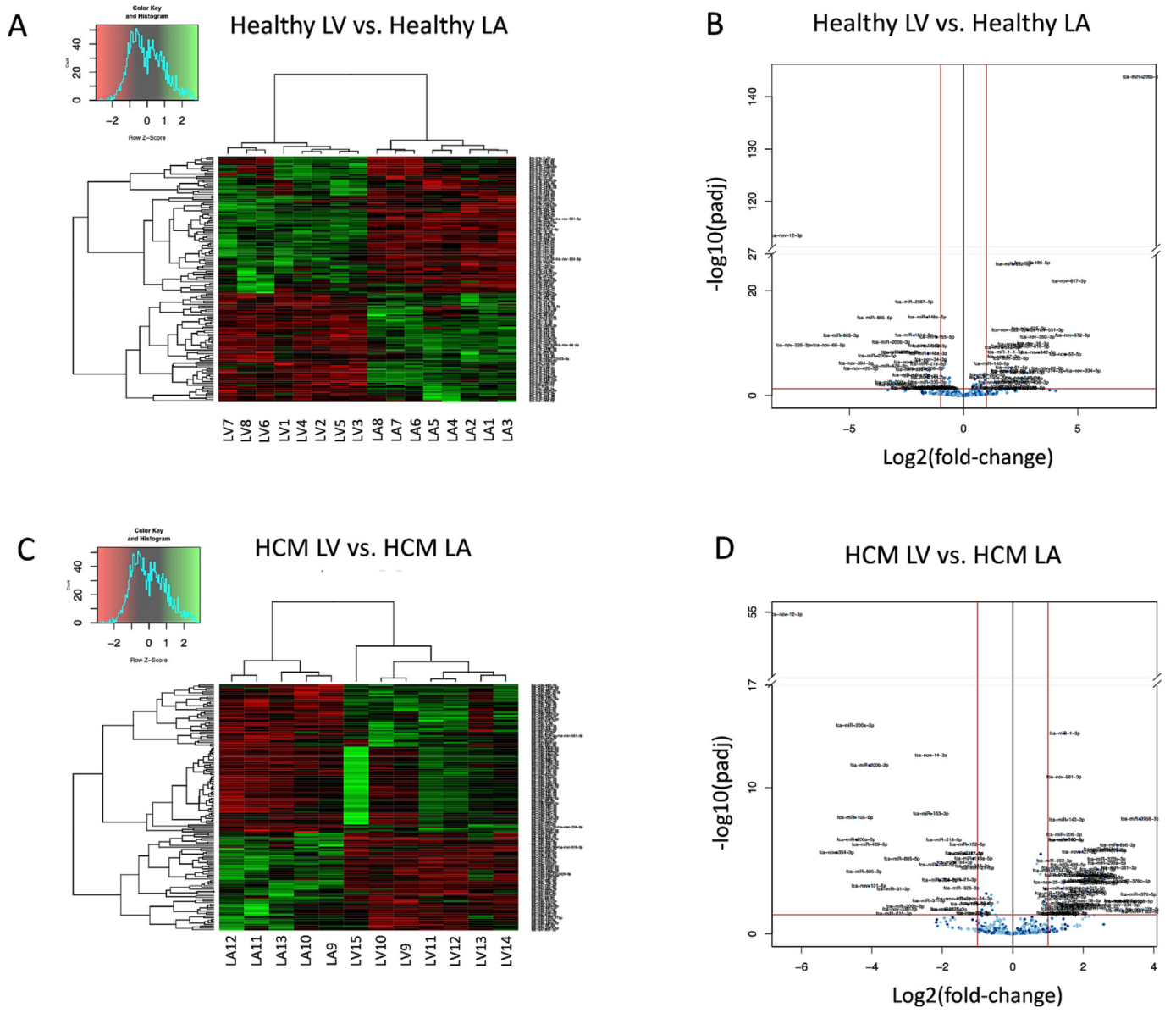


**Fig. 2.** Differentially expressed microRNA transcripts distinguish feline left ventricle (LV) and left atrium (LA) in healthy cats and cats with HCM.
Heatmaps of differentially expressed microRNAs and volcano plots distinguish the left ventricle (LV) from the left atrium (LA) samples in the healthy heart (A and B respectively; healthy LV: $n = 8$; healthy LA: $n = 8$) and the HCM heart (C and D respectively; HCM LV: $n = 7$; HCM LA: $n = 5$). Normalized read counts were filtered with the parameters Log2 Fold Change (Log2 FC) $\pm 1$ and False Discovery Rate (FDR) <0.01.

### *3.4. Pathways and networks are region-specific in the feline HCM heart*

Next, we investigated the biological function of the differentially expressed microRNAs in the HCM LV and LA. IPA and network analysis were conducted to identify positively or negatively affected pathways and regulators, along with their interactions.

Overall, more pathway activation was observed in the HCM LV than in the HCM LA when each region was compared to their healthy equivalent (Fig. 6A, B). Within the HCM LV, key pathways included granulocyte adhesion and diapedesis, agranulocyte adhesion and diapedesis, VDR/RXR (vitamin D receptor/retinoid x receptor) activation, role of macrophages, fibroblasts and endothelial cells, cardiac hypertrophy, growth, fibrosis and immune-modulatory pathways (Fig. 6A). Only LXR/RXR (liver x receptor/RXR) activation was found to have decreased expression in the HCM LV compared to the healthy LV.

In the HCM LA, the top pathway was AMPK (5′adenosine monophosphate activated protein kinase) signaling, followed by several structural, growth and fibrosis pathways, including the activated BEX2 (brain expressed x-lined 2), HIPPO, ERK/MAPK (extracellular signal regulated kinase/ mitogen activated protein kinase 1), ILK (integrin linked kinase), and PI3K/AKT (phosphoinositide 3-kinase/protein kinase B) pathways (Fig. 6B). Production of nitric oxide and reactive oxygen species in macrophages, hepatic fibrosis and STAT3 (signal transducer and activator of transcription 3) pathways were activated in both the HCM LV and LA. Further analysis of the biological networks identified IL1A (interleukin-1A) and TFNA (tumor necrosis factor-alpha) as key molecules in the HCM LV and LA (Fig. 7A, B). The HCM LV showed COX2 (cyclooxygenase 2): PTGS2 (prostaglandin-endoperoxide synthase) in a central position, in addition to activation of lipid metabolism, cell movement, growth of blood vessels, and mononuclear leukocytes (Fig. 7A). Within the HCM LA, IL1B, IL6, and endothelin-1 were centrally placed. Activated pathways included fibrogenesis and structural pathways, activation and adhesion of inflammatory cells, growth and cell movement (Fig. 7B).

**Table 2**
Differentially expressed microRNAs upregulated (UP) and downregulated (DOWN) in the left ventricle (LV) compared to the left atrium (LA) in the healthy heart (A) and the HCM heart (B). Similar regulated microRNAs in the regional comparison of the healthy and HCM are indicated in bold. Number of predicted mRNA targets for each microRNA from TargetScan based on human or mice (specified by mmu) homologs. Parameters p-adj < 0.05 and log2FC ±1 were applied. Fca: *Felis catus*; Mmu: *Mus musculus*.

| 2A | | |
|---|---|---|
| Known microRNAs | Healthy LV ($n$ = 8). Healthy LA ($n$ = 8) | |
| UP | log2FoldChange | Predicted target genes, # |
| fca-miR-208b-3p | 7.8 | 211 |
| **fca-miR-499-5p** | 3.01 | 270 (mmu) |
| **fca-miR-652-3p** | 2.86 | 17 |
| **fca-miR-652-5p** | 2.18 | 2413 |
| fca-miR-1-1-5p | 1.83 | 0 |
| fca-miR-34a-5p | 1.7 | 754 |
| **fca-miR-140-5p** | 1.22 | 434 |
| **fca-miR-190a-5p** | 1.09 | 223 |
| **fca-miR-365-3p** | 1.04 | 269 (mmu) |
| DOWN | | |
| **fca-miR-885-3p** | −5.36 | 3522 |
| **fca-miR-885-5p** | −3.89 | 3082 |
| **fca-miR-200a-5p** | −3.79 | 3225 |
| **fca-miR-429-3p** | −3.23 | 966 (mmu) |
| **fca-miR-200b-3p** | −3.17 | 1196 |
| **fca-miR-200b-5p** | −3.05 | 3225 |
| **fca-miR-200a-3p** | −2.8 | 905 |
| fca-miR-181c-3p | −2.7 | 1675 |
| fca-miR-96-5p | −2.42 | 1193 |
| fca-miR-181c-5p | −2.28 | 1371 |
| fca-miR-335-5p | −2.19 | 289 |
| fca-miR-181d-5p | −2.17 | 1371 |
| **fca-miR-2387-5p** | −2.17 | 0 |
| fca-miR-708-3p | −1.89 | 2978 |
| fca-miR-376a-2-5p | −1.68 | 2485 |
| fca-miR-708-5p | −1.66 | 240 |
| **fca-miR-148a-5p** | −1.6 | 3866 |
| **fca-miR-218-5p** | −1.56 | 1102 |
| fca-miR-148a-3p | −1.55 | 802 |
| **fca-miR-153-3p** | −1.53 | 886 |
| fca-miR-335-3p | −1.53 | 7318 |
| fca-miR-551b-3p | −1.38 | 8 |
| fca-miR-375-3p | −1.36 | 222 (mmu) |
| fca-miR-432-5p | −1.22 | 3902 |
| fca-miR-424-3p | −1.18 | 1772 |
| fca-miR-155-5p | −1.18 | 556 |
| fca-miR-493-5p | −1.08 | 796 |
| fca-miR-379-5p | −1 | 124 |

| 2B | | |
|---|---|---|
| Known microRNAs | HCM LV ($n$ = 7). HCM LA ($n$ = 5) | |
| UP | log2FoldChange | Predicted target genes, # |
| fca-miR-1185-5p | 3.62 | 2994 |
| fca-miR-3958-3p | 3.61 | 0 |
| fca-miR-370-5p | 3.56 | 319 (seed sequence with miR-1193) |
| fca-miR-3958-5p | 3.43 | 0 |
| fca-miR-376c-5p | 3.36 | 2618 |
| fca-miR-487a-5p | 3.15 | 3083 |
| fca-miR-381-5p | 3.14 | 826 |
| fca-miR-381-3p | 3 | 833 |
| fca-miR-656-3p | 2.98 | 5020 |
| fca-miR-409-5p | 2.72 | 136 |
| fca-miR-487b-3p | 2.69 | 16 |
| fca-miR-299a-5p | 2.67 | 186 (mmu) |
| fca-miR-323b-3p | 2.66 | 2873 |
| fca-miR-655-3p | 2.62 | 850 |
| fca-miR-3959-5p | 2.54 | 0 |
| fca-miR-758-3p | 2.49 | 271 |
| fca-miR-487b-5p | 2.47 | 3083 |

