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Biomarkers

The Circulating Level of FABP3 Is an Indirect Biomarker of MicroRNA-1

Francesca Varrone, PHD,* Barbara Gargano, PHD,* Pierluigi Carullo, BSC,†‡ Dario Di Silvestre, PHD,§ Antonella De Palma, PHD,§ Ludovica Grasso, MD,‖ Carolina Di Somma, MD,¶ Pierluigi Mauri, PHD,§ Louise Benazzi, PHD,§ Anna Franzone, MD,# Gloria Saccani Jotti, MD, PHD,** Marie-Louise Bang, PHD,†‡ Giovanni Esposito, MD, PHD,# Annamaria Colao, MD, PHD,‖ Gianluigi Condorelli, MD, PHD,†‡ Daniele Catalucci, PHD†‡

Milan, Naples, and Parma, Italy

Objectives This study sought to identify proteins from the cardiomyocyte (CM) secretome that are directly targeted by the muscle-specific microRNA-1 (miR-1), and thus reflect the pathophysiological state of the CM.

Background MicroRNAs play critical regulatory roles during myocardial remodeling and progression to heart failure. However, it remains unknown whether secreted microRNA-targeted proteins can be used as indicators of myocardial microRNA expression and function.

Methods A proteomic analysis based on multidimensional protein identification technology was performed on supernatants from cultured CMs overexpressing miR-1. Biochemical assays and an inducible cardiac-specific transgenic mouse model overexpressing miR-1 were used to demonstrate that heart-type fatty acid-binding protein-3 (FABP3) is a target of miR-1. Levels of miR-1 and FABP3 in cardiac tissue and plasma samples from mouse models as well as human patients were quantified by quantitative reverse-transcription polymerase chain reaction and enzyme-linked immunosorbent assay, respectively. The study included wild-type mice subjected to ventricular pressure overload or fasting, as well as patients diagnosed with ventricular hypertrophy due to valvular aortic stenosis, acromegaly, or growth hormone deficiency, conditions associated with altered miR-1 expression.

Results An inverse relationship between myocardial expression of miR-1 and circulating levels of FABP3 was found both in vitro and in vivo under various pathological conditions.

Conclusions Assessment of FABP3 plasma levels in human patients might be used for indirectly measuring cardiac miR-1 activity. (J Am Coll Cardiol 2013;61:88–95) © 2013 by the American College of Cardiology Foundation

Cardiomyocytes (CMs) produce a wide variety of bioactive molecules that regulate myocardial processes such as hypertrophic growth, excitation–contraction coupling as well as structural and metabolic remodeling. The proteins that are actively released into the interstitial space or the bloodstream are known to be important for homeostasis, not only of the heart, but also of the whole organism (1). Release may take place in response to either physiological stimuli or pathological stresses, and failure to adequately adjust to these changing conditions may lead to cardiac dysfunction. Many of the compensatory mechanisms associated with physiological or pathological processes in the heart may be related to alterations in the expression profile of specific microRNAs (miRs) (2). MiRs are small noncoding RNAs that negatively modulate the translation of target mRNAs by recognizing specific binding sites in the 3′ untranslated regions (UTRs) (2). In the heart, the striated muscle–specific miR-1 has been highlighted for its critical role in orchestrating myocardial development and function as well as structural remodeling associated with the progression of heart failure (HF). In a recent work, we demonstrated a feedback loop in which miR-1 and the insulin-like growth

From the *IRCCS Multimedica Hospital, Scientific and Technology Pole, Milan, Italy; †Milan Unit, Biomedical and Genetic Research Institute, National Research Council, Milan, Italy; ‡Humanitas Clinical and Research Center, Rozzano, Milan, Italy; §Institute of Biomedical Technologies, National Research Council, Segrate, Milan, Italy; ‖Department of Molecular and Clinical Endocrinology and Oncology, Section of Endocrinology, Federico II University Medical School of Naples, Naples, Italy; ¶IRCCS SDN Foundation Naples, Naples, Italy; #Department of Internal Medicine and Cardiovascular Sciences, University Federico II, Naples, Italy; and the **Department of Public Health, University of Parma, Parma, Italy. This work was supported by grants from the Italian Ministry of Health (GR-2007-682438, RFSDO-2007-627952, RFPS-2007-2-644709), Fondazione Cariplo 2007_5312 12431 and 12-4-5157157-31, aging project, Telethon (S07006TELA) (Italy), and the Fondation Leducq (France). The authors have reported that they have no relationships relevant to the contents of this paper to disclose. Dr. Varrone and Dr. Gargano contributed equally to this work.

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factor (IGF)-1 pathway are inversely regulated (3). In particular, we showed that IGF-1 as well its receptor are targets of miR-1, whose expression per se depends on, as recently supported (4), the activation state of the IGF-1 signal transduction cascade. This mechanism likely plays an important role in the modulation of cardiac and skeletal muscle development and hypertrophy. On the basis of this and increasing evidence demonstrating an important role of miR-1 in regulating cardiac function and disease, we hypothesized that miR-1 may also be involved in modulating the levels of proteins secreted by CMs (i.e., the CM secretome). Identifying miR targets that are actively secreted by a specific cell type might be useful for identifying indirect circulating biomarkers that indicate the pathophysiological state of the cell by reflecting the cellular levels of the targeting miR.

