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Review

Integrative System Biology Analyses Identify Seven Micrornas to Predict Heart Failure

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Abstract: Heart failure (HF) has several etiologies including myocardial infarction (MI) and left ventricular remodeling (LVR), but its progression remains difficult to predict in clinical practice. Systems biology analyses of LVR after MI predict molecular insights of this event such as modulation of microRNA (miRNA) that could be used as a signature of HF progression. To define a miRNA signature of LVR after MI, we use 2 systems biology approaches integrating either proteomic data generated from LV of post-MI rat induced by left coronary artery ligation or multi-omics data (proteins and non-coding RNAs) generated from plasma of post-MI patients from the REVE-2 study. The first approach predicts 13 miRNAs and 3 of these miRNAs were validated to be associated with LVR *in vivo*: miR-21-5p, miR-23a-3p and miR-222-3p. The second approach predicts 24 miRNAs among 1310 molecules and 6 of these miRNAs were selected to be associated with LVR *in silico*: miR-17-5p, miR-21-5p, miR-26b-5p, miR-222-3p, miR-335-5p and miR-375. We identified a signature of 7 microRNAs associated with LVR after MI that support the interest of integrative systems biology analyses to define a miRNA signature of HF progression.

Keywords: biomarkers; miRNAs; heart failure; system biology

1. Introduction

Heart failure (HF) is a major cause of mortality in occidental countries that is difficult to predict in clinical practice [1]. HF can be the consequence of left ventricle remodeling (LVR) induced by a myocardial infarction (MI) [2,3]. LVR is characterized by cardiac hypertrophy and reduction of LV wall. Although, LVR is an adaptive response early after MI, it is becoming deleterious in a long term [4]. Deciphering molecular events underlying LVR may offer new opportunities in the identification of early predictive biomarkers of LVR and HF. Omics approaches including transcriptomics, proteomics, and metabolomics have been extensively used to explore these mechanisms but the amount of complex generated data prevents from their comprehensive analysis.

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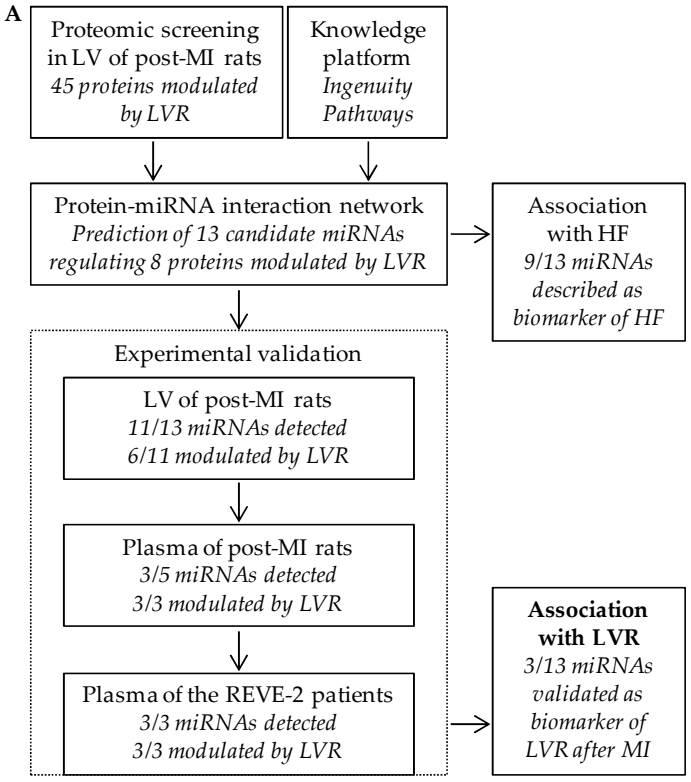
Recently, systems biology opened new opportunities to understand molecular networks and identify new targets involved in HF [5,6]. Among those potential targets, microRNAs (miRNAs) are non-coding RNAs of 19 to 23 nucleotides that regulate gene expression by targeting messenger RNAs [7]. MiRNAs are involved in many processes like cardiomyocyte hypertrophy, fibroblast to myofibroblast transformation and cell to cell communication [8–11]. The modulation of expression of a small set of miRNAs associated with LVR may define a miRNA signature to detect this process. The present study aims to define a signature of miRNAs associated with LVR after MI to predict HF. We use two systems biology analyses integrating either proteomic data generated from LV of post-MI rat induced by left coronary artery ligation [12-14] or multi-omics data (proteins and non-coding RNAs) generated from plasma of post-MI patients from the REVE-2 study and gathered in a molecular network of LVR.