**Table 2** (*continued*)

| 2B | | |
|---|---|---|
| Known microRNAs | HCM LV ($n$ = 7). HCM LA ($n$ = 5) | |
| UP | log2FoldChange | Predicted target genes, # |
| fca-miR-410-3p | 2.41 | 604 |
| fca-miR-411-3p | 2.41 | 310 |
| fca-miR-382-5p | 2.38 | 218 |
| fca-miR-323a-3p | 2.35 | 488 |
| fca-miR-411-5p | 2.31 | 90 (mmu) |
| fca-miR-654-3p | 2.14 | 3673 |
| fca-miR-889-3p | 2.13 | 4716 |
| fca-miR-382-3p | 2.1 | 271 |
| fca-miR-432-5p | 2.07 | 3902 |
| fca-miR-299a-3p | 2.06 | 90 (mmu) |
| fca-miR-134-5p | 1.97 | 197 |
| fca-miR-493-5p | 1.96 | 796 |
| fca-miR-127-3p | 1.92 | 25 |
| fca-miR-493-3p | 1.86 | 170 |
| fca-miR-543-3p | 1.85 | 532 (mmu) |
| fca-miR-329-5p | 1.73 | 4532 |
| fca-miR-380-3p | 1.59 | 4268 |
| fca-miR-379-5p | 1.58 | 124 |
| fca-miR-127a-5p | 1.58 | 0 |
| **fca-miR-499-5p** | 1.57 | 270 (mmu) |
| fca-miR-140-3p | 1.53 | 0 |
| **fca-miR-140-5p** | 1.51 | 434 |
| fca-miR-1-3p | 1.47 | 896 |
| fca-miR-206-3p | 1.46 | 665 (mmu) |
| fca-miR-329-3p | 1.41 | 359 |
| fca-miR-193b-5p | 1.39 | 4365 |
| fca-miR-495-3p | 1.36 | 870 |
| fca-miR-370-3p | 1.33 | 5022 |
| **fca-miR-652-5p** | 1.32 | 2413 |
| **fca-miR-365-3p** | 1.32 | 269 (mmu) |
| fca-miR-379-3p | 1.3 | 310 |
| fca-miR-376c-3p | 1.25 | 270 |
| **fca-miR-652-3p** | 1.18 | 17 |
| **fca-miR-190a-5p** | 1.17 | 223 |
| fca-miR-133a-5p | 1.11 | 3353 |
| DOWN | | |
| **fca-miR-200a-3p** | −4.48 | 905 |
| fca-miR-105-5p | −4.47 | 5146 |
| **fca-miR-200a-5p** | −4.44 | 3225 |
| **fca-miR-885-3p** | −4.22 | 3522 |
| **fca-miR-200b-3p** | −4.07 | 1196 |
| **fca-miR-429-3p** | −4.06 | 966 (mmu) |
| fca-miR-31-3p | −3.39 | 2387 |
| fca-miR-615-3p | −3.38 | 15 |
| **fca-miR-885-5p** | −3.14 | 3082 |
| **fca-miR-200b-5p** | −3.05 | 3225 |
| fca-miR-31-5p | −2.39 | 477 |
| **fca-miR-153-3p** | −2.32 | 886 |
| fca-miR-204-5p | −2.16 | 791 |
| fca-miR-204-3p | −2.16 | 5753 |
| **fca-miR-218-5p** | −2.07 | 1102 |
| fca-miR-375-3p | −1.81 | 222 (mmu) |
| fca-miR-184-3p | −1.65 | 19 (mmu) |
| fca-miR-21-3p | −1.49 | 3664 |
| fca-miR-326-3p | −1.46 | 335 (mmu) |
| **fca-miR-2387-5p** | −1.37 | 0 |
| fca-miR-152-5p | −1.29 | 3817 |
| fca-miR-129-5p | −1.26 | 735 |
| **fca-miR-148a-5p** | −1.12 | 3866 |
| fca-miR-98-3p | −1.01 | 5244 |
| fca-miR-21-5p | −1 | 384 |

### *3.5. RT-qPCR validation of microRNA sequencing data*

To validate the microRNA sequencing data, RT-qPCR of selected microRNA was carried out. Consistent with the sequencing results, the microRNA concentrations were higher in the HCM LV and LA compared the healthy LV and LA, respectively (Fig. 8A, B). The difference was significant for miR-185-5p and miR-208-3p in the HCM LV and for miR-132-3p in the HCM LA (Fig. 8A, B).

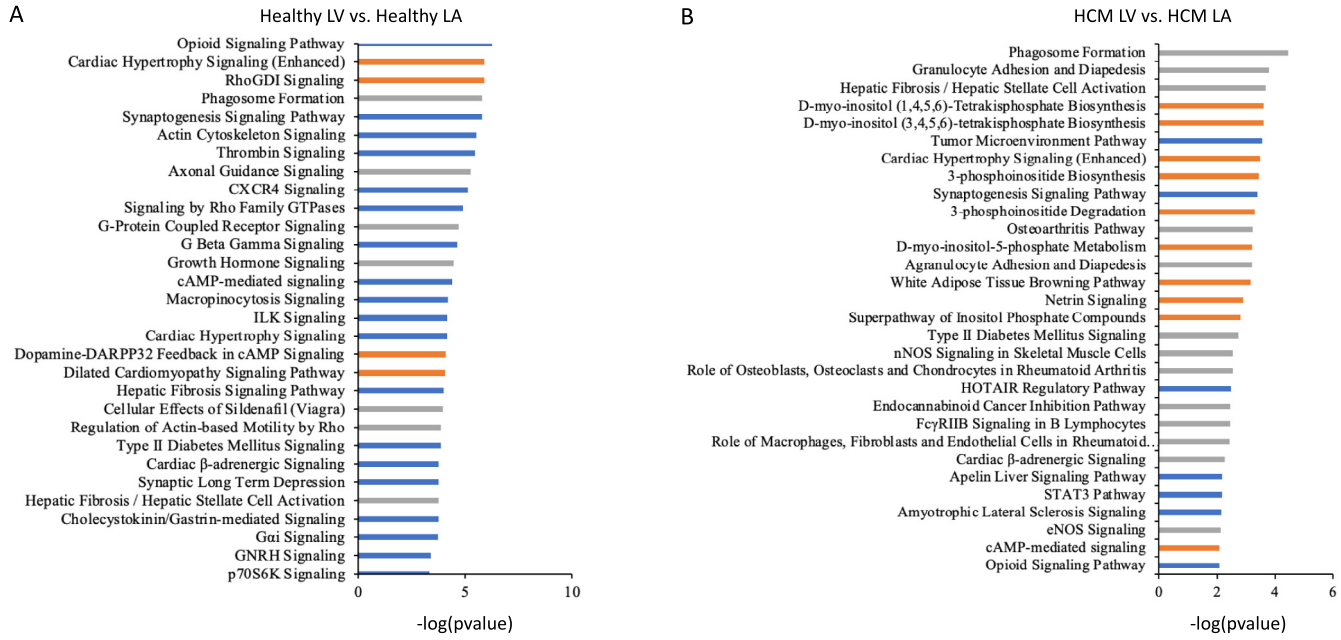


**Fig. 3.** Top canonical pathways expressed by microRNA targets in the LV and the LA of the healthy (A) and HCM (B) heart.
Healthy LV: $n = 8$; healthy LA: $n = 8$; HCM LV: $n = 7$; HCM LA: $n = 5$. All targets in the analysis were filtered $P$-value <0.05 and their positive or negative expression was identified based on their $Z$-score. Line colors indicate significantly increased expression (orange), significantly decreased expression (blue) and no direction (gray).

## 4. Discussion

This study is the first to identify the constitutive miRNome of the feline healthy LV and LA and the changes associated with HCM. Region-specific microRNA expression patterns were found for the LV and the LA, which were shown to be altered in HCM, revealing a distinct microRNA signature. Low intra-variability in the HCM LV and LA groups indicated that these microRNAs formed a consistent pattern.

The overlapping microRNAs we detected in the regional comparison of the healthy and HCM myocardium suggest an involvement of these microRNAs in the maintenance of myocardial structure and function. The determination of LV and LA specific microRNAs in HCM and healthy cat hearts indicates that several microRNAs have chamber specific expression under physiological conditions and in HCM, similar to what has been observed for humans [33–35]. Furthermore, overlapping microRNAs were found in the HCM LV and the LA suggesting an

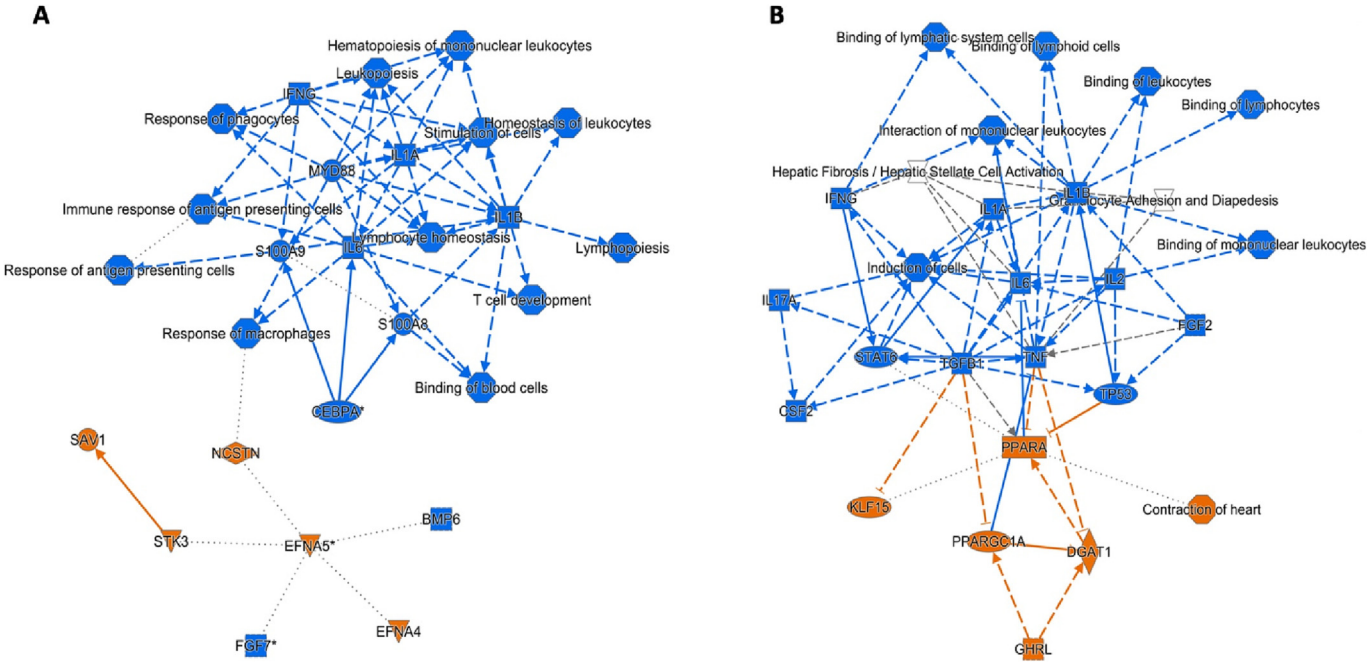


**Fig. 4.** Network analysis shows major pathways, regulators and processes within the LV compared to the LA of the healthy (A) and the HCM heart (B).
Healthy LV: $n = 8$; healthy LA: $n = 8$; HCM LV: $n = 7$; HCM LA: $n = 5$. Node and line colors indicate significantly increased expression (orange), significantly decreased expression (blue) and no direction (gray). For each function, z-scores were used to predict activation or inhibition. Lines and arrows between nodes represent direct interactions (solid) and indirect interactions (dashed) between molecules. Node shapes symbolize genes: enzymes (diamond), kinases (down pointing triangle), transcriptional regulators or modulators (oval), cytokines (rectangle), as well as functions (octagon) and complexes or groups (circle and hourglass).