Here, we demonstrate that miR-1 regulates the level of fatty acid-binding protein-3 (FABP3), a small cytoplasmic protein involved in cardiac metabolism and released into the bloodstream by CMs following ischemic episodes (5), and we identify circulating FABP3 as an indirect biomarker for miR-1 myocardial expression.

Methods

See the Methods section of the Online Appendix.

Results

FABP3 is a direct target of miR-1. CMs are known to secrete a variety of bioactive molecules involved in a plethora of autocrine/paracrine mechanisms. We therefore asked whether alterations in miR expression levels, in particular those related to the muscle-specific miR-1, might be involved in the modulation of the CM secretome. To this end, a proteomic analysis based on multidimensional protein identification technology (6) was performed on supernatants from in vitro neonatal CMs infected with either a miR-1–expressing adenoviral vector (AdmiR1) or empty vector (Adempty). Among the proteins that were differentially down-regulated in the AdmiR1-treated samples (Online Fig. 1) was FABP3. Due to the considerable interest in the identification of novel biomarkers for early detection of the molecular events underlying cardiac progression to HF, we focused our attention on FABP3, the secretion of which is associated with ischemic conditions (7). Findings of other differentially expressed proteins from the proteomics analysis will be published elsewhere.

MiR–mRNA interaction is initiated by base pairing of the miR's seed sequence, found at the 5′ end, to a binding site present on the 3′-UTR of the targeted mRNA. However, with web-based bioinformatics algorithms, we could not find any canonical binding site for the 5′ seed sequence of miR-1 on the 3′-UTR of FABP3 mRNA. Because growing evidence indicates that base pairing outside of the seed sequence can compensate for imperfect seed matching (8), we assessed whether FABP3 mRNA might represent a noncanonical miR-1 target (Fig. 1A). To this end, a dual-luciferase assay was performed on 293 cells that do not endogenously express miR-1. Briefly, either wild-type or mutant FABP3 3′-UTR cloned downstream of a luciferase reporter gene was transfected into 293 cells before transduction with either AdmiR1 or Adempty. In cultures exposed to AdmiR1, we found a significant decrease in luciferase activity with the construct containing wild-type FABP3 3′-UTR, whereas no effect was found with the construct containing a 3′-UTR mutated in the putative miR-1 binding site (Fig. 1B). Similar results were obtained using a synthetic miR-1 mimic and scramble oligos in the place of the adenoviral vectors (Online Fig. 2).

To further confirm that miR-1 binds to the FABP3 3′-UTR, immunoprecipitation of biotinylated miR-1 oligo was performed on homogenate from adult mouse heart followed by RNA extraction and PCR analysis for FABP3 mRNA. Consistent with the preceding results, a specific PCR signal for FABP3 was obtained, which was absent in the negative control (Fig. 1C). In addition, Western blot analysis of 293 cells transduced with AdmiR1 revealed a decrease in FABP3 protein levels upon miR-1 overexpression (Fig. 1D). All together, these results demonstrate that FABP3 mRNA is a direct target of miR-1.

MiR-1 regulates secreted levels of FABP3 in vitro and in vivo. Since FABP3 is a fatty acid (FA) binding protein involved in the cellular uptake of FAs, we used an enzyme-linked immunosorbent assay (ELISA) to assess whether miR-1 expression might be inversely correlated with the extracellular FABP3 protein level. First, we determined the FABP3 protein level in C2C12 muscle cells, a myogenic cell line in which we recently demonstrated up-regulation of endogenous miR-1 expression upon the switch from proliferation to differentiation (3). As expected, the levels of cytosolic (cFABP3) and secreted FABP3 (sFABP3) were significantly decreased when C2C12 cells were switched from growth to differentiation medium (Fig. 2A), whereas transfection of cells with a miR-1 inhibitor was sufficient to prevent with this reduction (Fig. 2A). Similar findings were obtained in neonatal CMs, in which overexpression or reduction of miR-1 was sufficient to induce an inverse modulation of both intra- and extracellular levels of FABP3 (Fig. 2B). Consistent with our previous studies demonstrating a role of the IGF-1 pathway in negatively regulating

Abbreviations and Acronyms

ACRO = acromegaly
AS = aortic valve stenosis
cFABP3 = cytosolic fatty acid-binding protein-3
CM = cardiomyocyte
dox = doxycycline
ELISA = enzyme-linked immunosorbent assay
FA = fatty acid
FABP3 = fatty acid-binding protein-3
GH = growth hormone
GHD = growth hormone deficiency
HF = heart failure
IGF = insulin-like growth factor
MiR = microRNA
sFABP3 = secreted fatty acid-binding protein-3
TAC = transaortic constriction
TAVI = transcatheter aortic valve implantation
Tg = transgenic
UTR = untranslated region

