MicroRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients

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Submitted 14 December 2009; accepted in final form 18 May 2010

MicroRNA signatures in peripheral blood mononuclear cells of chronic heart failure patients. Physiol Genomics 42: 420–426, 2010. First published May 18, 2010; doi:10.1152/physiolgenomics.00211.2009.—MicroRNAs (miRNAs) are noncoding RNAs that act as negative regulators of gene expression. Interestingly, specific alterations of miRNA expression have been found in failing hearts of different etiologies. The aim of this study was to identify the miRNA expression pattern of peripheral blood mononuclear cells (PBMCs) derived from chronic heart failure (CHF) patients affected by ischemic (ICM) and nonischemic dilated (NIDCM) cardiomyopathy. The expression profile of 257 miRNAs was assessed in 7 NIDCM patients, 8 ICM patients, and 9 control subjects by quantitative real-time PCR. Significantly modulated miRNAs were validated by using an independent set of 34 CHF patients (NIDCM = 19, ICM = 15) and 19 control subjects. Three miRNAs (miR-107, -139, and -142-5p) were downmodulated in both NIDCM and ICM patients versus control subjects. Other miRNAs were deregulated in only one of the CHF classes analyzed compared with control subjects: miR-142-3p and -29b were increased in NIDCM patients, while miR-125b and -497 were decreased in ICM patients. Bioinformatic analysis of miRNA predicted targets and of gene expression modifications associated with CHF in PBMCs indicated a significant impact of the miRNA signature on the transcriptome. Furthermore, miRNAs of both the NIDCM and the ICM signature shared predicted targets among CHF-modulated genes, suggesting potential additive or synergistic effects. The present study identified miRNAs specifically modulated in the PBMCs of NIDCM and ICM patients. Intriguingly, most of these miRNAs were previously reported as deregulated in human and/or mouse failing hearts. The identified miRNAs might have a potential diagnostic and/or prognostic use in CHF.

microRNA expression; leukocyte; ischemic cardiomyopathy; nonischemic dilated cardiomyopathy

Heart failure, a progressive pathological condition in which the heart fails to provide the organism with a sufficient amount of blood, can result from diverse acute and chronic insults and is one of the leading causes of mortality in industrialized countries (13). Chronic heart failure (CHF) is the functional consequence of different diseases such as ischemic cardiomyopathy (ICM), caused by coronary artery disease and myocardial infarction (26), or nonischemic dilated cardiomyopathy (NIDCM), characterized by left ventricular dilation and impaired left ventricular systolic function (24).

Biomarkers, such as brain natriuretic peptide (BNP), its prohormone proBNP (8), and C-reactive protein (CRP) (27), have a relevant complementary diagnostic/prognostic value for CHF, in addition to well-established clinical tools (12), highlighting the increasing interest in the development of efficient diagnostic and prognostic CHF biomarkers (10). In this respect, peripheral blood mononuclear cells (PBMCs) are a particularly attractive source for biological tests because of the accessibility of peripheral blood and the straightforward preparation. Furthermore, inflammation and the underlying cellular and molecular mechanisms seem to play a crucial pathological role in the progression toward CHF (28, 31, 37).

We recently reported a large-scale gene expression analysis of PBMCs of CHF patients. Our study revealed a CHF-specific mRNA signature and identified chemokine receptors and early growth response (EGR) family members as putative biomarkers (5, 28).

An emerging class of biomarkers is represented by microRNAs (miRNAs). These small, endogenous noncoding RNAs modulate gene expression by regulating the stability and/or translational efficiency of target messenger RNAs (35). Each miRNA can target multiple mRNAs, and, vice versa, individual mRNAs can be targeted by multiple miRNAs (35). Thus miRNAs hold the potential to modulate complex physiological or disease phenotypes by regulating entire functional networks (36). A steadily growing number of studies emphasize that miRNAs are crucial not only in the development and growth of the heart (9) but also in cardiac diseases (11, 25). A genomewide miRNA expression profiling in human heart tissue carried out in patients affected by ICM, dilated cardiomyopathy, and aortic stenosis showed distinct miRNA expression patterns for each disease (17). Furthermore, molecular oncology provided clear-cut examples of the diagnostic potential of miRNA profiling, showing that miRNA expression profiles allow successful classification of poorly differentiated tumors with a much higher accuracy compared with messenger RNA profiles (23).