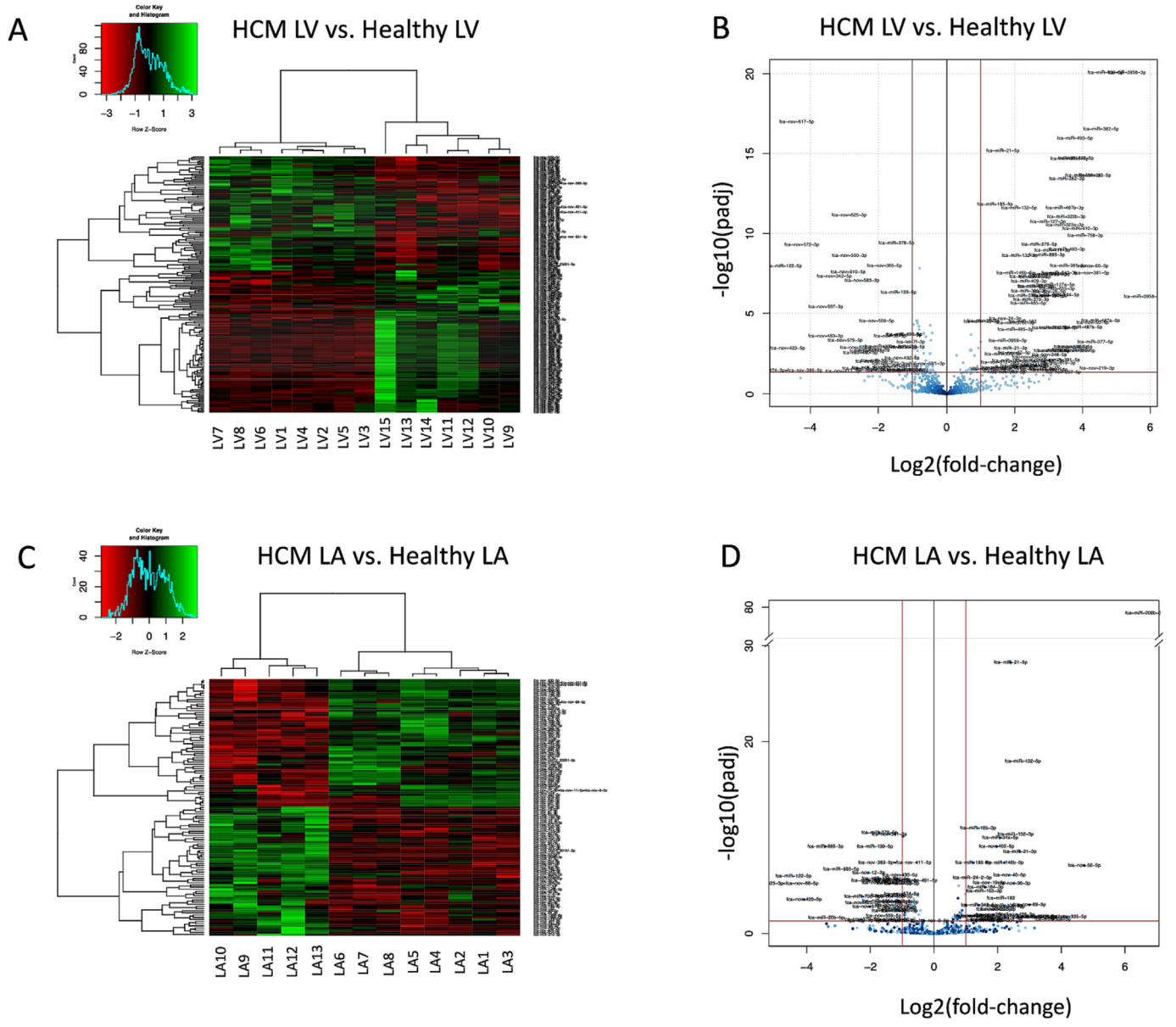


**Fig. 5.** Differentially expressed microRNAs distinguish between healthy and HCM in the cat left ventricle (LV) and left atrium (LA).
Heatmaps of differentially expressed microRNAs and volcano plots distinguish the healthy and HCM samples in the LV (A and B respectively) and the LA (C and D respectively) of the feline heart. Healthy LV: $n = 8$; healthy LA: $n = 8$; HCM LV: $n = 7$; HCM LA: $n = 5$. Normalized read counts were filtered with the parameters Log2 FC $\pm 1$ and FDR $< 0.01$.

association with the disease process independent of the cardiac region. There is a high similarity when comparing the feline LV and LA miRNome from our study with microRNA profiles obtained from human LV and LA [20,21]. However, for differentially expressed microRNA, variations were detected between our results and human HCM hearts. For the HCM LV, we identified 80 differentially expressed microRNAs, which is more than the 13 differentially expressed microRNAs observed for human LV HCM samples [20]. Of these, miR-96, miR-383 showed the same expression pattern in the cat and human LV, miR-204 and miR-708 were reduced in the cat and increased in the human HCM LV, and miR-34 was enriched in the human HCM LV and the cat HCM LA [20]. Recent bioinformatic studies looking into the pathogenesis of human HCM and selecting hypertrophy or HCM associated microRNAs identified key microRNAs including the top downregulated in the feline HCM heart, miR-122-5p. Additionally, miR-146-5p and miR-21-5p were found to be enriched in the cat HCM LV and LA, and the hub gene miR-144 was reduced in the HCM LV [21–23].

Little overlap in differentially expressed microRNAs was observed when we compared results from LA samples with human studies. This is consistent with studies looking at human samples only and indicates the differences in diseases and presence of disease specific microRNA profiles [33,35,40,41]. An overlap of our results with human atrial samples was observed for miR-21-5p, miR-155, miR-378, miR-486, and miR-708 [33,35,40–42].

Interestingly, several of the top differentially regulated microRNAs in the HCM heart, such as miR-3958 (top enriched in the HCM LV), miR-chrD4_30107-3p (in the HCM LA), miR-3548 (enriched in the HCM LV and LA) have not been reported for the heart so far. Little is known about the top enriched microRNAs in the HCM LV, miR-382-5p and miR-487a-5p, and we identified novel microRNAs (15 % of reads) that are not yet annotated for the feline heart. The dissimilarity to human microRNA profiles might either indicate species or disease stage differences, or the detection of relevant microRNAs that have not been considered previously and further investigations into their role are required.

**Table 3**
Differentially expressed microRNAs upregulated (UP) and downregulated (DOWN) in HCM compared to healthy in the left ventricle (LV; A) and the left atrium (LA; B). In the LV and LA similar regulated microRNA are indicated in bold. Number of predicted mRNA targets for each microRNA from TargetScan based on human or mice (specified by mmu) homologs. Parameters p-adj < 0.05 and log2FC ±1 were applied. Fca: *Felis catus*; Mmu: *Mus musculus*.

| 3A | | |
|---|---|---|
| Known microRNAs | HCM LV ($n$ = 7). Healthy LV ($n$ = 8) | |
| UP | log2FoldChange | Predicted target genes, # |
| fca-miR-3958-5p | 5.78 | 0 |
| fca-miR-3958-3p | 5.3 | 0 |
| **fca-miR-409-5p** | 4.68 | 136 |
| fca-miR-382-5p | 4.54 | 218 |
| fca-miR-487a-5p | 4.53 | 3083 |
| fca-miR-377-5p | 4.38 | 4588 |
| fca-miR-758-3p | 4.08 | 271 |
| fca-miR-487b-5p | 4.02 | 3083 |
| fca-miR-656-3p | 4.01 | 5020 |
| fca-miR-410-3p | 3.93 | 604 |
| fca-miR-380-5p | 3.85 | 1996 |
| fca-miR-432-5p | 3.8 | 3902 |
| fca-miR-493-5p | 3.77 | 796 |
| **fca-miR-3548-5p** | 3.67 | 0 |
| fca-miR-3959-5p | 3.67 | 0 |
| fca-miR-381-3p | 3.57 | 833 |
| fca-miR-493-3p | 3.54 | 170 |
| fca-miR-382-3p | 3.54 | 271 |
| fca-miR-323b-3p | 3.53 | 2873 |
| fca-miR-323a-3p | 3.48 | 488 |
| fca-miR-487b-3p | 3.48 | 16 |
| fca-miR-381-5p | 3.4 | 826 |
| fca-miR-134-5p | 3.38 | 197 |
| fca-miR-543-3p | 3.32 | 532 (mmu) |
| fca-miR-376c-5p | 3.28 | 2618 |
| fca-miR-655-3p | 3.25 | 850 |
| fca-miR-127a-5p | 3.2 | 0 |
| **fca-miR-96-5p** | 3.15 | 1193 |
| fca-miR-411-3p | 3.1 | 310 |
| fca-miR-654-3p | 3.1 | 3673 |
| fca-miR-370-5p | 3.09 | 319 (seed sequence with miR-1193) |
| fca-miR-329-5p | 3.04 | 4532 |
| fca-miR-380-3p | 3.03 | 4268 |
| fca-miR-127-3p | 2.98 | 25 |
| fca-miR-299a-5p | 2.97 | 186 (mmu) |
| fca-miR-889-3p | 2.95 | 4716 |
| fca-miR-889-5p | 2.95 | 3782 |
| fca-miR-379-5p | 2.72 | 124 |
| fca-miR-411-5p | 2.63 | 90 (mmu) |
| fca-miR-433-3p | 2.6 | 346 |
| fca-miR-376a-5p | 2.58 | 3220 |
| fca-miR-370-3p | 2.48 | 5022 |
| fca-miR-337-3p | 2.46 | 3310 |
| fca-miR-329-3p | 2.42 | 359 |
| fca-miR-409-3p | 2.42 | 316 |
| fca-miR-299a-3p | 2.41 | 90 (mmu) |
| fca-miR-485-5p | 2.4 | 448 |
| fca-miR-379-3p | 2.34 | 310 |
| **fca-miR-182** | 2.24 | 1329 (5p-broadly conserved) |
| **fca-miR-132-3p** | 2.15 | 474 |
| **fca-miR-132-5p** | 2.13 | 1246 |
| fca-miR-383-5p | 2.1 | 0 |
| fca-miR-376c-3p | 2.03 | 270 |
| **fca-miR-146b-5p** | 2.02 | 283 |
| fca-miR-495-3p | 2.02 | 870 |
| **fca-miR-21-3p** | 1.88 | 3664 |
| **fca-miR-183-5p** | 1.86 | 407 (mmu) |
| fca-miR-3959-3p | 1.8 | 0 |
| fca-miR-376a-2-5p | 1.72 | 2485 |
| **fca-miR-21-5p** | 1.65 | 384 |
| fca-miR-335-5p | 1.65 | 289 |
| fca-miR-487a-3p | 1.58 | 174 |
| **fca-miR-185-5p** | 1.42 | 385 |
| fca-miR-376a-3p | 1.33 | 258 |
| fca-miR-485-3p | 1.26 | 446 |
| fca-miR-140-3p | 1.02 | 0 |
| DOWN | | |
| **fca-miR-122-5p** | −4.78 | 226 |
| fca-miR-31-3p | −2.28 | 2387 |
| **fca-miR-885-5p** | −2.18 | 3082 |
| fca-miR-200a-3p | −1.84 | 905 |
| fca-miR-105-5p | −1.82 | 5146 |
| **fca-miR-378-5p** | −1.46 | 0 |
| **fca-miR-139-5p** | −1.4 | 432 |
| fca-miR-144-3p | −1.34 | 1048 |
| fca-miR-200b-3p | −1.29 | 1196 |
| fca-miR-1-1-5p | −1.25 | 0 |
| fca-miR-499-5p | −1.23 | 270 (mmu) |
| fca-miR-218-5p | −1.15 | 1102 |
| fca-let-7f-3p | −1.04 | 0 |
| fca-miR-204-5p | −1.03 | 791 |

| 3B | | |
|---|---|---|
| Known microRNAs | HCM LA ($n$ = 5). Healthy LA ($n$ = 8) | |
| UP | log2FoldChange | Predicted target genes, # |
| fca-miR-208b-3p | 6.62 | 211 |
| fca-miR-506-3p | 3.73 | 1325 |
| fca-miR-chrD4_30107-3p | 3.33 | 0 |
| **fca-miR-3548-5p** | 3.21 | 0 |
| **fca-miR-132-5p** | 2.79 | 1246 |
| **fca-miR-21-3p** | 2.7 | 3664 |
| **fca-miR-132-3p** | 2.56 | 474 |
| **fca-miR-21-5p** | 2.41 | 384 |
| fca-miR-105-5p | 2.22 | 5146 |
| **fca-miR-146b-5p** | 2.21 | 283 |
| **fca-miR-182** | 2.1 | 1329 (5p-broadly conserved) |
| fca-miR-34a-5p | 2.08 | 754 |
| **fca-miR-96-5p** | 1.83 | 1193 |
| fca-miR-184-3p | 1.61 | 19 (mmu) |
| **fca-miR-183-5p** | 1.58 | 407 (mmu) |
| fca-miR-153-3p | 1.57 | 886 |
| fca-miR-342-5p | 1.41 | 4139 |
| fca-miR-204-3p | 1.39 | 5753 |
| fca-miR-185-3p | 1.38 | 5145 |
| **fca-miR-409-5p** | 1.33 | 136 |
| **fca-miR-185-5p** | 1.23 | 385 |
| fca-miR-24-2-5p | 1.2 | 1923 |
| DOWN | | |
| **fca-miR-122-5p** | −4.42 | 226 |
| fca-miR-885-3p | −3.41 | 3522 |
| fca-miR-205-5p | −3.39 | 593 |
| **fca-miR-885-5p** | −2.9 | 3082 |
| fca-miR-708-3p | −2.25 | 2978 |
| fca-miR-708-5p | −2.03 | 240 |
| **fca-miR-139-5p** | −1.86 | 432 |
| fca-miR-378-5p | −1.71 | 0 |
| fca-miR-139-3p | −1.7 | 3145 |
| fca-miR-181c-3p | −1.66 | 1675 |
| fca-miR-206-3p | −1.25 | 665 (mmu) |
| fca-miR-181d-5p | −1.17 | 1371 |
| fca-miR-486-3p | −1.08 | 5264 |
| fca-miR-486-5p | −1.05 | 174 |
| **fca-miR-378-3p** | −1.04 | 0 |