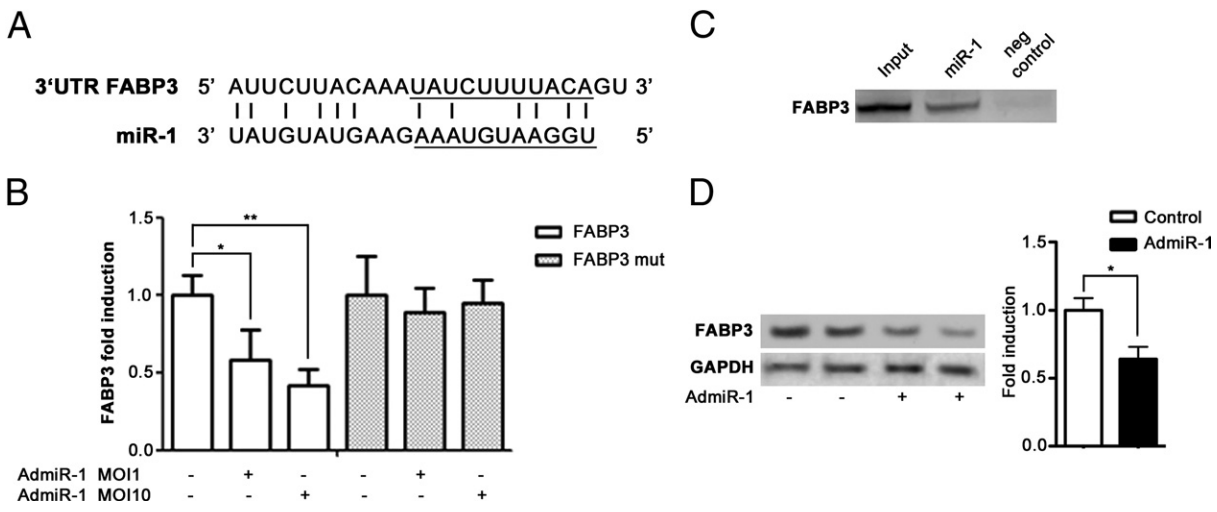

Figure 1 **FABP3 Is a Direct Target of MiR-1**

(A) Sequence alignment of mature miR-1 and the 3′-UTR of FABP3 mRNA. **(B)** Luciferase activity in 293 cells infected with an adenoviral vector expressing miR-1 (AdmiR1) and transfected with a luciferase reporter gene linked to wild-type (FABP3) or mutated (FABP3 mut) FABP3 mRNA 3′-UTR (n = 4). *p < 0.05, **p < 0.01. **(C)** Representative PCR analysis (n = 3) of FABP3 mRNA co-immunoprecipitated with biotinylated-miR-1 in left ventricular homogenate. Input = pre-immunoprecipitation homogenate (positive control); neg control = no oligo (negative control). **(D)** Representative Western blot and densitometric analysis (n = 4 per group) for FABP3 and GAPDH on 293 cells infected with either an adenoviral vector expressing miR-1 (AdmiR1) or an empty vector (control) (MOI 10). FABP3 = fatty acid-binding protein-3; MiR = microRNA; MOI = multiplicity of infection; UTR = untranslated region.

miR-1 expression in cultured CMs (3), both cFABP3 and sFABP3 protein levels were found to be up-regulated in response to IGF-1 exposure (Fig. 2B).

Finally, to further confirm whether miR-1 plays a direct role in controlling the secreted levels of FABP3 in vivo, we used a gain-of-function approach by generating a transgenic (Tg) mouse model engineered for an inducible and cardiac-specific expression of miR-1. Induction of miR-1 expression was obtained by feeding Tg mice with food supplemented with doxycycline (dox). As shown in Figure 2C (left), dox administration to Tg mice resulted in a time-dependent increase in myocardial miR-1 expression and a correspondent significant reduction in sFABP3 levels in Tg compared with control mice (Fig. 2C, right). No apparent effect of dox administration to control mice was observed (Fig. 2C, Online Fig. 3). The Tg mouse line, where the expression of the miR is fine-tuned through the dose and time of dox administration, could represent a valuable tool for miR-1 target identification, and its detailed characterization will be published elsewhere. For the purpose of the current study, the obtained results confirmed an inverse relationship between miR-1 and FABP3 expression, and suggest the presence of an IGF-1/miR-1/FABP3 signaling axis.

Inverse relationship between miR-1 expression and FABP3 levels in pressure-overloaded mouse. We (3,10) and others (11) have previously reported that cardiac hypertrophy induced by transverse aortic constriction (TAC) is associated with decreased miR-1 expression levels and increased levels of IGF-1 (3). On the basis of this evidence, we hypothesized that TAC might be associated with corresponding changes in cFABP3 and sFABP3 levels. In fact, compared with sham-operated mice, animals subjected to TAC had increased levels of FABP3 (Fig. 3, Online Fig. 3). Moreover, to assess whether counteracting pressure-overload–induced miR-1 down-regulation has an effect on the level of sFABP3, we subjected miR-1 Tg mice to TAC and found decreased sFABP3 levels compared with wild-type mice (Fig. 3, Online Fig. 3).

All together, these results confirm the inverse relationship between miR-1 and FABP3 in a mouse model of cardiac pathology.