The aim of the present study was to identify specific miRNAs that are differentially expressed in PBMCs derived from CHF patients affected by ICM or NIDCM, thus providing potential novel disease biomarkers.

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We recently reported a large-scale gene expression analysis of PBMCs of CHF patients. Our study revealed a CHF-specific mRNA signature and identified chemokine receptors and early growth response (EGR) family members as putative biomarkers (5, 28).

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The aim of the present study was to identify specific miRNAs that are differentially expressed in PBMCs derived from CHF patients affected by ICM or NIDCM, thus providing potential novel disease biomarkers.
**Materials and Methods**

**Patient characteristics.** Blood samples derived from Caucasian patients with stable CHF classified as New York Heart Association (NYHA) functional class III/IV for ≥6 mo with a mean left ventricular ejection fraction (LVEF) ≤36% were collected and subdivided into two different classes according to heart failure etiology. The ICM class included all patients with a history of myocardial infarction and coronary atherosclerosis with stenosis >70% in at least one major coronary artery branch. The NIDCM class included patients with no history of myocardial infarction and angiographically normal coronary arteries. Echocardiographic images in 2D and M mode were used to determine LVEF and left ventricular internal diastolic diameter (LVIDd, mm). During the 6 mo before recruitment for this study and the blood collection, the patients had no infections, malignancies, autoimmune disorders, diabetes, pulmonary disease, myocardial infarction, unstable angina, or myocarditis. The selection of the ICM and NIDCM patients was conducted by the Cardiology Unit of the Catholic University of Rome. As controls, peripheral blood of individuals showing no overt cardiac disease, dyslipidemia, or leukocytosis and that proved negative for inflammatory indexes [such as CRP and erythrocyte sedimentation rate (ESR)], were collected at Istituto Dermoepatico dell’Immacolata (IDI-IRCCS), Rome.

Two patient cohorts were analyzed, the “profiling set” (7 NIDCM patients, 8 ICM patients, and 9 control subjects) and the “validation set” (19 NIDCM patients, 15 ICM patients, and 19 control subjects). Clinically relevant parameters of the two sets of CHF patients are summarized in Tables 1 and 2, respectively. One-way analysis of variance (ANOVA) analysis showed no significant age differences between control subjects, ICM patients, and NIDCM patients in both sets. Similarly, no significant difference in sex distribution between control subjects, ICM patients, and NIDCM patients was found by Fisher’s exact test (P > 0.05 in both tests). The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the Catholic University of Rome, and all subjects gave written informed consent.

**Sample preparation.** PBMCs were isolated from 20 ml of whole blood by Histopaque Ficoll (Sigma Diagnostics, St. Louis, MO) gradient centrifugation as described previously (5). Briefly, whole blood, diluted 1:1 with PBS, was gently overlaid on Ficoll and then centrifuged, resulting in the separation of the blood into its components. The PBMCs, consisting of subpopulations of monocytes, T lymphocytes, and B lymphocytes, trapped in the interphase (“buffy coat”) between plasma and Ficoll, were collected, and total RNAs were prepared.