### *4.1. Constitutive microRNA expression and associated pathways and networks in the feline LV and LA*

To determine microRNAs that might be involved in myocardial homeostasis, we identified microRNAs that showed a similar pattern in regional comparison of the healthy and HCM heart. The top enriched microRNAs in the healthy LV included miR-208b (top enriched in the healthy LV),

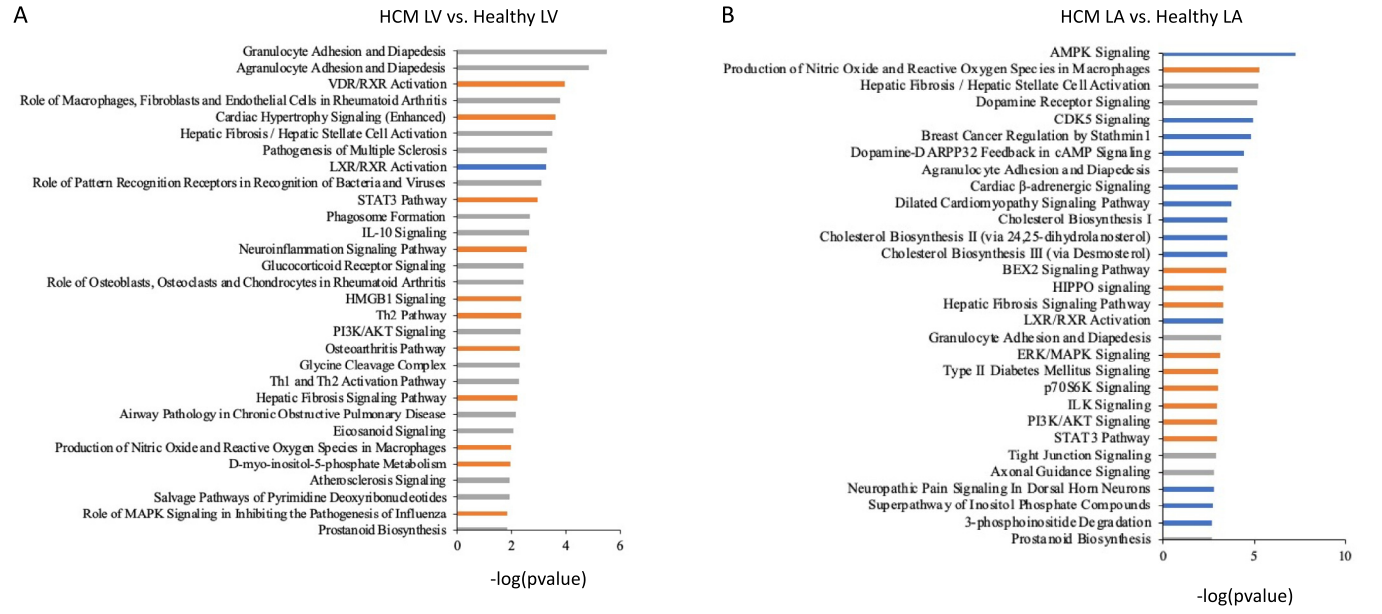


**Fig. 6.** Top canonical pathways expressed by microRNA targets in the HCM LV (A) and LA (B) compared to healthy heart.
Healthy LV: $n = 8$; healthy LA: $n = 8$; HCM LV: $n = 7$; HCM LA: $n = 5$. All targets in the analysis were filtered $P$-value <0.05 and their positive or negative expression was identified based on their $Z$-score. Line colors indicate significantly increased expression (orange), significantly decreased expression (blue) and no direction (gray).

miR-499-5p, miR-1-1-5p, and miR-34a. MiR-208b, miR-499, and miR-1 are considered cardiac enriched microRNAs with miR-208b being preferentially expressed in the LV [25,33,42–46]. Similarly, miR-34 is highly expressed in mouse hearts [47,48]. These microRNAs are involved in cardiomyocyte proliferation, differentiation and maintenance of the differentiated stage ([25,26,33,42–48], Table 4A).

Enriched microRNAs in the healthy LV that overlapped with the HCM LV were miR-140-5p, miR-190a-5p, miR-365-3p, miR-652-3p and miR-652-5p. These microRNAs are activated in hypoxia induced injured cardiomyocyte cell culture and suppress inflammatory markers; they are also found with cardiac hypertrophy, increased collagen mRNA and protein production in mouse hearts ([49–53], Table 4A).

The top enriched microRNAs in the healthy LA were miR-885-3p and -5p, miR-200a-5p, miR-200b-3p and -5p, miR-429-3p (all overlapping with the HCM LA) [Table 4A]. MiR-885 was reported to improve cell viability and reduced apoptotic rates [54]. Higher miR-885 concentrations reduced inflammatory cytokine production in a rat model [55]. MiR-429 belongs to the miR-200 family ([56], Table 4A). This miR family is involved in tissue fibrosis [56], cardiomyocyte proliferation and survival ([57,58], Table 4A). Considering the same expression pattern in the healthy and

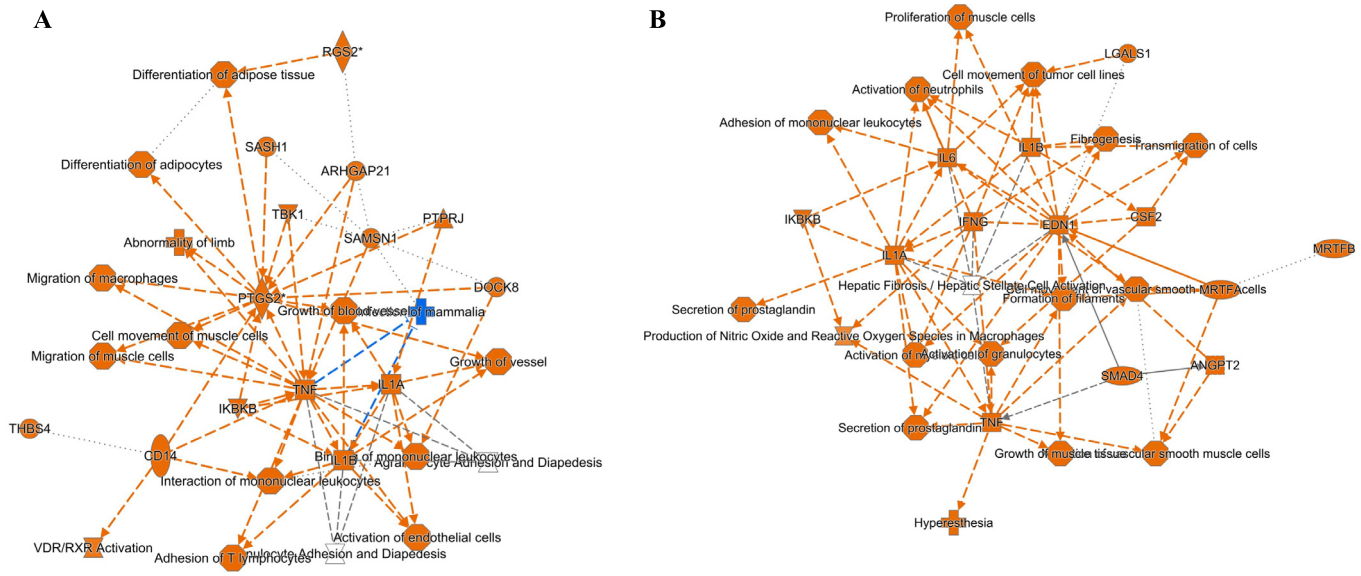


**Fig. 7.** Network analysis shows major pathways, regulators and processes differentially affected in the HCM heart LV (A) and LA (B) compared to the healthy heart.
Healthy LV: $n = 8$; healthy LA: $n = 8$; HCM LV: $n = 7$; HCM LA: $n = 5$. Node and line colors indicate significantly increased expression (orange), significantly decreased expression (blue) and no direction (gray). For each function, z-scores were used to predict activation or inhibition. Lines and arrows between nodes represent direct interactions (solid) and indirect interactions (dashed) between molecules. Node shapes symbolize genes: enzymes (diamond), kinases (down pointing triangle), phosphatases (triangle), transcriptional regulators or modulators (oval), cytokines (rectangle), as well as diseases (cross), functions (octagon) and complexes or groups (circle and hourglass).

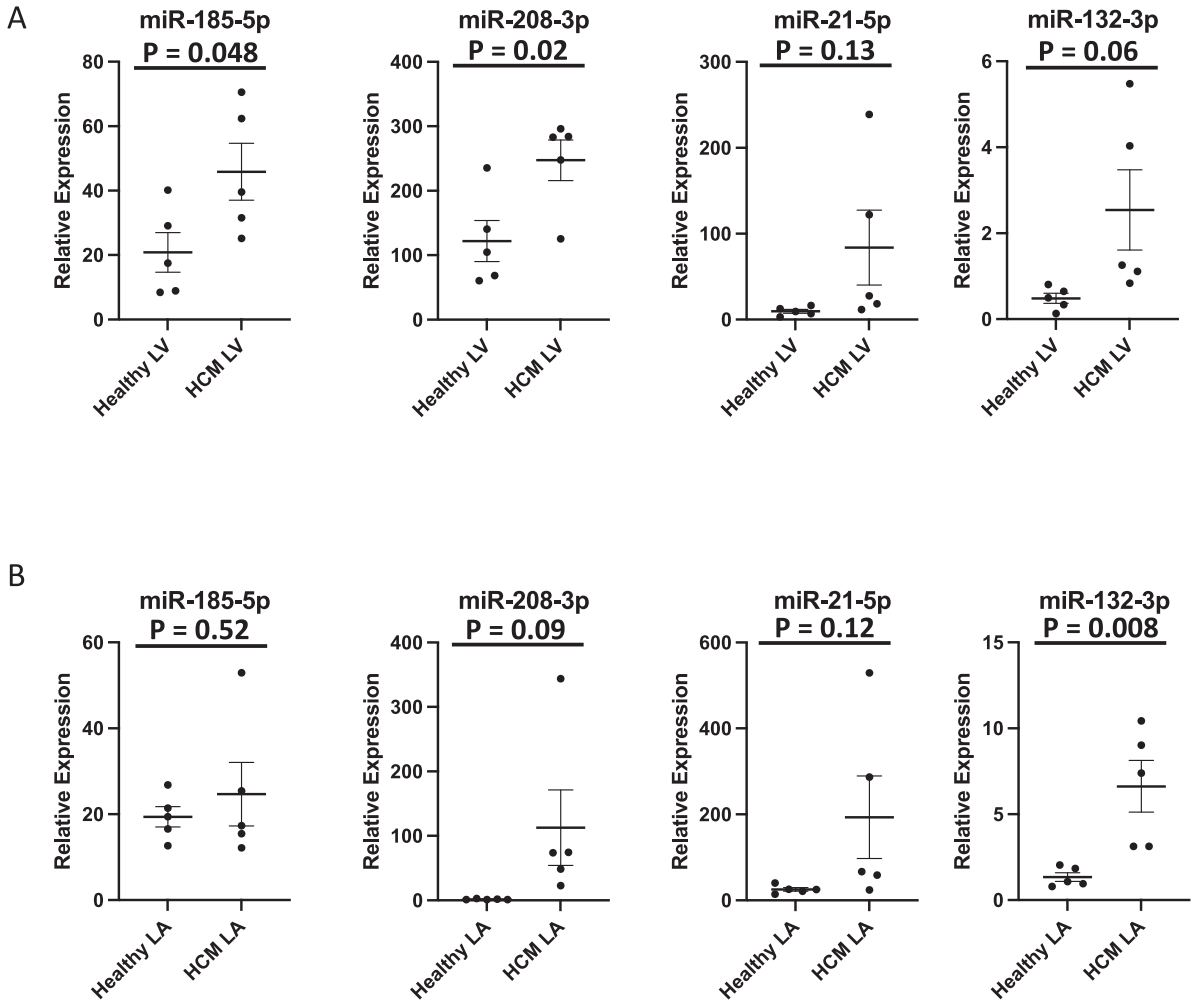


**Fig. 8.** Verification of microRNAs by RT-qPCR. (A) Expression of microRNAs in the healthy LV: $n = 5$ and HCM LV: $n = 5$. (B) Expression of microRNAs in healthy LA: $n = 5$ and HCM LA: $n = 5$. An unpaired *t*-test was used to compare groups. Mean ± SEM values are indicated and the single expression values against reference gene RNAU6 have been plotted.