Fasting inversely modulates miR-1 expression and sFABP3 level in mice. Reduction of circulating IGF-1 and elevation of FAs released from the breakdown of triglycerides are 2 events occurring in the adaptive metabolic phase during fasting conditions (12). Therefore, if the inverse relationship between miR-1 and FABP-3 holds true in different pathophysiological conditions, we hypothesized that dietary restriction might induce changes in miR-1 expression that could eventually affect the sFABP3 level. To assess this, we measured cFABP3 levels and ventricular miR-1 expression in wild-type mice subjected to 48 h of fasting. As shown in Figures 4A and 4B, we found significant decreased sFABP3 levels and increased miR-1 expression levels in fasted mice. By contrast, fasting of miR-1 Tg mice caused no additional increase in the already elevated miR-1 expression level and consequently no further decrease in sFABP3 level, which was already low (Figs. 4A and 4B, Online Fig. 4).

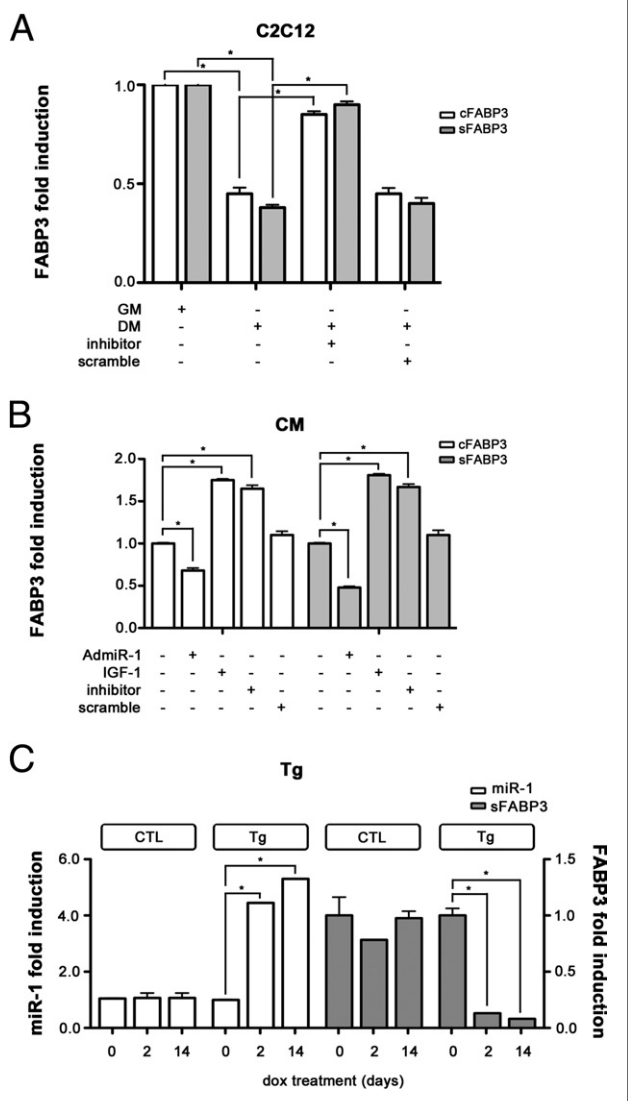

Figure 2 **Inverse Relationship Between MiR-1 and FABP3**

Enzyme-linked immunosorbent assay (ELISA) assay for levels of cellular and secreted FABP3 in **(A)** C2C12 cells, **(B)** neonatal mouse cardiomyocytes (CMs), and **(C)** heart lysate and plasma from control (CTL) and inducible, cardiac-specific miR-1 transgenic (Tg) mice. CTL and Tg mice were subjected to doxycycline (dox) administration. MiR-1 expression levels were determined by quantitative PCR (qPCR). *$p < 0.05$. cFABP3 = cytoplasmic FABP3; DM = differentiating medium; GM = growth medium; IGF = insulin-like growth factor; sFABP3 = secreted FABP3; other abbreviations as in Figure 1.

Next, to support the evidence that the circulating level of FABP3 is directly regulated by the IGF-1/miR-1 regulatory pathway, fasted wild-type mice were injected with IGF-1 in order to re-establish its levels and, consequently, repress miR-1 expression. As shown in Figures 4C and 4D, injection of IGF-1, not only reduced miR-1 levels in fasted mice to those found in fed mice, but also increased the levels of circulating FABP3.

Thus, down-modulation of the IGF-1 pathway in vivo, which occurs physiologically during dietary restriction, results in an up-regulation of miR-1 expression leading to a decrease in circulating levels of FABP3.