**miRNA quantification.** miRNA levels were analyzed with the TaqMan Real Time Assay [quantitative RT-PCR (qRT-PCR), 3.4 ng/assay] and quantified with the 7900HT Fast Real-Time PCR System (Applied Biosystems). Raw data, expressed as threshold cycle (Ct) values, were computed with the Applied Biosystems RQ Manager 1.2 software. Relative expression was calculated with the comparative Ct method (2^−ΔΔCt). For miRNA profiling, 257 miRNAs and positive and negative controls were assayed in a 96-well format, and samples were normalized to the median Ct value. The data set was filtered for outliers with a zeta score >2 and analyzed. First, the distribution within each group of the profiling set was checked for normality by the Kolmogorov-Smirnov test (K-S test). Significance of the differences in the relative expression of normally distributed data sets was determined by ANOVA followed by Dunnett’s posttest. Data sets not normally distributed were analyzed by Kruskal-Wallis test followed by Dunn’s posttest. Statistical analysis was performed with GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA). A P value ≤0.05 (test signature) was deemed statistically significant.

Validation of the test signature was performed by measuring relevant miRNA levels by qRT-PCR as described above. Data were normalized to miR-16 levels that profiling experiments indicated to be readily detectable and not affected by the analyzed conditions. Subsequent statistical analysis was performed as specified before.

NIDCM and ICM miRNA signature scores were calculated as follows: The patients belonging to the profiling set and to the validation set were pooled, constituting the “aggregation set” (26 NIDCM, 23 ICM, 28 control). The mean fold change of all the miRNAs belonging to each specific signature (ICM signature or NIDCM signature) was then calculated for each patient. Since the NIDCM

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**Table 1. Profiling set patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>NIDCM (n = 7)</th>
<th>ICM (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr [mean (range)]</td>
<td>66.6 ± 2.1 (59–74)</td>
<td>66.1 ± 2.0 (60–77)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>5/2</td>
<td>7/1</td>
</tr>
<tr>
<td>NYHA class</td>
<td>III–IV</td>
<td>III–IV</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>27.6 ± 2.2</td>
<td>35.4 ± 2.9</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>71.3 ± 4.9</td>
<td>59.8 ± 2.7</td>
</tr>
<tr>
<td>Therapy, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Antagonist</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>86</td>
<td>50</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>57</td>
<td>75</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>14</td>
<td>50</td>
</tr>
<tr>
<td>Statins</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Digitoxin</td>
<td>86</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are means ± SE for n subjects. ICM, ischemic cardiomyopathy; NIDCM, nonischemic dilated cardiomyopathy; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; LVIDd, left ventricular internal diastolic diameter; ACE, angiotensin-converting enzyme.

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**Table 2. Validation set patient characteristics**

<table>
<thead>
<tr>
<th></th>
<th>NIDCM (n = 19)</th>
<th>ICM (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr [mean (range)]</td>
<td>63.8 ± 1.8 (54–80)</td>
<td>67.0 ± 1.8 (54–77)</td>
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<tr>
<td>Sex, M/F</td>
<td>13/6</td>
<td>13/2</td>
</tr>
<tr>
<td>NYHA class</td>
<td>III (n = 9)</td>
<td>III (n = 8)</td>
</tr>
<tr>
<td></td>
<td>III–IV (n = 10)</td>
<td>III–IV (n = 7)</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>25.7 ± 1.8</td>
<td>29.4 ± 2.3</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>68.8 ± 1.6</td>
<td>69.1 ± 2.0</td>
</tr>
<tr>
<td>Therapy, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Antagonist</td>
<td>89</td>
<td>80</td>
</tr>
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<td>ACE inhibitors</td>
<td>63</td>
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<td>Loop diuretics</td>
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<td>93</td>
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<tr>
<td>Aldosterone</td>
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<tr>
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<td>Digitoxin</td>
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<td>7</td>
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<tr>
<td>Statins</td>
<td>32</td>
<td>80</td>
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</table>

Values are means ± SE for n subjects.
signature comprises up- and downmodulated miRNAs, the sign of each value of the significantly upregulated miRNAs was inverted before calculating the mean. The significance of the NIDCM and ICM signature score differences in patients versus control subjects was calculated by two-tailed, unpaired t-test.