HCM hearts, these microRNAs with similar transcription activity in the healthy and HCM cat heart might be involved in the maintenance of myocardial structure and function.

To explore the biological functions of the microRNAs in the healthy heart, predicted pathway targets and networks were identified using IPA network analysis. Activated pathways in the healthy feline LV were cardiac hypertrophy, RhoGDI signaling and dilated cardiomyopathy, which is consistent with predicted pathways we had obtained from mRNA sequencing [39] and is similar to what has been reported for human hearts. The activated SAV1 and STK3 are part of the HIPPO pathway, which is known to be involved in cardiomyocyte proliferation and cardiac repair [59,60]. EFNA4 and EFNA5 belong to the family of tyrosine kinase receptors and allow cell to cell communication, which activates signaling pathways that affect the cellular cytoskeleton. Ephrin-Eph signaling has further been shown to regulate inflammation, reorganizing the actin cytoskeleton [61]. Interestingly, MyD88 (myeloid differentiation primary response 88) is centrally placed in our network analyses and was more abundant in the healthy LA in comparison to the healthy LV. Little is known about the role of MyD88 in the heart; however, it is an intracellular adapter protein that coordinates proinflammatory signaling [62]. MyD88 promotes inflammation and repair following myocardial infarction, and IL1 and interferon-gamma (IFNG), both activated in the LA compared to the LV, and in the HCM heart, signal through MyD88 [62]. The activation of STK3, SAV1, EFNA4 and EFNA5 signaling in the healthy LV and the central role and activation of MyD88 in the healthy LA indicate chamber-specific processes in myocardial homeostasis.

### *4.2. Region specific microRNA profiles and associated pathways and networks in the HCM heart*

To determine the microRNA profiles specific for the LV and the LA in HCM hearts, we identified the microRNAs that are present only in the HCM LV and the HCM LA, when compared with the LV and LA from healthy cat hearts respectively. Most abundantly expressed LV microRNAs were miR-3958-3p and -5p, miR-382-5p and miR-487a-5p. MiR-3958-3p and miR-3958-5p have not been reported in the heart so far and little is known about miR-382-5p and miR-487a-5p ([63–65], Table 4B). Being the top upregulated in the HCM LV indicates their relevance and further investigations into the role of these microRNAs is required. MiR-31-3p and miR-200a-3p were found to be the top downregulated LV specific microRNAs, and both have been reported to be cardioprotective when reduced in expression [66,67].

Top enriched microRNAs in the HCM LA were miR-208b-3p, miR-506-3p and miR-chrD4_30107-3p. MiR-205-5p, miR-708-3p and miR-708-5p were reduced compared to the healthy LA [Table 4B]. Interestingly, miR-208b is a microRNA that is preferentially expressed in the healthy LV in both cats and humans [33]. The upregulation in the HCM LA is most likely a response to cardiac stresses such as pressure overload [25]. A recent study

**Table 4**
Source, roles, function and according references of selected microRNAs.

4A. Regional differentially expressed microRNAs comparing the left ventricle (LV) with the corresponding left atrium (LA)

Healthy LV specific enriched microRNA compared to the healthy LA

| MicroRNA | Samples | Roles/function | References |
|---|---|---|---|
| MiR-208b-3p (Also top enriched in HCM LA) | Cardiac enriched microRNAs, LV preferential | Cardiogenesis<br>Cardiac repair and regeneration<br>Increase in response to cardiac stress Association with cardiac metabolism<br>Enriched in hypoxia exposed cardiomyocyte cell line<br>Inhibition was associated with reduced apoptosis, increased cell viability<br>Circulating marker for myocardial hypertrophy | 25,26,33 |
| miR-1-1-5p | Cardiac enriched microRNAs | Cardiogenesis<br>Cardiac repair and regeneration | 42–45 |
| miR-34 | Highly expressed in mouse hearts | Key regulator of the heart's repair and regeneration capacity | 47,48 |

Overlapping enriched microRNA in the LV (healthy and hypertrophic cardiomyopathy [HCM]) compared to the corresponding LA

| MicroRNA | Samples | Roles/function | References |
|---|---|---|---|
| fca-miR-140-5p | Hypoxia injured cardiomyocyte cell culture<br>mouse hearts | Associated with cell injury and apoptosis Cardiac hypertrophy, increased collagen mRNA and protein production | 52 |
| fca-miR-190a-5p | Cardiomyocyte injury | Inhibit profibrotic genes, hypoxia induced cardiomyocyte injury<br>Suppresses inflammatory markers | 53 |
| fca-miR-365-3p | Cardiomyocytes (in vitro, in vivo) – rodent models | Upregulated in hypertrophic cardiomyocytes<br>Pro-hypertrophic | 50,51 |
| fca-miR-499-5p | Cardiomyocytes (in vitro, in vivo – mouse hearts) | Reduces apoptosis, inhibits profibrotic genes<br>Cardiomyocyte hypertrophy<br>Suppresses inflammatory marker | 25,42–44,46 |
| fca-miR-652 | Myocardium (mouse model) | Increased in diseased heart<br>Silencing reduces cardiac fibrosis, apoptosis, improves cardiac function | 49 |

Overlapping enriched microRNA in the LA (HCM and healthy) compared to the corresponding LV

| MicroRNA | | Samples | Roles/function | References |
|---|---|---|---|---|
| fca-miR-148a-5p | | Mouse model of cardiac pressure overload<br>MI rodent model | Increased in concentric hypertrophy, reduced in eccentric cardiac remodeling<br>Increase reduces myocardial apoptosis | 111 |
| fca-miR-153-3p | | Ischemia/reperfusion mouse model | Inhibition reduced myocardial inflammation and oxidative stress | 112 |
| miR-200 family | fca-miR-200a-3p | Mouse model cardiomyocytes | Increased in MI, increases cardiomyocyte apoptosis | 67,110 |
| | fca-miR-200a-5p | Cell culture, Endothelial cells | Tissue fibrosis, cardiomyocyte proliferation and fibrosis | 56 |
| | fca-miR-200b-3p<br>fca-miR-200b-5p | Cell culture, Endothelial cells | Increase caused endothelial damage, suppressed angiogenic response<br>Reduction of miR-200b results in upregulation of GATA-4: supports cell growth differentiation, survival | 56,107-109 |
| | fca-miR-429-3p | MI Mouse model cardiomyocytes<br>Hypoxia cardiomyocyte cell culture (rodent, human) | Reduced in MI<br>Reduction provided cytoprotective effects against injury, reduces apoptosis | 57,58 |
| fca-miR-218-5p | | Myocardial fibroblast (MI mouse model) | Decreased in myocardial fibroblasts in MI<br>Increase inhibits myocardial fibrosis | 106 |
| fca-miR-885 | | Hypoxia cardiomyocyte cell culture<br>Rodent hearts | Reduced in injured cardiomyocytes<br>Increase improved cell viability, reduced apoptotic rates<br>Reduced inflammatory cytokine production | 54,55 |
| fca-miR-2387-5p | | | Unknown | |

4B. Differentially expressed microRNA in HCM compared to healthy

HCM LV specific microRNA compared to the healthy LV

| Enriched microRNA | Samples | Roles/function | References |
|---|---|---|---|
| fca-miR-382-5p | MI mouse model<br>Hypoxia cardiomyocyte cell culture | Increased in MI hearts<br>Inhibition reduces apoptosis | 63 |
| fca-miR-487a-5p | Human HCM hearts<br>Endothelial cell culture | Downregulated in human HCM<br>Regulates fibroblast growth<br>Induced by disturbed flow<br>Promotes endothelial proliferation | 64,65 |
| fca-miR-3958-5p | | Unknown | |
| fca-miR-3958-3p | | Unknown | |

| Reduced microRNA | Samples | Roles/function | References |
|---|---|---|---|
| fca-miR-31-3p | MI rodent models | Upregulated in hypoxia, oxidative stress – promotes remodeling and cardiac dysfunction<br>Reduction protects cardiac injury | 67,105 |
| fca-miR-105-5p | MI rodent models | Downregulated in rodent models of myocardial infarction and in cultured cardiomyocyte<br>Increase was associated with improved cell viability and suppression of cell death pathways | 104 |

HCM LA specific microRNA compared to the healthy LA

| Enriched microRNA | Samples | Roles/function | References |
|---|---|---|---|
| fca-miR-105-5p | See above | | |
| fca-miR-208b-3p | See above | | |
| fca-miR-506-3p | Cardiac ischemia/reperfusion mouse model | Reduced in ischemia<br>Increase has positive effect on myocardial injury | 103 |
| fca-miR-chrD4_30107-3p | | Unknown | |

| Reduced microRNA | Samples | Roles/function | References |
|---|---|---|---|
| fca-miR-205-5p | Cardiac ischemia/reperfusion mouse model | Increased in ischemia: reduced oxidative stress and apoptosis | 102 |

**Table 4** (*continued*)

| | | | |
|---|---|---|---|
| 4B. Differentially expressed microRNA in HCM compared to healthy | | | |
| fca-miR-708 | Healthy and HCM LA and LV<br>Hypoxia exposed cardiomyocyte cell line (H9c2) | Increased in HCM LV<br>overexpression associated with reduced apoptosis and increased cell viability | 20,35,101 |
| Overlapping LV and LA HCM microRNA compared to healthy LV and LA | | | |
| Enriched microRNA | Samples | Roles/function | References |
| fca-miR-21 | Heart (human)<br>Cardiac fibroblasts | Key microRNA hypertrophy pathogenesis<br>Human HCM with fibrosis and end stage heart failure<br>Enriched in cardiac fibroblasts<br>Contributes to cardiac hypertrophy in mouse models human HCM with fibrosis and end stage heart failure | 21,24,26,43,87,88 |
| fca-miR-132 | Cardiomyocytes<br>Human hypertrophic heart disease | Potential therapeutic option<br>Drives pathological cardiomyocyte growth, disrupt autophagy, impairs calcium handling and contractility | 75–77 |
| fca-miR-146b-5p | Heart (canine, rodent)<br>Fibroblasts | Promotes fibrosis<br>Pro-proliferative effects on vascular smooth muscle cells<br>Associated with increased concentrations of proinflammatory cytokines | 45,89-91 |
| Mir 183 cluster fca-miR-96-5p | Human LV<br>Endothelial cells (human, murine) | Enriched in human HCM LV<br>Enriched in endothelial cells<br>Angiogenic potential of endothelial cells | 20,78 |
| fca-miR-183-5p | Endothelial cells (human, murine) | Aortic banding mouse model down-regulated, overexpression: anti-hypertrophic effect<br>Enriched in endothelial cells | 78,100 |
| fca-miR-182 | Endothelial cells (human, murine)<br>Angiogenesis-induced cardiac hypertrophy mouse model | Enriched in human and murine endothelial cells Upregulated during the development of hypertrophy<br>Facilitate polarization of macrophages from M1 to M2 in an inflammatory | 79,98 |
| fca-miR-185-5p | LV samples (human, mouse)<br>Cardiac fibroblasts (mouse model)<br>Cardiomyocyte cell culture | Enriched with myocardial fibrosis (LV)<br>Increased expression stimulated collagen deposition<br>Aortic banding mouse model, miR-185 down-regulated, overexpression: anti-hypertrophic effects | 80,99,100 |
| fca-miR-409-5p | Hypoxia cardiomyocyte cell line | Reduced in hypoxia<br>Mimics inhibited inflammatory response, increased cell viability, reduced apoptosis | 96 |
| fca-miR-3548-5p | Pancreatic tumor cells<br>liver rats<br>not reported in the heart | Influence on lipid metabolism in liver. | 97 |
| Reduced microRNA | Samples | Roles/function | References |
| fca-miR-122-5p | Cardiac disease rat models<br>human HCM | Involvement in myocardial hypertrophy, fibrosis and inflammation.<br>Overexpression: pro-hypertrophy<br>Inhibition: anti-inflammatory and anti-fibrotic effects<br>Potential hub-miRNA | 23,92,93 |
| fca-miR-139-5p | Myocardium of HCM patients<br>Cultured neonatal rat cardiomyocytes | Downregulated HCM hearts. Increase causes increase in cell surface area (cardiomyocytes culture)<br>Overexpression reduced inflammatory cytokines | 84 |
| fca-miR-378-3p | Human cardiomyocytes | Cardiac enriched<br>Increased in CHF<br>Reduced in pressure overload mouse models and in human cardiomyocyte cell cultures<br>Mimics reduce myocardial hypertrophy and inhibited cardiac fibroblast proliferation and collagen production.<br>Regulates cardiac miRNA and mRNA, involved in metabolic remodeling | 35,40,44,83 |
| fca-miR-885-5p | See above | | |

identified miR-208b in blood samples from patients with HCM, indicating its potential use as circulating marker for myocardial hypertrophy [68]. Similar to our results, miR-708 was found in the healthy and HCM human LV and LA, however not as a differentially expressed microRNA [20,35]. Of note, the third highest microRNA in the HCM LA, miR-chrD4_30107-3p, has not been reported in the heart and its role is entirely unknown.