The circulating level of sFABP3 inversely reflects myocardial miR-1 expression in patients with aortic valve stenosis. To address whether the aforementioned inverse relationship between miR-1 and sFABP3 levels is significant also in the clinical setting, we next determined miR-1 expression and sFABP3 levels in heart biopsies and plasma from patients with left ventricular hypertrophy due to aortic valve stenosis (AS). In agreement with results from the TAC pressure overload model (Fig. 3, Online Fig. 3), myocardial miR-1 expression was decreased whereas the circulating sFABP3 level was increased in AS patients compared with healthy subjects (Fig. 5A, Online Table 1). We also compared sFABP3 in AS patients before and after transcatheter aortic valve implantation (TAVI) and found, as expected, that after the significant decrease of myocardial wall stress after TAVI (Fig. 5B, Online Table 1), the level of sFABP3 decreased also to a value comparable to that of control individuals (Fig. 5A, Online Table 1). Along this line, the increased level of circulating IGF-1 in pre-TAVI AS patients was significantly blunted by the procedure (Fig. 5C).

Despite the limitation of a small patient cohort, these results strengthen the hypothesis of a clinically significant relationship between the IGF-1/miR-1 pathway and circulating FABP3 levels, and serve as a proof of concept that the measurements of a specific secreted miR target can be used as an indirect biomarker of the cardiac miR level.

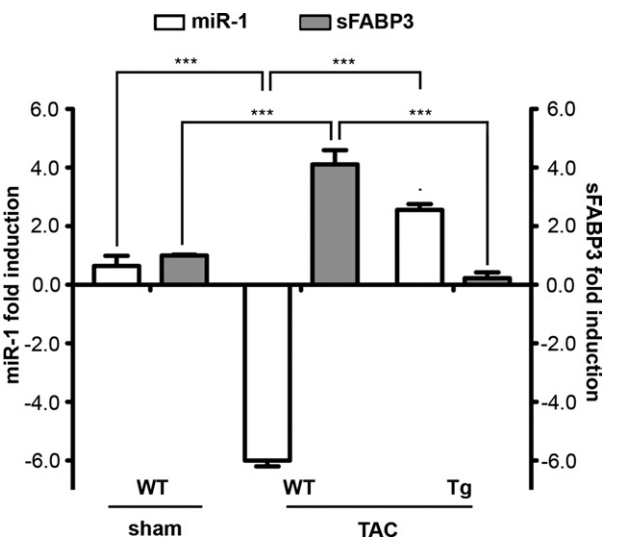

Figure 3 **Inverse Relationship Correlation Between miR-1 and FABP3 In Vivo During Pathological Cardiac Hypertrophy in Mice**

FABP3 levels and myocardial miR-1 expression in a mouse pressure-overload model of pathological cardiac hypertrophy. Control and Tg mice were subjected to doxycycline (dox) administration. (n = 8). ***$p < 0.001$. Abbreviations as in Figures 1 and 2.

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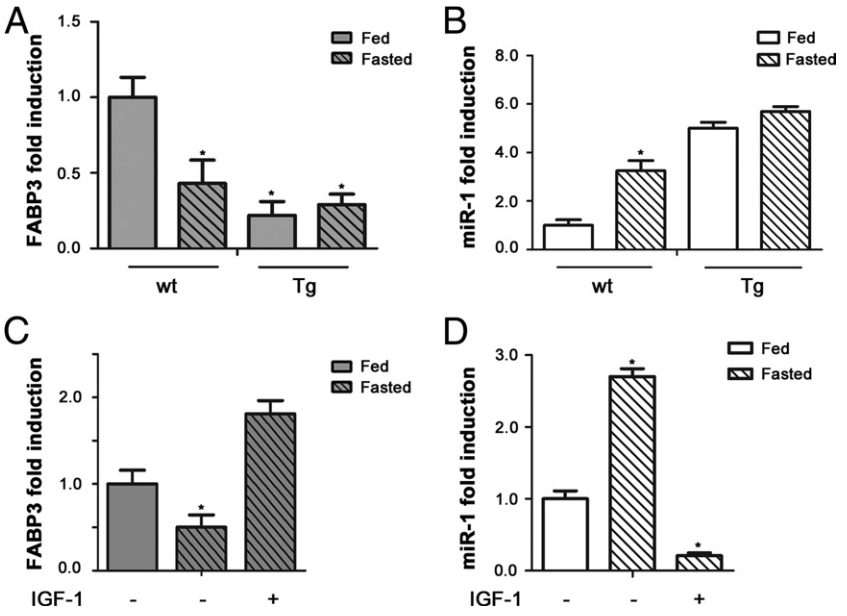

Figure 4 **Fasting Modulates MiR-1 Expression and sFABP3 Levels in Mice**

ELISA **(A and C)** and qPCR **(B and D)** analyses for sFABP3 and miR-1, respectively. Following 2 weeks of induction with doxycycline, transgenic miR-1 mice were injected with IGF-1 or phosphate-buffered saline, and either fed or fasted for 48 h (n = 6). *p < 0.05. wt = wild-type mice; other abbreviations as in Figures 1 and 2.

sFABP3 in patients with acromegaly and growth hormone deficiency. Growth hormone (GH) plays an important role in the maintenance of normal cardiac function and structure (13). Its effect on myocardial tissue is mediated by the autocrine/paracrine action of IGF-1, which upon secretion from the anterior pituitary gland contributes to myocardial tissue growth, anabolic effects, increased lipolysis, and protein synthesis (14), as well as modulation of miR-1 expression (3). Perturbation of the IGF-1 axis is associated with several cardiovascular pathologies (15), including acromegaly (ACRO), a condition in which excessive GH secretion results in elevated levels of circulating IGF-1 (16). Congestive HF associated with high levels of IGF-1 is a common complication in ACRO patients (17,18).