2. Results

2.1. Analysis of the protein-miRNA network derived from post-MI rats identified circulating miR-21-5p, miR-23a-3p and miR-222-3p to be associated with LVR after MI

The proteomic screening in LV of post-MI male rats [12], previously published [13,14], allowed the identification of 45 proteins modulated by LVR. Using the Qiagen's Ingenuity Pathway knowledge base, we built a protein-miRNA interaction network highlighting 13 candidate miRNAs which were prioritized to identify candidate miRNAs to detect LVR after MI (**Figure 1**).



B

Candidate miRNAs	Post-MI		
	Rat model		REVE-2 patients
	LV	Plasma	
miR-21-3p	↕	X	
miR-21-5p	↕	↕	↕
miR-23a-3p	↕	↕	↕
miR-29b-3p	↔		
miR-122	X		
miR-133a	↔		
miR-145-5p	↔		
miR-210	X		
miR-222-3p	↕	↕	↕
miR-320a	↕		
miR-338-3p	↔		
miR-377-5p	↕	X	
miR-483-3p	↔		

Figure 1. Identification of miR-21-5p, miR-23a-3p and miR-222-3p to detect left ventricular remodeling (LVR) after myocardial infarction (MI) and to predict heart failure (HF). (**A**) Design and (**B**) experimental validation of the 13 candidate miRNAs predicted from the proteomic data obtained in LV of post-MI rats by the Ingenuity Pathway knowledge platform. Quantification of candidate miRNAs in LV of post-MI rats and in plasma of post-MI rats and REVE-2 patients are normalized with miR-423-3p and *Caenorhabditis elegans* Cel-39 respectively. ↕ indicates a significant modulation of miRNAs ($p < 0.05$) detected between sham- and post-MI rats / between patients with no and high

LVR, ↔ indicates no modulation, X indicates a lack of detection. MiRNAs remaining after the validation process are in bold.

The 13 candidate miRNAs are predicted to interact with 8 out of the 45 proteins modulated by LVR [10], testifying that they are involved in LVR after MI. Moreover, 9 out of the 13 candidate miRNAs have been described as biomarkers of HF: miR-21-3p, miR-21-5p, miR-23a-3p, miR-29b-3p, miR-122, miR-133a, miR-145-5p, miR-222-3p and miR-320a [10,15-19], confirming their potential as targets to predict HF (**Figure 1A**). To prioritize candidate miRNAs with high relation specificity with LVR, we evaluated *in vivo* the association of the 13 candidate miRNAs with LVR after MI (**Figure 1B**). First, we excluded 7 candidate miRNAs which were not detected (miR-122 and miR-210) or not modulated by LVR in LV of post-MI rats (miR-29b-3p, miR-133a, miR-145-5p, miR-338-3p and miR-483-3p). Second, we excluded 2 candidate miRNAs which were not detected (miR-21-3p and miR-377-5p) or not measurable (miR-320a) in plasma of post-MI rats. To date, we validated the 3 candidate miRNAs: miR-21-5p, miR-23a-3p and miR-222-3p for their association with LVR in plasma of post-MI patients, especially in men, from the REVE-2 study [10] confirming their potential as circulating biomarkers of adverse LVR after MI to predict HF.