To investigate the biological function of the microRNAs in the HCM heart, IPA analysis was performed. The predicted network analysis identified the inflammatory genes TNFA and IL1A at central positions in the HCM LV and LA. For the HCM LV, the COX2 pathway was centrally placed. For the HCM LA, inflammatory and fibrotic pathways were together with IL1B, IL6, and endothelin-1 in central positions. COX2 is induced in response to stress and has cardioprotective effects in late phases of ischemia and enables chronic healing [69–71]. Interestingly, myocardin-related transcription factor (MRTF)-A and -B (a transcription factor involved in cardiac structure and fibrosis) were shown to be activated in the HCM LA. MRTFs are essential for development as well as the maintenance of cardiac structure and function [72–74]. In cardiac disease, MRTF-A is involved in promoting fibrosis by transducing biomechanical and humoral signals, stimulating the transformation of cardiac fibroblasts to myofibroblasts, and activating a fibrotic gene program [72,73]. These results further support the involvement of inflammation in the LV and LA remodeling process in feline HCM, an activation of cardioprotective pathways in the HCM LV, and the role of fibrosis and structural adjustments in the HCM LA of cats [36,38,39].

### *4.3. Region independent HCM associated microRNA with similar enrichment pattern in the HCM LV and LA*

To detect which microRNAs were associated with HCM independent of cardiac region, we looked for microRNAs that were simultaneously enriched in the HCM LV and LA. We found 11 microRNAs upregulated in both the HCM LV and LA: miR-21-3p, miR-21-5p, miR-132-3p, miR-132-5p, miR-146b-5p, miR-96-5p, miR-182, miR-183-5p, miR-185-5p, miR-409-5p, miR-3548-5p; 4 microRNAs were downregulated in the HCM LV and LA: miR-122-5p, miR-885-5p, miR-378-5p and miR-139-5p [Table 4B].

Of these, miR-21-5p, miR-122-5p, miR-146b-5p have been reported as potentially relevant key microRNAs for the HCM pathogenesis [21,23]. As observed for other species, our results suggest that these microRNAs could play role in myocardial fibrosis and inflammation in feline HCM [Table 4B]. MiR-96 and miR-409-5p, the third highest microRNA in the HCM LV, were also found in the human HCM LV [20]. MiR-3548, has not been reported in the heart so far.

The enrichment of miR-132 is of particular interest for its potential as a future therapeutic option. Studies investigating miR-132 inhibition as treatment for heart failure and prevention of heart failure progression in human hypertrophic heart disease are ongoing [75–77]. Further enriched in the HCM LV and LA were the members of the miR-183 cluster: miR-96, miR-182, miR-183-5p, and miR-185-5p [Table 4B]. The common candidate pathways for miR-96 and miR-183 are the HIPPO pathway, the PI3K/AKT/FOXO signaling pathway, and the regulation of the actin cytoskeleton [78]. The HIPPO, PI3K/AKT and MRTF-A signaling pathways were activated in the HCM LA, similar to what has been observed in human LA samples with atrial fibrillation [39]. MiR-182 was reported to facilitate polarization of macrophages from M1 to M2 in an inflammatory environment [79], and miR-185 was found to be enriched in LV samples from humans with myocardial fibrosis [80]. The enrichment of these microRNAs in the feline HCM myocardium indicates their potential role in cardiac hypertrophy, fibrosis, angiogenesis and macrophage polarization, which was further supported by the results obtained from the network analysis. These findings are further consistent with the increase of macrophages and fibrosis, attempts of angiogenesis and endothelial nitric oxide synthase transcription in the feline HCM LV we observed in previous studies [39,81,82].

The top downregulated microRNAs for the HCM LV and LA were miR-122-5p and miR-885-5p; miR-378-5p and miR-139-5p were also downregulated in both the HCM LV and LA [Table 4B]. The reduction of the LA enriched miR-885 in both the HCM LV and LA, might be consistent with disease associated hypoxia [54,55]. MiR-378, a cardiac enriched microRNA [83], and miR-139-5p were found to be downregulated in the myocardium of HCM patients ([84], Table 4B). The reduction of these microRNAs was consistent with the pro-fibrotic, pro-hypertrophic and inflammatory environment indicated by the pathway analysis, network analysis and our previous findings in the feline HCM heart [36,38,39,81,82].

Limitations of this study include the age difference between control cats and cats with HCM. The influence of age on cardiomyocyte maturation and cardiac remodeling is well known, and differences between neonatal and adult mouse cardiac microRNA signatures have been reported [47,78,85,86]. Therefore, some results might be caused by age and not HCM. However, including a young homogenous control group allowed the identification of the constitutive microRNA profile in the healthy cat heart. To obtain directly applicable information, we study pet cats rather than animal models. For ethical reasons, healthy adult cats are not euthanized. Euthanasia of adult cats is carried out for medical reasons such as progressed systemic diseases that might influence myocardial microRNA profiles and would preclude the differentiation from microRNA profiles associated with HCM [36]. Echocardiography of cats with HCM was obtained at different time points prior to euthanasia. These data do not reflect the stage of heart disease at the time point of death and were therefore not included into the study. Hearts from the young control cats were donated and echocardiography was not part of their assessment, although no cardiac abnormalities were observed on gross and histopathological examination. The sample size was small; however, it was sufficient for the sequencing analysis and the statistical power was increased by the inclusion of paired (matched) tissue samples from the 8 healthy control cats and 3 individual HCM hearts. Furthermore, the cats with HCM had advanced disease, and some of the microRNAs identified might be associated with heart failure and not primarily HCM [44,94]. However, as only cats with HCM were included in the study, the results obtained will still be specific for HCM.

## 5. Conclusions

Our study identified microRNAs likely involved in cardiac homeostasis, which included well known cardiac enriched microRNAs. Additionally, we distinguished region-specific microRNAs and microRNAs associated with the disease. Interestingly, several of the top enriched microRNAs: miR-3958, miR-382-5p, miR-487a-5p (top enriched in the HCM LV), miR-chrD4_30107-3p (in the HCM LA), miR-3548 (enriched in the HCM LV and LA) have either not been reported in the heart or only little is known. Similarly, the enriched miR-21, miR-146b, and reduced miR-122-5p have recently been suggested as key microRNAs for the HCM pathogenesis. Further investigations into the relevance of the unknown and potential hub microRNAs might be of interest. MiR-132, enriched in the HCM LV and LA, could provide future therapeutic approaches. MiR-208b, top enriched in the HCM LA, might be of interest as potential circulating marker for HCM associated changes in the LA.

The microRNA profiles observed in the present study support our previous findings that indicate a central role for ischemia and microvascular alterations in the feline HCM disease process [81,82]. We also observed the involvement of inflammation and growth pathways in the LV and LA, fibrosis and structural adjustments in the LA, and the activation of cardioprotective pathways in the LV [39]. Our study that addresses naturally occurring HCM is of particular interest, as most information about microRNAs in cardiac disease is obtained from mouse models and cell culture, which are both limited in applicability for the naturally occurring disease [95]. The results we obtained are directly applicable to cats. Additionally, the similarity of the feline and human form of HCM indicates the translational relevance of our research to human HCM.

## Declaration of competing interest

The authors have no conflicts of interest to disclose.

## Acknowledgements

The authors sincerely thank Dr. Marco Baron Toaldo, University of Zurich, for the donation of HCM cat hearts, the animal health team at Kingfisher International Inc. for the donation of control cat hearts and Prof. Brandon Lillie, Department of Pathobiology, University of Guelph, who supervised the postmortem examinations of the control cats.

## Funding

This study has received financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC) discovery grant RGPIN-2017-04399.

## References

[1] Payne JR, Brodbelt DC, Luis Fuentes V. Cardiomyopathy prevalence in 780 apparently healthy cats in rehoming centres (the CatScan study). J Vet Cardiol. Dec 1 2015;17:S244–57.
[2] Fox PRDM, Liu SKD, Maron BJ. Echocardiographic assessment of spontaneously occurring feline hypertrophic cardiomyopathy: an animal model of human disease. Circulation. Nov 1995;92(9):2645–51.
[3] Maron BJ, Fox PR. Hypertrophic cardiomyopathy in man and cats. J Vet Cardiol. Dec 1 2015;17:S6–9.
[4] Kittleson MD, Meurs KM, Harris SP. The genetic basis of hypertrophic cardiomyopathy in cats and humans. J Vet Cardiol Off J Eur Soc Vet Cardiol. Dec 2015;17(Suppl. 1):S53–73.
[5] Maron BJ, Maron MS. Hypertrophic cardiomyopathy. The Lancet. Jan 19 2013;381(9862):242–55.
[6] O'Donnell K, Adin D, Atkins CE, DeFrancesco T, Keene BW, Tou S, et al. Absence of known feline MYH7 and MYBPC3 variants in a diverse cohort of cats with hypertrophic cardiomyopathy. Anim Genet. 2021;52(4):542–4.
[7] Meurs KM, Sanchez X, David RM, Bowles NE, Towbin JA, Reiser PJ, et al. A cardiac myosin binding protein C mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy. Hum Mol Genet. Dec 1 2005;14(23):3587–93.
[8] Meurs KM, Norgard MM, Ederer MM, Hendrix KP, Kittleson MD. A substitution mutation in the myosin binding protein C gene in ragdoll hypertrophic cardiomyopathy. Genomics. Aug 1 2007;90(2):261–4.
[9] Meurs KM, Williams BG, DeProspero D, Friedenberg SG, Malarkey DE, Ezzell JA, et al. A deleterious mutation in the ALMS1 gene in a naturally occurring model of hypertrophic cardiomyopathy in the Sphynx cat. Orphanet J Rare Dis. Feb 27 2021;16(1):108.
[10] Schipper T, Van Poucke M, Sonck L, Smets P, Ducatelle R, Broeckx BJG, et al. A feline orthologue of the human MYH7 c.5647G>A (p.(Glu1883Lys)) variant causes hypertrophic cardiomyopathy in a domestic shorthair cat. Eur J Hum Genet. 2019 Nov;27(11):1724–30.
[11] Wess G, Schinner C, Weber K, Küchenhoff H, Hartmann K. Association of A31P and A74T polymorphisms in the myosin binding protein C3 gene and hypertrophic cardiomyopathy in Maine Coon and other breed cats. J Vet Intern Med. Jun 2010;24(3):527–32.
[12] Carlos Sampedrano C, Chetboul V, Mary J, Tissier R, Abitbol M, Serres F, et al. Prospective echocardiographic and tissue Doppler imaging screening of a population of Maine