We have previously demonstrated that in ACRO patients, cardiac hypertrophy and increased levels of circulating IGF-1 are inversely correlated with myocardial miR-1 expression (3). We therefore hypothesized that, as a general mechanism, the level of sFABP3 may also be increased in ACRO patients. ELISA revealed that sFABP3 was in fact increased in the plasma of ACRO patients, and moreover, that medical treatment of ACRO patients, which usually results in normalization of circulating IGF-1, was accompanied by down-regulation of sFABP3 to levels comparable to those of healthy individuals (Fig. 6A, Table 1).

To further strengthen the relationship between IGF-1 and FABP3 levels in human pathologies, we analyzed patients with growth hormone deficiency (GHD), a condition opposite to acromegaly in many aspects (19). As a matter of fact, in GHD, the body does not produce enough GH, a situation eventually leading to ventricular dysfunction and abnormalities in heart structure and function, such as low cardiac output and reduced left ventricular mass. In addition, GHD is often associated with various cardiovascular risk factors, including an abnormal lipid profile and insulin resistance, which may contribute to increased atherosclerotic risk. GHD patients have lower IGF-1 plasma levels compared with healthy subjects, and correspondingly, we found decreased levels of sFABP3 in GHD patients (Fig. 6B, Table 1). Furthermore, treatment of these patients, which results in an increased IGF-1 serum level, was accompanied by a significant rise in sFABP3 (Fig. 6B, Table 1). Finally, a linear relationship between sFABP3 levels and left ventricular mass index was found in both ACRO and GHD patients (Fig. 6C).

Discussion

Accumulating evidence from studies on mouse models of cardiomyopathy and human heart pathology suggest that miR-1 is an important regulator of cardiac development and function, and is closely associated with HF progression (20). In particular, alterations in miR-1 levels have been associated with the control of CM hypertrophy, cell cycle and development, membrane excitability, and apoptosis (3,21–23). We report here that miR-1 plays a role in regulating the cardiac secretome. More specifically, we demonstrate that FABP3 is a direct target of miR-1 and that the circulating level of FABP3 is inversely related to the CM expression of miR-1.

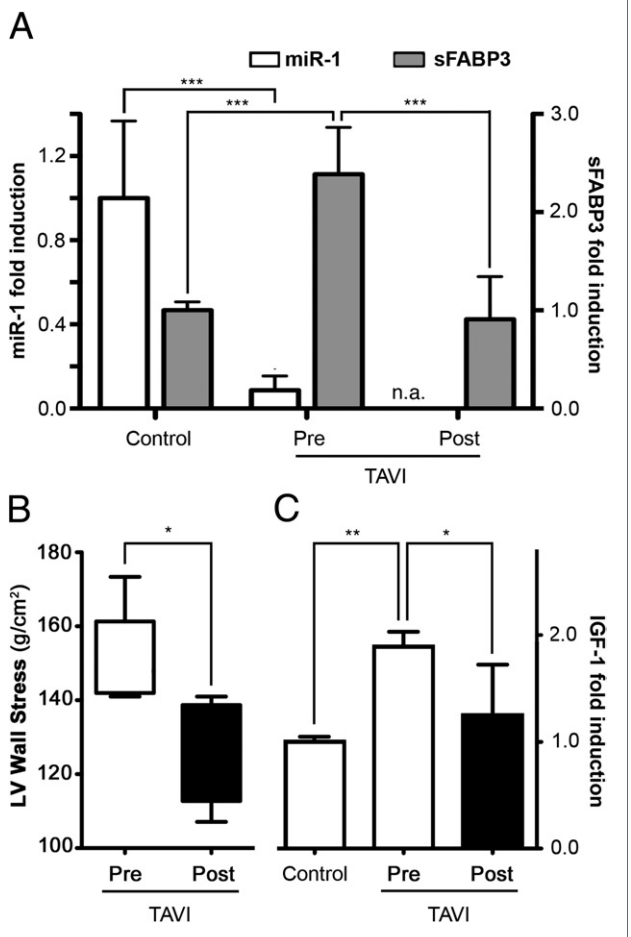

Figure 5 **Inverse Relationship Between miR-1 and FABP3 In Vivo During Pathological Cardiac Hypertrophy in Patients**

(A) Plasma and biopsies from patients with pressure overload left ventricular hypertrophy due to aortic valve stenosis before and after TAVI (n = 5). **(B)** Left ventricular (LV) wall stress and **(C)** IGF-1 levels in patients with pressure overload left ventricular hypertrophy due to aortic valve stenosis before and after transcatheter aortic valve implantation (n = 5). *p < 0.05, **p < 0.01, ***p < 0.005. n.a. = not available; TAVI = transcatheter aortic valve implantation; other abbreviations as in Figures 1 and 2.