2.2. Analysis of the REVE-2 network identified miR-21-5p, miR-222-3p, miR-335-5p, miR-26b-5p, miR-375 and miR-17-5p to detect LVR after MI

The REVE-2 molecular data generated by the measurement of 24 variables (including miR-21-5p, miR-23a-3p and miR-222-3p) and the EdgeLeap's knowledge platform EdgeBox were used to build the REVE-2 molecular interaction network described in detail elsewhere [20]. The REVE-2 network contains 1310 molecules, including 24 miRNAs which were prioritized to identify candidate miRNAs to detect LVR after MI (**Figure 2**).

Fourteen out of the 24 candidate miRNAs are decribed to be associated with HF: miR-21-5p, miR-222-3p, miR-423-5p, miR-26b-5p, miR-23a-3p, miR-744-5p, miR-133a-3p, miR-17-5p, miR-29c-3p, miR-145-5p, miR-29b-3p, let-7g-5p, miR-143-3p and miR-451a [10,15,17,18,21-24], confirming they are interesting targets to predict HF (**Figure 2A-B**). To prioritize candidate miRNAs with high relation specificity with LVR, we evaluated *in silico* the association of the 24 candidate miRNAs with LVR after MI through 2 criteria: active modules and betweeness centrality (**Figure 2A**). To avoid the selection of miRNAs associated with mechanisms not specific of LVR such as inflammation [20], we excluded the 15 candidate miRNAs active only at baseline. However, it could be interesting to analyze the miRNAs only active at baseline as potential biomarker of early LVR. To avoid the selection of miRNAs less significant in LVR, we excluded 13 miRNAs that were not in the top 50 molecules with the highest centrality. When combining these 2 criteria, among the 24 candidate miRNAs, only 6 remained: miR-21-5p, miR-222-3p, miR-335-5p, miR-26b-5p, miR-375 and miR-17-5p (**Figure 2B**). To date, only miR-21-5p and miR-222-3p, also identified by the first approach, were validated *in vivo* to detect LVR after MI [10], testifying that the signature defined by the 6 last candidate miRNAs may be used as circulating biomarker of adverse LVR after MI to predict HF.

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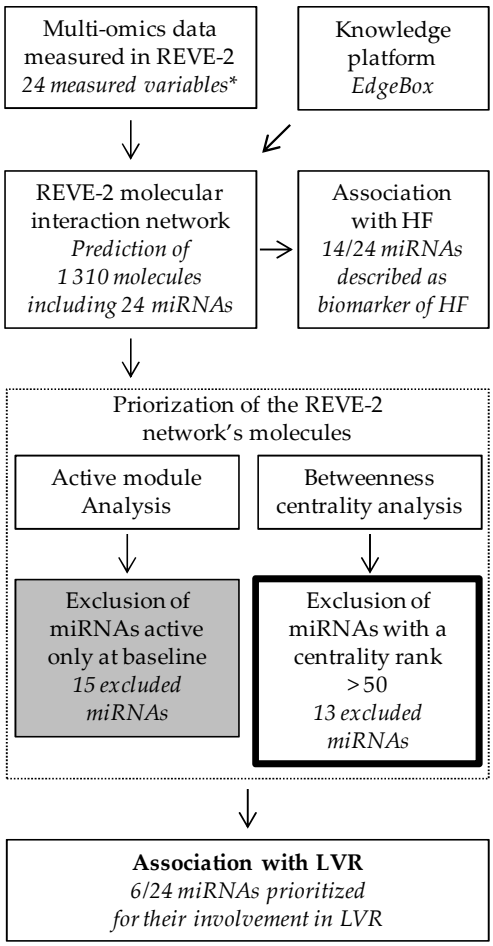
A