Coon cats tested for the A31P mutation in the myosin-binding protein C gene: a specific analysis of the heterozygous status. J Vet Intern Med. Feb 2009;23(1):91–9.
[13] Longeri M, Ferrari P, Knafelz P, Mezzelani A, Marabotti A, Milanesi L, et al. Myosin-binding protein C DNA variants in domestic cats (A31P, A74T, R820W) and their association with hypertrophic cardiomyopathy. J Vet Intern Med. 2013;27(2):275–85.
[14] Mary J, Chetboul V, Sampedrano CC, Abitbol M, Gouni V, Trehiou-Sechi E, et al. Prevalence of the MYBPC3-A31P mutation in a large European feline population and association with hypertrophic cardiomyopathy in the Maine Coon breed. J Vet Cardiol. Dec 1 2010;12(3):155–61.
[15] Trehiou-Sechi E, Tissier R, Gouni V, Misbach C, Petit Am p, Balouka D, et al. Comparative echocardiographic and clinical features of hypertrophic cardiomyopathy in 5 breeds of cats: a retrospective analysis of 344 cases (2001 − 2011). J Vet Intern Med. 2012;26(3):532–41.
[16] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. Jan 23 2004;116(2):281–97.
[17] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. Jan 23 2009; 136(2):215–33.
[18] Roma-Rodrigues C, Raposo LR, Fernandes AR. MicroRNAs based therapy of hypertrophic cardiomyopathy: the road traveled so far. Biomed Res Int. 2015;2015:983290.
[19] Quiat D, Olson EN. MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. J Clin Invest. Jan 2 2013;123(1):11–8.
[20] Kuster DWD, Mulders J, ten Cate FJ, Michels M, dos Remedios CG, da Costa Martins PA, et al. MicroRNA transcriptome profiling in cardiac tissue of hypertrophic cardiomyopathy patients with MYBPC3 mutations. J Mol Cell Cardiol. Dec 1 2013;65:59–66.
[21] Osmak G, Baulina N, Kiselev I, Favorova O. MiRNA-regulated pathways for hypertrophic cardiomyopathy: network-based approach to insight into pathogenesis. Genes. Dec 18 2021;12(12):2016. https://doi.org/10.3390/genes1212201634946964. PMC8701189.
[22] Li J, Wu Z, Zheng D, Sun Y, Wang S, Yan Y. Bioinformatics analysis of the regulatory lncRNA-miRNA-mRNA network and drug prediction in patients with hypertrophic cardiomyopathy. Mol Med Rep. Jul 2019;20(1):549–58. https://doi.org/10.3892/mmr.2019.10289.
[23] Chen JY, Xie ZX, Dai JZ, Han JY, Wang K, Lu LH, et al. Reconstruction and analysis of potential biomarkers for hypertrophic cardiomyopathy based on a competing endogenous RNA network. BMC Cardiovasc Disord. Sep 22 2022;22(1):422. https://doi.org/10.1186/s12872-022-02862-9.
[24] Bauersachs J. Regulation of myocardial fibrosis by microRNAs. J Cardiovasc Pharmacol. Nov 2010;56(5):454–9.
[25] Piubelli C, Meraviglia V, Pompilio G, D'Alessandra Y, Colombo GI, Rossini A. microRNAs and cardiac cell fate. Cells. Aug 5 2014;3(3):802–23.
[26] Tang R, Long T, Lui KO, Chen Y, Huang ZP. A roadmap for fixing the heart: RNA regulatory networks in cardiac disease. Mol Ther Nucleic Acids. Jun 5 2020;20:673–86.
[27] Wang L, Lu F, Xu J. Identification of potential miRNA-mRNA regulatory network contributing to hypertrophic cardiomyopathy (HCM). Front Cardiovasc Med. May 31 2021; 8:660372. https://doi.org/10.3389/fcvm.2021.66037234136543. PMC8200816.
[28] Kozomara A, Birgaoanu M, Griffiths-Jones S. miRBase: from microRNA sequences to function. Nucleic Acids Res. Jan 8 2019;47(D1):D155–62.
[29] Laganà A, Dirksen WP, Supsavhad W, Yilmaz AS, Ozer HG, Feller JD, et al. Discovery and characterization of the feline miRNAome. Sci Rep. Aug 23 2017;7(1):9263.
[30] Weber K, Rostert N, Bauersachs S, Wess G. Serum microRNA profiles in cats with hypertrophic cardiomyopathy. Mol Cell Biochem. Apr 1 2015;402(1):171–80.
[31] Palacín M, Reguero JR, Martín M, Díaz Molina B, Morís C, Alvarez V, et al. Profile of microRNAs differentially produced in hearts from patients with hypertrophic cardiomyopathy and sarcomeric mutations. Clin Chem. Nov 2011;57(11):1614–6.
[32] Wang C, Wang S, Zhao P, Wang X, Wang J, Wang Y, et al. MiR-221 promotes cardiac hypertrophy in vitro through the modulation of p27 expression. J Cell Biochem. 2012; 113(6):2040–6.
[33] Kakimoto Y, Tanaka M, Kamiguchi H, Hayashi H, Ochiai E, Osawa M. MicroRNA deep sequencing reveals chamber-specific miR-208 family expression patterns in the human heart. Int J Cardiol. May 2016;211:43–8.
[34] Johnson EK. Regional differences in mRNA and lncRNA expression profiles in non-failing human atria and ventricles. Sci Rep. Sep 17 2018;8(1):13919. https://doi.org/10.1038/s41598-018-32154-230224797. PMC6141608.
[35] Hsu J, Hanna P, Van Wagoner DR, Barnard J, Serre D, Chung MK, et al. Whole genome expression differences in human left and right atria ascertained by RNA sequencing. Circ Cardiovasc Genet. Jun 2012;5(3):327–35. https://doi.org/10.1161/CIRCGENETICS.111.961631.
[36] Fonfara S, Kitz S, Monteith G, Hahn S, Kipar A. Myocardial transcription of inflammatory and remodeling markers in cats with hypertrophic cardiomyopathy and systemic diseases associated with an inflammatory phenotype. Res Vet Sci. May 1 2021;136: 484–94.
[37] Fonfara S, Hetzel U, Hahn S, Kipar A. Age- and gender-dependent myocardial transcription patterns of cytokines and extracellular matrix remodelling enzymes in cats with non-cardiac diseases. Exp Gerontol. Dec 2015;72:117–23.
[38] Colpitts ME, Caswell JL, Monteith G, Joshua J, O'Sullivan ML, Raheb S, et al. Cardiac gene activation varies between young and adult cats and in the presence of hypertrophic cardiomyopathy. Res Vet Sci. Dec 2022;152:38–47.
[39] Joshua J, Caswell J, O'Sullivan ML, Wood G, Fonfara S. Feline myocardial transcriptome in health and in hypertrophic cardiomyopathy-a translational animal model for human disease. PLoS One. Mar 16 2023;18(3):e0283244. https://doi.org/10.1371/journal.pone.0283244.
[40] Doñate Puertas R, Jalabert A, Meugnier E, Euthine V, Chevalier P, Rome S. Analysis of the microRNA signature in left atrium from patients with valvular heart disease reveals their implications in atrial fibrillation. PLoS One. May 3 2018;13(5):e0196666. https://doi.org/10.1371/journal.pone.0196666.
[41] Liu H, Chen GX, Liang MY, Qin H, Rong J, Yao JP, et al. Atrial fibrillation alters the microRNA expression profiles of the left atria of patients with mitral stenosis. BMC Cardiovasc Disord. Jan 25 2014;14:10. https://doi.org/10.1186/1471-2261-14-10.
[42] Matkovich SJ, Hu Y, Dorn GW. Regulation of cardiac microRNAs by cardiac microRNAs. Circ Res. Jun 21 2013;113(1):62–71.
[43] Vacchi-Suzzi C, Hahne F, Scheubel P, Marcellin M, Dubost V, Westphal M, et al. Heart structure-specific transcriptomic atlas reveals conserved microRNA-mRNA interactions. PLoS One. 2013;8(1):e52442. https://doi.org/10.1371/journal.pone.0052442.
[44] Wojciechowska A, Braniewska A, Kozar-Kamińska K. MicroRNA in cardiovascular biology and disease. Adv Clin Exp Med Off Organ Wroclaw Med Univ. Aug 2017;26(5): 865–74.
[45] Koenig EM, Fisher C, Bernard H, Wolenski FS, Gerrein J, Carsillo M, et al. The beagle dog MicroRNA tissue atlas: identifying translatable biomarkers of organ toxicity. BMC Genomics. Aug 17 2016;17:649.
[46] Shi Y, Han Y, Niu L, Li J, Chen Y. MiR-499 inhibited hypoxia/reoxygenation induced cardiomyocytes injury by targeting SOX6. Biotechnol Lett. Jul 2019;41(6–7):837–47.
[47] Boon RA, Iekushi K, Lechner S, Seeger T, Fischer A, Heydt S, et al. MicroRNA-34a regulates cardiac ageing and function. Nature. Mar 7 2013;495(7439):107–10.
[48] Yang Y, Cheng HW, Qiu Y, Dupee D, Noonan M, Lin YD, et al. MicroRNA-34a plays a key role in cardiac repair and regeneration following myocardial infarction. Circ Res. Aug 14 2015;117(5):450–9.
[49] Bernardo BC, Nguyen SS, Winbanks CE, Gao X, Boey EJH, Tham YK, et al. Therapeutic silencing of miR-652 restores heart function and attenuates adverse remodeling in a setting of established pathological hypertrophy. FASEB J. Dec 2014;28(12):5097–110.
[50] Jentzsch C, Leierseder S, Loyer X, Flohrschütz I, Sassi Y, Hartmann D, et al. A phenotypic screen to identify hypertrophy-modulating microRNAs in primary cardiomyocytes. J Mol Cell Cardiol. Jan 2012;52(1):13–20. https://doi.org/10.1016/j.yjmcc.2011.07.010.
[51] Wu H, Wang Y, Wang X, Li R, Yin D. MicroRNA-365 accelerates cardiac hypertrophy by inhibiting autophagy via the modulation of Skp2 expression. Biochem Biophys Res Commun. Mar 4 2017;484(2):304–10. https://doi.org/10.1016/j.bbrc.2017.01.108.
[52] Xing B, Li QJ, Li H, Chen SS, Cui ZY, Ma J, et al. miR-140-5p aggravates hypoxia-induced cell injury via regulating MLK3 in H9c2 cells. Biomed Pharmacother. Jul 2018;103:1652–7.
[53] Li Y, Li Z, Liu J, Liu Y, Miao G. miR-190-5p alleviates myocardial ischemia-reperfusion injury by targeting PHLPP1. Dis Markers. 2021;2021:8709298.
[54] Meng X, Mei L, Zhao C, Chen W, Zhang N. miR-885 mediated cardioprotection against hypoxia/reoxygenation-induced apoptosis in human cardiomyocytes via inhibition of PTEN and BCL2L11 and modulation of AKT/mTOR signaling. J Cell Physiol. Nov 2020;235(11):8048–57.
[55] Zhang X, Gu H, Wang L, Huang F, Cai J. MiR-885-3p is down-regulated in peripheral blood mononuclear cells from T1D patients and regulates the inflammatory response via targeting TLR4/NF-κB signaling. J Gene Med. Jan 2020;22(1):e3145. https://doi.org/10.1002/jgm.314531763742. [Epub 2019 Dec 16].
[56] Sundararajan V, Burk UC, Bajdak-Rusinek K. Revisiting the miR-200 family: a clan of five siblings with essential roles in development and disease. Biomolecules. Jun 3 2022;12(6):781.
[57] Xu H, Jin L, Chen Y, Li J. Downregulation of microRNA-429 protects cardiomyocytes against hypoxia-induced apoptosis by increasing Notch1 expression. Int J Mol Med. Jun 2016;37(6):1677–85.
[58] Zhu Q, Hu F. Antagonism of miR-429 ameliorates anoxia/reoxygenation injury in cardiomyocytes by enhancing MO25/LKB1/AMPK mediated autophagy. Life Sci. Oct 15 2019;235:116842.
[59] Mia MM, Singh MK. The hippo signaling pathway in cardiac development and diseases. Front Cell Dev Biol. 2019;7:211.
[60] Zhou W, Zhao M. How hippo signaling pathway modulates cardiovascular development and diseases. J Immunol Res. 2018;2018:3696914.
[61] O'Neal WT, Griffin WF, Dries-Devlin JL, Kent SD, Chen J, Willis MS, et al. Ephrin-Eph signaling as a potential therapeutic target for the treatment of myocardial infarction. Med Hypotheses. Jun 2013;80(6):738–44.
[62] Bayer AL, Alcaide P. MyD88: at the heart of inflammatory signaling and cardiovascular disease. J Mol Cell Cardiol. Dec 2021;161:75–85.
[63] Zhang L, Zhu H, Teng X, Sheng X, Yu B. Modulation of miR-382-5p reduces apoptosis of myocardial cells after acute myocardial infarction. Autoimmunity. Jun 2021;54(4): 195–203. https://doi.org/10.1080/08916934.2021.1910812.
[64] Wang WL, Chen LJ, Wei SY, Shih YT, Huang YH, Lee PL, et al. Mechanoresponsive Smad5 enhances MiR-487a processing to promote vascular endothelial proliferation in response to disturbed flow. Front Cell Dev Biol. Apr 20 2021;9:647714. https://doi.org/10.3389/fcell.2021.647714.
[65] Gao J, Collyer J, Wang M, Sun F, Xu F. Genetic dissection of hypertrophic cardiomyopathy with myocardial RNA-Seq. Int J Mol Sci. Apr 25 2020;21(9):3040. https://doi.org/10.3390/ijms21093040.
[66] Deng H, Chu X, Song Z, Deng X, Xu H, Ye Y, et al. MicroRNA-1185 induces endothelial cell apoptosis by targeting UVRAG and KRIT1. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol. 2017;41(6):2171–82.
[67] Wang Y, Men M, Yang W, Zheng H, Xue S. MiR-31 downregulation protects against cardiac ischemia/reperfusion injury by targeting protein kinase C epsilon (PKCε) directly. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol. 2015;36(1): 179–90.
[68] Lin LR, Hu XQ, Lu LH, Dai JZ, Lin NN, Wang RH, et al. MicroRNA expression profiles in familial hypertrophic cardiomyopathy with myosin-binding protein C3 (MYBPC3) gene mutations. BMC Cardiovasc Disord. Jun 18 2022;22(1):278. https://doi.org/10.1186/s12872-022-02714-6.
[69] Zhu L, Xu C, Huo X, Hao H, Wan Q, Chen H, et al. The cyclooxygenase-1/mPGES-1/endothelial prostaglandin EP4 receptor pathway constrains myocardial ischemia-reperfusion injury. Nat Commun. Apr 23 2019;10(1):1888.