Intersection of miRNA and mRNA expression data. The validated NIDCM and ICM miRNA signatures were compared with gene expression profiles, recently identified by our group, obtained by Affymetrix using PBMCs of NIDCM and ICM patients and control subjects recruited according to the same criteria used in this study (5). For this purpose, the “Class comparison between groups of arrays” function of BRB-Array Tools Version 3.8.0-Beta_1 Release (developed by Dr. Richard Simon and the BRB-Array Tools Development Team, March 2009) was adopted. Using the list of expressed genes obtained by the Affymetrix study, the “Gene Set Comparison Tool” evaluates which miRNA shows a set of predicted target genes containing more differentially expressed genes than would be expected by chance, computing P values for LS permutation and Efron-Tibshirani’s GSA maxmean test. miRNAs corresponding to gene sets with a P value ≤0.05 for either of the tests were intersected with the miRNA signature identified by miRNA expression profiling, assuming a direct mRNA and miRNA interaction.

Target prediction. Bioinformatic prediction of miRNA target genes sites was performed with the following algorithms: miRBase Targets database of the Wellcome Trust Sanger Institute (14), TargetScan Human 5.1 (12, 15, 22), and PicTar (last update: March 26, 2007) (19).

mRNA quantification. mRNA levels were analyzed with the SYBR Green PCR system (Applied Biosystems, 7.5 ng/assay) and quantified by 10.220.33.1 on June 14, 2017 http://physiolgenomics.physiology.org/ Downloaded from

**Fig. 1.** Nonischemic dilated cardiomyopathy (NIDCM) and ischemic cardiomyopathy (ICM) microRNA (miRNA) signatures. A: validation of the miRNA test signature derived from the profiling set was carried out by quantitative RT-PCR (qRT-PCR) on an independent set of patients, named the validation set [19 NIDCM and 15 ICM vs. 19 control (Ctrl) subjects]. Statistically significant miRNA modulations are shown (*P < 0.05, **P < 0.01). Error bars indicate SE. B: patients belonging to the profiling set and to the validation set were analyzed together (aggregation set = 26 NIDCM, 23 ICM, 28 control). Significant positive and negative miRNA modulations are shown (*P < 0.05, **P < 0.01, and ***P < 0.001). Error bars indicate SE. C: miRNA signature scores. Mean fold change of all the miRNAs belonging to each specific signature (NIDCM miRNA signature score and ICM miRNA signature score) was calculated for each patient of the aggregation set (****P < 5 × 10⁻⁸).
as described previously (5). The primers were designed with Primer3 software (v.0.4.0). Average C\textsubscript{i} values of GAPDH and L13 housekeeping genes were used for data normalization. Statistical analysis was performed as described above.

RESULTS

miRNA expression profiling in PBMCs of CHF patients.

Total RNA was extracted from PBMCs derived from seven NIDCM patients, eight ICM patients, and nine control subjects (profiling set, Table 1), and the expression of 257 different miRNA species was determined by qRT-PCR (Supplemental Tables S1 and S2).\(^1\) In this assay, 159 miRNAs displayed an average C\textsubscript{i} value <32.0 in control PBMCs and were considered as expressed (Supplemental Table S3). Analysis of miRNAs showing statistically significant differential expression in at least one of the CHF classes compared with the control subjects identified a test signature of 12 candidate miRNAs (Table 3). Conversely, no miRNA was significantly modulated in NIDCM compared with ICM patients (not shown).

To validate this miRNA test signature, independent cohorts of 19 NIDCM patients, 15 ICM patients, and 19 control subjects (validation set, Table 2) were analyzed by qRT-PCR. Specifically, miRNAs displaying statistically significant differences in the profiling set were analyzed. The possibility that certain miRNA deregulations may not reach statistical significance because of the limited size of the profiling set was also considered. Thus validation was extended to those miRNAs that displayed statistically significant changes in one class of patients and nonsignificant changes in the same direction in another class. For instance, miR-139 was validated in both NIDCM and ICM patients since it was significantly decreased in ICM patients but displayed a nonsignificant decrease in NIDCM patients as well. Furthermore, miR-142-5p was also validated, since this miRNA originates from the same precursor as miR-142-3p, and this miRNA was significantly modulated in ICM patients.