B

Priorized candidate miRNAs (centrality rank)		REVE-2 timepoint (months)			
		0	1	3	12
1	**miR-21-5p***	√		√	
2	**miR-222-3p***	√		√	
3	miR-423-5p*	√			
5	**miR-335-5p**		√	√	
6	**miR-26b-5p**	√	√	√	
9	miR-23a-3p*	√			
10	miR-124-3p	√			
18	miR-744-5p	√			
21	miR-133a-3p*	√			
35	**miR-375**	√	√	√	√
42	**miR-17-5p**			√	
100	miR-29c-3p			√	
235	miR-455-3p	√			
244	miR-145-5p	√			
277	miR-29b-3p	√			
849	let-7g-5p	√			
895	miR-143-3p	√			
917	miR-138-5p	√			
946	miR-451a			√	
1047	miR-519d-3p	√			
1065	miR-519c-3p	√			
1071	miR-519a-3p	√			
1092	miR-338-3p			√	
1304	miR-424-3p	√			

Figure 2. Identification of miR-21-5p, miR-222-3p, miR-335-5p, miR-26ba-3p, miR-375 and miR-17-5p to detect left ventricular remodeling (LVR) after myocardial infarction (MI) and to predict heart failure (HF). (**A**) Selection process and (**B**) priorization analysis of the 24 miRNAs predicted from the multi-omic data obtained in REVE-2 study by the EdgeBox knowledge platform. √ indicates that miRNA is predicted to be active at the corresponding timepoint: baseline (0), 1 month, 3 month and 12 months after MI. MiRNAs only active at baseline (grey) and with a betweenness centrality rank lower than 50 (inside the thick line) were excluded from further investigation because they are not expected to be highly involved in LVR after MI. * indicates REVE-2 variables. MiRNAs remaining after the selection process are in bold.

2.3. Gene ontology analysis of the 7 miRNAs targets predicted with a high relation specificity with processes involved in LVR after MI

An analysis of the 7 miRNAs targets was performed using the ClueGO [25] and CluePedia [26] applications of Cytoscape (version 3.4.0). The applications used the miRecords database to identify the experimentally validated targets of each miRNA which were submitted to Gene Ontology enrichment (**Figure 3**).

Thirty-one targets were predicted to interact with 5 out of 7 miRNAs (miR-21-5p, miR-222-3p, miR-23a-3p, miR-375 and miR-17-5p) (**Figure 3A**). The 2 remaining miRNAs (miR-335-5p and miR-26b-5p) had no validated target in miRecords. We can observe that the miRNA's targets are involved in pathways involved in LVR development such as fibroblast proliferation, regulation of reactive oxygen species metabolism and intrinsic apoptotic signaling pathways, but also embryonic heart development as embryonic heart tube development and aorta development (**Figure 3B**). These results testify of the involvement of the 7 miRNAs in cardiac development and LVR processes.

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(A)

MicroRNA	Experimentally validated target	UniProt Accession number
miR-21-5p	SLC16A10	Q8TF71
	GLCCI1	Q86VQ1
	CFL2	Q9Y281
	FAM3C	Q92520
	CDKN1A	P38936
	TOPORS	Q9NS56
	CDK6	Q00534
	RASGRP1	O95267
	HIPK3	Q9H422
	PPIF	P30405
miR-222-3p	SOD2	P04179
	PPP2R2A	P63151
	MMP1	P03956
	ESR1	P03372
	CDKN1C	P49918
miR-335-5p		
miR-26b-5p		
miR-23a-3p	C21orf33	P30042
	ATAT1	Q5SQI0
	CXCL12	P48061
	POU4F2	Q12837
	HES1	Q14469
miR-375	YWHAZ	P63104
	YAP1	P46937
miR-17-5p	MAPK9	P45984
	PRKD1	Q15139
	MEF2D	Q14814
	JAK1	P23458
	CXCL8	P10145
	PRKD2	Q9BZL6
	GAB1	Q13480
	S1PR1	P21453
	CDKN1A	P38936
	BCL2L11	O43521

(B)