FABP3 is a small protein that belongs to a multigene family of FA-binding proteins. Among the members of this family, FABP3 is the most abundantly expressed in the heart, where it accounts for ~4% to 5% of total cellular proteins. Its main role is to mediate the intracellular uptake of FAs and their subsequent transport toward the mitochondrial β-oxidation system (5). FAs account for ~70% of the energy source in a healthy heart, but a number of myocardial pathologies, such as cardiac hypertrophy, dilated cardiomyopathy, myocardial infarction, and HF, are associated with perturbations of energy metabolism and a shift in substrate utilization toward either more FA or more glucose. Albeit the role of sFABP3 in the pathophysiology of HF is still a matter of debate and beyond the scope of this study, we here provide evidence for a previously unknown inverse relationship between cellular levels of a miR-1 and the circulating level of its target FABP3, suggesting the potential use of FABP3 as an indirect biomarker that mirror the myocardial activity of miR-1 (Fig. 7).

The relationship between miR-1 and FABP3 is tightly controlled by the IGF-1 axis. In response to ventricular pressure overload (TAC in mice or AS in patients), the myocardium switch to a high metabolic energy demand follows the hypertrophic response of the myocardium. This is paralleled by an increase in the level of IGF-1 (3,24) and a down-regulation of miR-1 expression (10) (Figs. 5A and 5C). As shown in this study, the reduction in miR-1 relieves its negative regulatory control over FABP3 protein synthesis, leading to a prompt release of the protein into the bloodstream (Figs. 3 and 5). Accordingly, exposure of primary CMs to IGF-1 leads to a release of high amounts of FABP3 into the extracellular medium, which can be prevented by overexpression of miR-1 (Fig. 2B).

By contrast, fasting is associated with alterations in circulating molecules, such as a decrease in IGF-1 and an elevation of circulating FAs released through the breakdown of triglycerides (12). In addition, a reduction in serum IGF-1 has been suggested to play a role in metabolic adaptation during malnutrition (25). Although further stud-

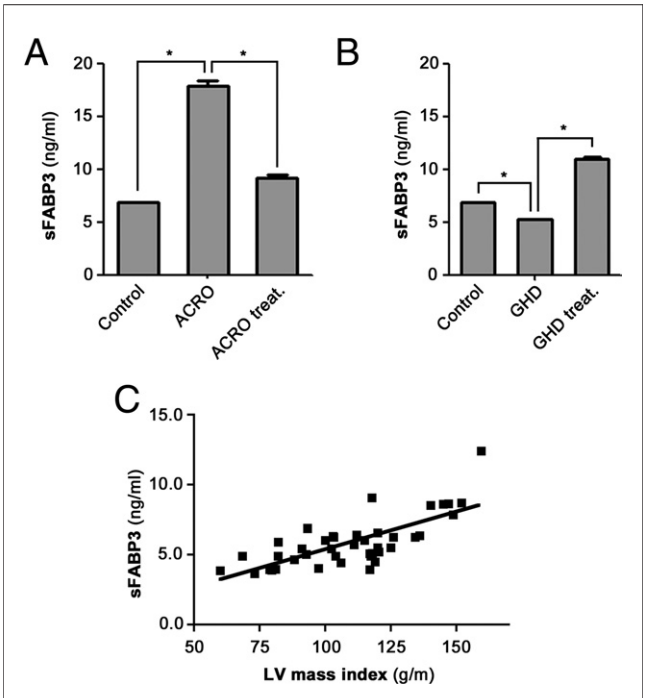

Figure 6 **Circulating Levels of FABP3 in ACRO and GHD**

ELISA for sFABP3 performed on plasma samples from patients with **(A)** acromegaly and **(B)** growth hormone deficiency before and after treatment. **(C)** Pearson product-moment correlation between left ventricular (LV) mass index and levels of sFABP3 measured in patients with acromegaly and growth hormone deficiency (p < 0.0001, r = 0.7226). A scatterplot with regression line is shown. Samples from healthy donors were used as control. *p < 0.05. ACRO = acromegaly; GHD = growth hormone deficiency; treat. = treatment; other abbreviations as in Figures 1 and 2.

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Table 1 Clinical and Echocardiographic Characteristics of ACRO and GHD Patients

	Study Group				
		ACRO		GHD	
Variable	Control (n = 18)	Pre-Treatment (n = 20)	Post-Treatment (n = 20)	Pre-Treatment (n = 10)	Post-Treatment (n = 10)
Female/male	12/16	13/7	13/7	3/7	3/7
Age, yrs	39.6 ± 10.8	48 ± 17.4	48 ± 17.4	62 ± 17.5	62 ± 17.5
LV ejection fraction, %	66.2 ± 1.2	56.2 ± 1.0†	63.7 ± 1.5*	58.2 ± 1.2†	62.7 ± 1.6*
LV mass index, g/m	86.3 ± 2.7	132.1 ± 3.7†	118.9 ± 4.5*	98.5 ± 3.0†	122.6 ± 6.3*
IGF-1, ng/ml	248.65 ± 20.1	800.3 ± 80.9†	292.1 ± 33.2*	59.7 ± 15.3†	105.7 ± 22.3*
FABP3, ng/ml	6.8 ± 1.5	17.8 ± 2.5†	9.2 ± 2.2*	5.23 ± 0.5†	10.9 ± 2.5*