It was found that seven miRNAs displayed significantly different expression levels in the validation set. In NIDCM patients, we found miR-107, -139, and -142-5p to be significantly less expressed in pathological than in control samples, whereas miR-142-3p and miR-29b were expressed at higher levels (Fig. 1A). In the ICM group of patients, validation revealed a significant downmodulation of miR-107, -125b, -139, -142-5p, and -497 (Fig. 1A). No significantly upregulated miRNAs could be confirmed in this disease class. Certain miRNAs indicated by profiling set analysis as significantly different in only one of the two diagnostic classes revealed in the validation set a significant differential expression in both classes, NIDCM and ICM. To confirm that this apparent discrepancy was caused by the smaller size of the profiling set of patients, values obtained from both the profiling set and the validation set were aggregated (aggregation set) and statistical significance of differences was analyzed. Aggregation set analysis confirmed all seven miRNAs identified by the analysis of the validation set (Fig. 1B). Additionally, the miRNA expression levels of the complete NIDCM and ICM signatures of the aggregation set were summarized in scores and then investigated for significant differential expression. Both the NIDCM signature and the ICM signature scores displayed significant differences in NIDCM and ICM patients, respectively, compared with the control subjects (Fig. 1C).

**Significant impact of CHF signature miRNAs on transcriptome.** To further validate the NIDCM and ICM miRNA signatures we identified, the predicted targets of each miRNA were compared with the significantly modulated messenger RNAs (mRNAs) in the PBMCs of NIDCM and ICM patients. To this end, we took advantage of a gene expression analysis we recently published (5). In that study we analyzed the transcriptome of PBMCs derived from NIDCM and ICM patients and control subjects recruited with the same criteria used for this study at the same facilities. Predicted targets for each miRNA were compared with opposite modulated genes in CHF patients by applying “Class comparison between groups of arrays” of the BRB-Array Tools, which uses the miRBase prediction algorithm and provides a statistical analysis of the degree of overlap. It was found that in the NIDCM class there was a significant degree of overlap for miR-107, -139, -142-3p, and -142-5p. In the ICM group, a significant impact of miR-107 and miR-497 on the transcriptome was found (Table 4).

**Predicted target analysis.** mRNAs can be targeted by more than one miRNA (7). Thus we wanted to assess whether two or more miRNAs belonging to either NIDCM or ICM signatures shared predicted targets among CHF signature genes (5), suggesting potential additive or synergistic effects and further validating our findings. In this case, we were able to take advantage not only of miRBase algorithm but also, given their reported specificity, of PicTar and TargetScan prediction software (16). Figure 2A shows that miR-107, -139, and -142-5p display 21, 16, and 29 potential targets among the NIDCM signature mRNAs, respectively. Intriguingly, many of these, for example, SON DNA binding protein (SON), were targeted by more than one miRNA. Similarly, a high level of overlap between the targets of the ICM signature miRNAs was observed (Fig. 2B), with two mRNAs, SON and the human homolog of yeast Ubc6p ubiquitin-conjugating enzyme (UBE2J1), targeted by three ICM signature miRNAs. Assuming a direct interaction between the modulated miRNAs and their predicted target, we confirmed by qRT-PCR the significant upregulation not only of SON in NIDCM and ICM patients but also of UBE2J1 in ICM patients (Fig. 3). The induction of glutaredoxin (thioltransferase-GLRX), which was predicted to be targeted by two ICM signature miRNAs, was validated as well (Fig. 3B).

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Class</th>
<th>Targets</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-107</td>
<td>NIDCM</td>
<td>360</td>
<td>0.028</td>
</tr>
<tr>
<td>hsa-miR-107</td>
<td>ICM</td>
<td>360</td>
<td>0.005</td>
</tr>
<tr>
<td>hsa-miR-139</td>
<td>NIDCM</td>
<td>406</td>
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<tr>
<td>hsa-miR-142-3p</td>
<td>NIDCM</td>
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<tr>
<td>hsa-miR-142-5p</td>
<td>NIDCM</td>
<td>474</td>
<td>0.005</td>
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<tr>
<td>hsa-miR-497</td>
<td>ICM</td>
<td>377</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Targets, number of interactions between miRBase-predicted targets and reciprocally modulated genes. P values were calculated with LS permutation or Efron-Tibshirani GSA statistical tests.