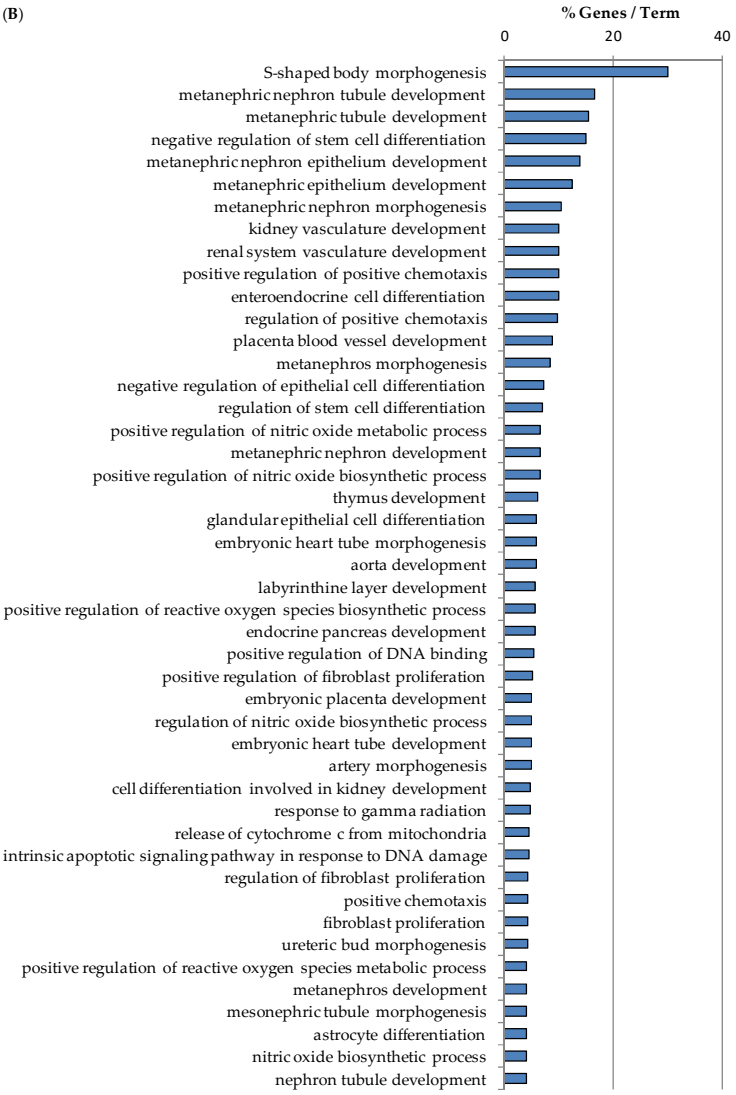

Figure 3. Functional annotation enrichment analysis of the 7 miRNA's targets. (**A**) Targets of miRNAs were predicted by miRecords database. No targets have been described for miR-335-5p and miR-26b-5p. (B**b**) Biological processes of miRNA's target genes were predicted by Cytoscape plugin ClueGO and Cluepedia applications ($p < 0.05$).

3. Conclusion and Perspectives

Candidate miRNAs associated with LVR identified from model of post-MI rats must be analyzed carefully and required a human validation. Indeed, Vegter et al. showed that circulating miRNA profiles in HF rat models do not reflect human profile [27]. Moreover, candidate miRNAs associated with LVR selected only by plasma detection may be misleading, knowing that some miRNAs such as miR-21-3p were not detected in the plasma of our model of post-MI rats, but could be detected in small EVs from rat cardiac fibroblasts [8]. These results testify of the interest of extracellular transporter study to detect candidate miRNAs associated with LVR after MI.