[70] Shinmura K, Tang XL, Wang Y, Xuan YT, Liu SQ, Takano H, et al. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. Proc Natl Acad Sci U S A. Aug 29 2000;97(18):10197–202.
[71] Timmers L, Sluijter JPG, Verlaan CWJ, Steendijk P, Cramer MJ, Emons M, et al. Cyclooxygenase-2 inhibition increases mortality, enhances left ventricular remodeling, and impairs systolic function after myocardial infarction in the pig. Circulation. Jan 23 2007;115(3):326–32.
[72] Parmacek MS. Myocardin-related transcription factor-A. Circ Res. Jul 23 2010;107(2): 168–70.
[73] Münch J, Abdelilah-Seyfried S. Sensing and responding of cardiomyocytes to changes of tissue stiffness in the diseased heart. Front Cell Dev Biol. Feb 26 2021;9:642840. https://doi.org/10.3389/fcell.2021.64284033718383. PMC7952448.
[74] Mokalled MH, Carroll KJ, Cenik BK, Chen B, Liu N, Olson EN, et al. Myocardin-related transcription factors are required for cardiac development and function. Dev Biol. Oct 15 2015;406(2):109–16.
[75] Foinquinos A, Batkai S, Genschel C, Viereck J, Rump S, Gyöngyösi M, et al. Preclinical development of a miR-132 inhibitor for heart failure treatment. Nat Commun. Jan 31 2020;11(1):633.
[76] Hinkel R, Batkai S, Bähr A, Bozoglu T, Straub S, Borchert T, et al. AntimiR-132 attenuates myocardial hypertrophy in an animal model of percutaneous aortic constriction. J Am Coll Cardiol. Jun 15 2021;77(23):2923–35.
[77] Täubel J, Hauke W, Rump S, Viereck J, Batkai S, Poetzsch J, et al. Novel antisense therapy targeting microRNA-132 in patients with heart failure: results of a first-in-human phase 1b randomized, double-blind, placebo-controlled study. Eur Heart J. Jan 7 2021;42(2):178–88.
[78] Castellan RF, Vitiello M, Vidmar M, Johnstone S, Iacobazzi D, Mellis D, et al. miR-96 and miR-183 differentially regulate neonatal and adult postinfarct neovascularization. JCI Insight. Jul 23 2020;5(14):134888.
[79] Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. Cardiovasc Res. Jun 1 2019;115(7):1205–16.
[80] Lin R, Rahtu-Korpela L, Szabo Z, Kemppi A, Skarp S, Kiviniemi AM, et al. MiR-185-5p regulates the development of myocardial fibrosis. J Mol Cell Cardiol. Apr 2022;165: 130–40.
[81] Rodríguez JMM, Fonfara S, Hetzel U, Kipar A. Feline hypertrophic cardiomyopathy: reduced microvascular density and involvement of CD34+ interstitial cells. Vet Pathol. Mar 1 2022;59(2):269–83.
[82] Kitz S, Fonfara S, Hahn S, Hetzel U, Kipar A. Feline hypertrophic cardiomyopathy: the consequence of cardiomyocyte-initiated and macrophage-driven remodeling processes? Vet Pathol. Jul 1 2019;56(4):565–75.
[83] Yuan J, Liu H, Gao W, Zhang L, Ye Y, Yuan L, et al. MicroRNA-378 suppresses myocardial fibrosis through a paracrine mechanism at the early stage of cardiac hypertrophy following mechanical stress. Theranostics. 2018;8(9):2565–82.
[84] Ming S, Shui-yun W, Wei Q, Jian-hui L, Ru-tai H, Lei S, et al. miR-139-5p inhibits isoproterenol-induced cardiac hypertrophy by targetting c-Jun. Biosci Rep. Mar 9 2018;38(2):BSR20171430.
[85] Guo Y, Pu WT. Cardiomyocyte maturation: new phase in development. Circ Res. Apr 10 2020;126(8):1086–106.
[86] Yusifov A, Woulfe KC, Bruns DR. Mechanisms and implications of sex differences in cardiac aging. J Cardiovasc Aging. 2022;2:20. https://doi.org/10.20517/jca.2022.0135419571. PMC9004711. [Epub 2022 Mar 16].
[87] Scolari FL, Faganello LS, Garbin HI, Mattos Piva E, B, Biolo A. A systematic review of microRNAs in patients with hypertrophic cardiomyopathy. Int J Cardiol. Mar 15 2021;327:146–54.
[88] Dong Y, Xu S, Liu J, Ponnusamy M, Zhao Y, Zhang Y, et al. Non-coding RNA-linked epigenetic regulation in cardiac hypertrophy. Int J Biol Sci. 2018;14(9):1133–41.
[89] Wang J, Wang Y, Han J, Li Y, Xie C, Xie L, et al. Integrated analysis of microRNA and mRNA expression profiles in the left atrium of patients with nonvalvular paroxysmal atrial fibrillation: role of miR-146b-5p in atrial fibrosis. Heart Rhythm. May 2015;12 (5):1018–26.
[90] Zhang H, Zhang Q, Liu Y, Xue T. miR-146a and miR-146b predict increased restenosis and rapid angiographic stenotic progression risk in coronary heart disease patients who underwent percutaneous coronary intervention. Ir J Med Sci. May 2020;189(2): 467–74.
[91] Ye Q, Liu Q, Ma X, Bai S, Chen P, Zhao Y, et al. MicroRNA-146b-5p promotes atrial fibrosis in atrial fibrillation by repressing TIMP4. J Cell Mol Med. Nov 2021;25(22): 10543–53.
[92] Song G, Zhu L, Ruan Z, Wang R, Shen Y. MicroRNA-122 promotes cardiomyocyte hypertrophy via targeting FoxO3. Biochem Biophys Res Commun. Nov 19 2019;519(4): 682–8.
[93] Liu Y, Song JW, Lin JY, Miao R, Zhong JC. Roles of microRNA-122 in cardiovascular fibrosis and related diseases. Cardiovasc Toxicol. Oct 2020;20(5):463–73.
[94] Zhang X, Schulze PC. MicroRNAs in heart failure: non-coding regulators of metabolic function. Biochim Biophys Acta BBA - Mol Basis Dis. Dec 2016;1862(12):2276–87.
[95] Vegter EL, Ovchinnikova ES, Silljé HHW, Meems LMG, Pol A van der, Velde AR van der, et al. Rodent heart failure models do not reflect the human circulating microRNA signature in heart failure. PLoS One. May 5 2017;12(5):e0177242.
[96] Xue Q, Yang D, Zhang J, Gan P, Lin C, Lu Y, et al. USP7, negatively regulated by miR-409-5p, aggravates hypoxia-induced cardiomyocyte injury. APMIS Acta Pathol Microbiol Immunol Scand. Mar 2021;129(3):152–62.
[97] Guo S, Yan K, Fang X, Ni Y, Ma W, Zhao R. α-Lipoic acid alleviates hepatic lipid deposition by inhibiting FASN expression via miR-3548 in rats. Nutrients. Jul 8 2021;13(7): 2331.
[98] Li N, Hwangbo C, Jaba IM, Zhang J, Papangeli I, Han J, et al. miR-182 modulates myocardial hypertrophic response induced by angiogenesis in heart. Sci Rep. Feb 18 2016; 6:21228.
[99] Kim JO, Song DW, Kwon EJ, Hong SE, Song HK, Min CK, et al. miR-185 plays an anti-hypertrophic role in the heart via multiple targets in the calcium-signaling pathways. PLoS One. 2015;10(3):e0122509.
[100] Gong FH, Chen XL, Zhang Q, Xiao XQ, Yang YS, Song BJ, et al. MicroRNA-183 as a novel regulator protects against cardiomyocytes hypertrophy via targeting TIAM1. Am J Hypertens. Jan 5 2022;35(1):87–95.
[101] Qu Y, Zhang J, Zhang J, Xiao W. MiR-708-3p alleviates inflammation and myocardial injury after myocardial infarction by suppressing ADAM17 expression. Inflammation. Jun 2021;44(3):1083–95.
[102] Xu Y, Guo W, Zeng D, Fang Y, Wang R, Guo D, et al. Inhibiting miR-205 alleviates cardiac ischemia/reperfusion injury by regulating oxidative stress, mitochondrial function, and apoptosis. Oxid Med Cell Longev. 2021;2021:9986506.
[103] Zhang M, Wang JY, Li L, Li GM. MiR-506 alleviates myocardial ischemia-reperfusion injury via targeting PI3K/AKT. Eur Rev Med Pharmacol Sci. Dec 2020;24(24): 12896–903.
[104] Shin S, Choi JW, Moon H, Lee CY, Park JH, Lee J, et al. Simultaneous suppression of multiple programmed cell death pathways by miRNA-105 in cardiac ischemic injury. Mol Ther Nucleic Acids. Jan 10 2019;14:438–49.
[105] Martinez EC, Lilyanna S, Wang P, Vardy LA, Jiang X, Armugam A, et al. MicroRNA-31 promotes adverse cardiac remodeling and dysfunction in ischemic heart disease. J Mol Cell Cardiol. Nov 2017;112:27–39.
[106] Sun B, Zhao C, Mao Y. MiR-218-5p mediates myocardial fibrosis after myocardial infarction by targeting CX43. Curr Pharm Des. 2021;27(44):4504–12.
[107] Yao CX, Wei QX, Zhang YY, Wang WP, Xue LX, Yang F, et al. miR-200b targets GATA-4 during cell growth and differentiation. RNA Biol. Apr 2013;10(4):465–80.
[108] Chan YC, Khanna S, Roy S, Sen CK. miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. J Biol Chem. Jan 21 2011;286(3):2047–56. https://doi.org/10.1074/jbc.M110.15879021081489. PMC3023502. [Epub 2010 Nov 16].
[109] Zhang F, Cheng N, Du J, Zhang H, Zhang C. MicroRNA-200b-3p promotes endothelial cell apoptosis by targeting HDAC4 in atherosclerosis. BMC Cardiovasc Disord. Apr 12 2021;21 (1):172. https://doi.org/10.1186/s12872-021-01980-033845782. PMC8042726.
[110] Wang Y, Jiang Y, Sun X, Shen X, Wang H, Dong C, et al. Downregulation of miR-200a protects cardiomyocyte against apoptosis. Biomed Pharmacother Biomed Pharmacother. Mar 2020;123:109303.
[111] Raso A, Dirkx E, Philippen LE, Fernandez-Celis A, De Majo F, Sampaio-Pinto V, et al. Therapeutic delivery of miR-148a suppresses ventricular dilation in heart failure. Mol Ther J Am Soc Gene Ther. Mar 6 2019;27(3):584–99.
[112] Hou W, Zhu X, Liu J, Ma J. Inhibition of miR-153 ameliorates ischemia/reperfusion-induced cardiomyocytes apoptosis by regulating Nrf2/HO-1 signaling in rats. Biomed Eng Online. Mar 6 2020;19(1):15.