\(^1\) Supplemental Material for this article is available online at the Journal website.
DISCUSSION

In the present study, for the first time, miRNA expression profiles of PBMCs of CHF patients were analyzed and miRNAs that were specifically modulated in NIDCM and ICM patients versus control subjects were identified. There is no single diagnostic test available for CHF, but the levels of cardiac biomarkers together with the chest X-ray, the electrocardiogram, and Doppler echocardiography are considered for the diagnosis (20). The identification of a distinct miRNA expression pattern in the PBMCs of CHF patients could contribute to...

Fig. 2. Common predicted targets among chronic heart failure (CHF) signature miRNAs. Predicted targets of both NIDCM and ICM signature miRNAs were determined with miRBase, Pictar and Targetscan algorithms. Predicted targets of each miRNA were then compared with significantly modulated miRNAs in peripheral blood mononuclear cells (PBMCs) of NIDCM and ICM patients that displayed reciprocal regulation. The number of targets for each miRNA is indicated in parentheses. Venn diagrams display the number of overlapping targets among different miRNAs belonging to either the NIDCM (A) or the ICM (B) signature.

Fig. 3. Expression validation of CHF signature miRNAs potential targets. mRNA levels of genes found to be modulated in the Affymetrix study and predicted as targets for the significantly modulated miRNAs were determined by qRT-PCR in 9 NIDCM patients, 10 ICM patients, and 10 control subjects selected from both the profiling set and the validation set. A: SON expression is increased in both NIDCM and ICM patients (*P < 0.05). B: UBE2J1 and GLRX are upregulated in ICM patients compared with control subjects (*P < 0.05, **P < 0.01).
the development of a noninvasive and easily measurable test that might enhance the accuracy of clinical diagnosis and prognosis (25). Indeed, numerous indications exist that miRNAs may constitute excellent disease biomarkers. miRNAs seem to be less sensitive to degradation compared with mRNAs (18) and can be detected via several methods, such as microarrays and bead-based arrays, and only nanograms of total RNA are necessary for qRT-PCR. As a matter of fact, miRNA-based diagnostic assays have been already developed and approved for certain neoplastic diseases (www.rosettagenomics.com; www.asuragen.com). On the other hand, clinical experience with this relatively new molecule class is very limited and further extensive studies are granted.

We found that, miR-107, -139 and -142-5p were downmodulated in both patient classes. Conversely, miR-142-3p and -29b were significantly increased only in NIDCM patients. Indeed, these miRNAs were significantly upregulated in the profiling and aggregation sets of ICM patients as well. However, their increase failed to reach statistical significance in the validation set of ICM patients. Thus it is possible that recruiting of a larger patient cohort may allow further validation of these miRNAs in ICM patients. Similarly, miR-125b and -497 were significantly decreased only in ICM patients. However, their expression was downmodulated in the aggregation set of NIDCM patients as well, albeit not significantly (not shown). Thus, while further studies are needed, these data may explain why none of the identified miRNAs could be used to distinguish between NIDCM and ICM patients.

One surprising observation was the opposite modulation of miR-142-5p and miR-142-3p in NIDCM patients, since these miRNAs derive from the same precursor. However, miRNAs are strongly modulated at the posttranscriptional level, and opposite modulations of the two possible miRNAs generated by the two branches of the same precursor may derive from different leading/passenger strand selection in different conditions (3, 38). Differential degradation of the mature products is another potential mechanism.