In this study, we described two systems biology analyses integrating either proteomic data generated from LV of post-MI rats induced by left coronary artery ligation or multi-omics data generated from plasma of post-MI patients from the REVE-2 study to identified a miRNA signature of LVR after MI to predict HF. The first approach predicted 13 candidate miRNAs and 3 of these miRNAs were validated to be associated with LVR *in vivo*: miR-21-5p, miR-23a-3p and miR-222-3p. The second approach predicted 24 candidate miRNAs among 1310 molecules and 6 of these miRNAs were selected to be associated with LVR *in silico*: miR-17-5p, miR-21-5p, miR-26b-5p,

miR-222-3p, miR-335-5p and miR-375. Altogether, these two integrative systems biology analyses identified a signature of 7 microRNAs associated with LVR after MI and more especially with mechanisms underlying LVR, such as apoptosis, oxidative stress and fibroblasts proliferation.

To date, we validated 3 candidate miRNAs miR-21-5p, miR-23a-3p and miR-222-3p for their association with LVR *in vivo* in LV and in plasma but not in extracellular transporter of miRNAs. Four candidate miRNAs are not yet experimentally validated: miR-335-5p, miR-26b-5p, miR-375 and miR-17-5p. MiR-335-5p has not been yet linked with cardiovascular diseases and it has been described as a potential biomarker of osteosarcoma in children [28] and of osteoporosis [29]. MiR-375 has been shown to be mainly expressed in the developing heart and to a lower extent in the adult heart [30] and it is also increased in the plasma of pregnant women with fetal congenital heart defects [31]. MiR-26b-5p and miR-17-5p are well known to be involved in the cardiovascular system. MiR-26b-5p has been shown to be modulated in plasma of patients presenting major cardiovascular events [23]. MiR-17-5p was shown to be increased in the plasma of patients with hypertrophic cardiomyopathy and diffuse myocardial fibrosis [24]. These results testify that miR-320a, miR-335-5p, miR-26b-5p, miR-375 and miR-17-5p may be interesting targets to predict HF and suggest they should be taken into account to define more accurately the circulating miRNA signature of LVR to predict HF.

In conclusion, we highlighted the interest of integrative systems biology analyses to define a miRNA signature of LVR to predict HF. However, even though the building of molecular networks relies on experimental data, we showed that miRNA signature still need to be experimentally validated to be relevant *in vivo*.

4. Methods

4.1. Experimental model of HF in rats

MI was induced by left anterior descending coronary artery ligation in 10 weeks old Wistar male rats. Hemodynamic and echocardiographic measurements were performed at 7 days and 2 months after surgery, followed by heart excision and plasma sampling as previously described [12]

4.2. The REVE-2 study

The REVE-2 study is a prospective multicenter study aiming to analyze the association between circulating biomarkers and LVR. The study has been previously detailed [3], and has included 226 patients with a first anterior wall Q-wave MI. Echocardiographic studies were performed at hospital discharge (baseline), 3 months and 12 months after MI, to assess LVR. Serial blood samples were taken at 4 time point: baseline, 1 month, 3 months and 12 months. Twenty-four molecular variables were measured in the REVE-2 plasma at 1 to 4 time points.

4.3. Functional analysis of miRNAs targets

MiRNA target prediction was performed using the ClueGO (version 2.5.2 http://apps.cytoscape.org/apps/cluego) [25] and CluePedia (version 1.5.2 http://apps.cytoscape.org/apps/cluepedia) applications [26] of Cytoscape software (version 3.4.0 http://www.cytoscape.org/). Only validated miRNAs targets from the miRecords database were selected. A Gene Ontology (GO) enrichment analysis for Biological Processes was performed for all the miRNAs targets using the ClueGO application of Cytoscape software. *P*-value was set at 0.05, and corrected for multiple testing using Benjamini-Hochberg adjustment.

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Author Contributions: F.P. and C.B. conceived and designed the experiments; H.C., M.C., E.D.D. and P.M. performed the experiments; H.C., M.C., E.D.D., P.M., V.R., C.B. and F.P. analyzed the data; H.C., M.C., E.D.D., C.B. and F.P. wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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