Values are n/n or mean ± SD. *$p < 0.05$ post-treatment versus pre-treatment for each pathological-condition; †$p < 0.05$ compared with control.
ACRO = acromegaly; Control = healthy subjects; FABP3 = fatty acid-binding protein-3; GHD = growth hormone deficiency; IGF = insulin-like growth factor; LV = left ventricular.

ies are required to ultimately understand the functional relevance of circulating FABP3 activity, we found that the molecular events that are activated under fasting lead to increased miR-1 expression that in turn results in a decreased circulating level of FABP3. Consistent with our previous report in which we demonstrated that miR-1 is negatively regulated by the IGF-1 pathway (3), administration of IGF-1 to fasted mice was sufficient to induce down-regulation of miR-1 and, consequently, to increase the level of circulating FABP3 (26,27).

Of interest is the positive relationship between circulating FABP3 and levels of IGF-1 in ACRO and GHD patients. These are pathological conditions in which either hyper- or hyposecretion of GH and IGF-1 lead to an increase in cardiovascular, cerebrovascular, and metabolic morbidity. Furthermore, despite the limitations that a replacement therapy might have in regard to over- or undertreatment, we found that alterations in circulating levels of IGF-1 as a result of medical interventions in these patients lead to a corresponding variation in circulating FABP3 concentration.

Recently, several lines of evidence have supported the clinical relevance of FABP3 as a biomarker for myocardial injury (26,27). In agreement with this, our analysis of an in vivo mouse model of cardiac damage revealed release of FABP3 in response to injury, which was inversely related to the cellular levels of miR-1 (Fig. 3). Interestingly, an inverse relationship was also found in patients with different cardiac pathological conditions (Figs. 5A and 6).

In conclusion, we report here a novel role of the muscle-specific miR-1 in modulating the cellular and secreted levels of FABP3 and demonstrate its potential use as an indirect biomarker in the plasma, reflecting the cellular activity of

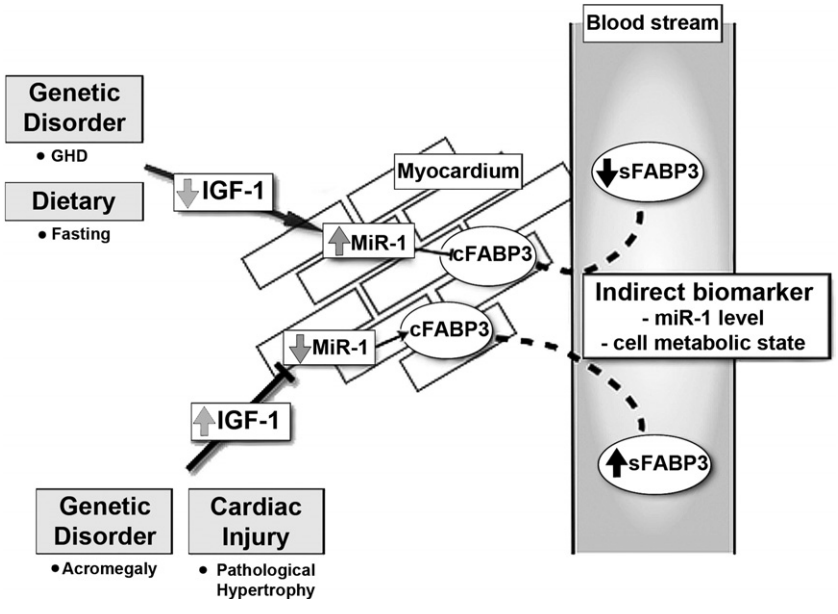

Figure 7 Working Model of sFABP3 as an Indirect Biomarker, Reflecting the Cellular Activity of MiR-1

Abbreviations as in Figures 1 and 2.

miR-1. This might overcome the difficulties in directly measuring the cellular miR-1 levels in humans (Fig. 7).

Study limitations. Despite the relevance of our finding, a limitation of the study is that the pathophysiological relevance of variations in circulating FABP3 remains unsolved. In fact, it is still a matter of debate: 1) whether and how FABP3 may affect cardiac metabolism during remodeling stress; 2) whether changes in circulating FABP3 may discriminate between different types of remodeling (concentric, eccentric, with/without left ventricular dysfunction); and 3) whether it may confer any prognostic information. Answering these questions will require further additional studies.

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Reprint requests and correspondence: Dr. Daniele Catalucci, Milan Unit, Biomedical and Genetic Research Institute (IRGB), National Research Council, via Fantoli, 16/15, 20138, Milan, Italy. E-mail: daniele.catalucci@cnr.it. OR Dr. Gianluigi Condorelli, Humanitas Clinical and Research Center, Via Manzoni 56, 20089, Rozzano, Milano, Italy. E-mail: gianluigi.condorelli@humanitasresearch.it.

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Key Words: biomarker ■ cardiovascular diseases ■ FABP3 ■ microRNA ■ miR-1.

APPENDIX

For the study methods and a supplementary table and figures, please see the online version of this paper.