A number of recent studies have uncovered a crucial role of miRNAs not only in heart growth and development but also in cardiac diseases, like hypertrophy, fibrosis, and heart failure (9, 17, 33, 34, 36). Many of the CHF miRNAs we identified in PBMCs were previously reported to be deregulated in biopsies derived from failing hearts. In accordance with our findings, miR-107 and miR-29b are down- and upregulated, respectively, in the heart of NIDCM patients (34). In the ICM class, miR-497 was expressed at significantly lower amounts than in healthy individuals, as also observed in end-stage human failing hearts (33).

Intriguingly, several studies report that miR-125b is upregulated in the heart of HF patients (17, 31, 37), while we found that this miRNA was downmodulated in the PBMCs of ICM patients.

miR-139 and miR-142-3p, which were down- and upregulated, respectively, in PBMCs of CHF patients, have not been reported as deregulated in failing human hearts so far. However, miR-139 is decreased and miR-142-3p is increased during the later stage of hypertrophy in a mouse model of heart hypertrophy induced by transverse aortic constriction (29, 32). Furthermore, in another study on cardiac hypertrophy, miR-107 and miR-139, showing a significant downregulation in both ICM and NIDCM signatures, were identified as significantly downmodulated in mouse heart tissues after aortic banding at 28 and 14 days, respectively (6). It is tempting to speculate that miRNA modulations observed in failing hearts and in PBMCs may be due to common systemic signals.

Along with their ability to inhibit target gene translation, miRNAs can also induce the exonucleolytic degradation of target mRNAs (30). Thus miRNA deregulation is expected to have a direct impact on the transcriptome as much as on the proteome. miRNAs bind to their targets according to complex and yet not fully understood rules (4). Thus complex algorithms have been developed to predict miRNA-mRNA pairs, although none of these prediction tools displays a completely satisfactory sensitivity and specificity (2). Nevertheless, we were able to identify a statistically significant interaction between four NIDCM miRNAs and two ICM miRNAs and opposite modulated transcripts in these diseases. In this case, our analysis was limited to the use of the miRBase prediction software by “BRB-Array tool” technical limitations. However, when we assessed whether different miRNAs of the same signature shared common predicted targets among NIDCM and ICM signature genes (5), we took advantage also of Pictar and Targetscan algorithms to increase sensitivity (16). We found that numerous common potential targets were shared by both NIDCM and ICM signature miRNAs, suggesting potential additive effects. Investigation for specific modulation of some of these potential targets in the PBMCs of CHF patients yielded valuable further insight. For instance, SON, a gene involved in the regulation of cell proliferation and/or apoptosis (1), predicted target for three ICM signature miRNAs (miR-107, -497, and -142-5p) and also for two NIDCM signature miRNAs (miR-107 and -142-5p), was significantly upregulated in the CHF samples. In addition, we demonstrated a significantly higher expression of UBE2J1 in ICM, a predicted target shared by three ICM-signature miRNAs (miR-107, -125b, and -497). This E2 enzyme is localized in the cytoplasmic surface of the endoplasmic reticulum (ER) and participates in ER-associated degradation of misfolded proteins including the α-subunit of the T-cell receptor (21). Interestingly, the upmodulation of GLRX, predicted target of two miRNAs of the ICM class (miR-497 and -142-5p) could also be confirmed. GLRX, featuring oxidoreductase activity, is involved in the cellular protection against oxidative damage (22). While the functional relevance of these miRNA-mRNA interactions needs further investigation, these results contribute to validate the novel miRNA signatures identified in the present study.

In conclusion, we found that miRNA expression is altered in PBMCs derived from both NIDCM and ICM patients versus control subjects, suggesting a possible discrimination between healthy individuals and CHF patients, by measuring a small number of miRNAs. Further studies are needed to assess whether the identified miRNAs may constitute a useful tool for patient diagnosis and treatment.

GRANTS
This work was partly supported by Ministero della Salute (RF06 and RF07).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES
1. Ahn EY, Yan M, Malakhova OA, Lo MC, Boyapati A, Ommen HB, Hines R, Hokland P, Zhang DE. Disruption of the NHR4 domain...


