Physical Exercise and MicroRNAs: New Frontiers in Heart Failure

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Abstract

Although the impact of exercise on survival of patients with heart failure has been recently questioned, exercise training improves quality of life, functional capacity, inflammation, endothelial and autonomic function. In recent years, interest has increased regarding a group of small non-protein coding RNAs called microRNAs.

Studies have shown that the expression of these molecules changes in several pathological conditions, such as myocardial infarction, myocardial ischemia and heart failure, and when clinical improvement occurs, they seem to normalize. With the potential for practical applicability, markers that may be useful in diagnostic and prognostic assessment of heart failure have been identified, such as miR-423-5p. In addition, results of experimental studies have indicated that there are potential therapeutic effects of microRNAs.

MicroRNAs are involved in the regulation of gene expression during fetal development and in adult individuals, increasing or decreasing in the heart in response to physiological stress, injury or hemodynamic overload. Thus, the study of the behavior of these molecules in physical exercise has brought important information about the effects of this therapeutic modality and represents a new era in the understanding of heart failure.

This review aims to integrate the evidence on microRNAs in heart failure with greater relevance in the study of physical exercise.

Introduction

Several advances in the understanding of the physiopathology of heart failure (HF) allowed the development of new therapeutic modalities, or even optimized them, with a consequent increase in survival. Nevertheless, HF is still a condition with high mortality and morbidity, being the leading cause of hospitalization due to cardiovascular disease in Brazil[1].

Keywords

Exercise; microRNAs; heart rate / physiopathology.

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In spite of recent evidence questioning the impact of exercise on survival[2], physical training improves quality of life[3], functional capacity[4], inflammation[5], and autonomic[4] and endothelial function[4]. The mechanisms involved in this phenomenon are not yet fully understood. In recent years, interest has increased in the area of research involving aspects related to the body's response to exercise in the genetic scenario, in which microRNAs appear to play a key role.

It has become increasingly evident that microRNAs play a critical role in many biological processes, and research in cancer and cardiovascular diseases have been the main focus in translational medicine. Recent studies have evaluated the expression of several microRNAs in myocardial hypertrophy, myocardial infarction and heart failure, which will improve the understanding of the physiopathology of these clinical conditions, in addition to their promising use in the diagnosis and prognosis of major cardiovascular diseases[6,7]. Moreover, the possibility of mimicking microRNAs with reduced expression or antagonizing those of which increased expression would be causally linked to a specific pathology, represent a therapeutic potential and new paradigm in the management of heart diseases.

Implicated in the regulation of gene expression during fetal development and in adult individuals[8,9], many of these microRNAs increase or decrease in the heart in response to physiological stress, injury or hemodynamic overload[10]. The study of the behavior of these molecules in physical exercise therefore represents a great potential for new discoveries of this type of therapeutic modality in HF.

This review aims to integrate the evidence for microRNAs in heart failure with greater relevance in the study of physical exercise.

MicroRNAs

In response to external stimuli such as exercise, gene expression can be modulated by different mechanisms, including gene silencing by small RNAs, including the microRNAs (miRNAs)[11].

The miRNAs, initially described in 1993 when studying the development of nematodes[12], are characterized as a group of small non-protein-coding RNAs, with approximately 19-25 nucleotides in length. Unlike the wide range of RNAs encoded by the human genome, this variety of RNA has been noted for its unique ability to modulate an enormous and complex regulatory network of gene expression[13].

It is currently known that, in general, miRNAs are synthesized from specific genes or certain genetic regions that are not associated with protein production (introns)[11]. The maturation process of miRNAs involves a complex metabolic pathway that begins in the nucleus and extends to the cell cytoplasm (Fig. 1). The first step in the miRNA maturation is the transcription of a long primary miRNA strand (pri-miRNA) from a certain gene[14]. The pri-miRNA is cleaved by an enzyme complex called *Drosha*, releasing precursor regions called pre-miRNAs, which will form distinct miRNAs[15]. In the cytoplasm, after being exported from the nucleus by exportin-5[16], the pre-miRNA is cleaved by the *Dicer* enzyme, forming a double-stranded RNA with approximately 22 nucleotides[17]. The two strands are then separated; however, only one of them will potentially act as a functional miRNA, while the other is generally degraded[18].

In its mature form, miRNAs, with the help of an enzyme complex called RNA-Induced Silencing Complex (RISC), binds to the target messenger RNA (mRNA). This binding prevents the ribosomes from accessing the genetic information contained in the mRNA, resulting in decreased protein synthesis of the target gene[19,20].

In general, the function of miRNAs is to act as a recognition component for RISC, as they bind to mRNA, identifying it as a target. The complexity of protein expression regulation by miRNA is illustrated by the fact that a single miRNA can regulate hundreds of distinct target genes, and otherwise cooperate to control a single target gene [2,13].

More than 700 human miRNAs have been identified and there seems to be 150 to 200 of them expressed in the heart[10]. This number is expected to grow even further, due to the recent development of sequencing technologies and computational prediction methods[19,20]. Although the biological functions of miRNAs are not fully understood, it is believed that 30% to 60% of the protein-coding genes are regulated by miRNAs[11].

Studies have shown that although miRNAs are expressed in various cell types, specific miRNAs are synthesized exclusively in certain tissues or cell groups[19,20].

MicroRNAs and heart failure

In recent years, it has been shown that many miRNAs are expressed in a specific way in some cardiovascular

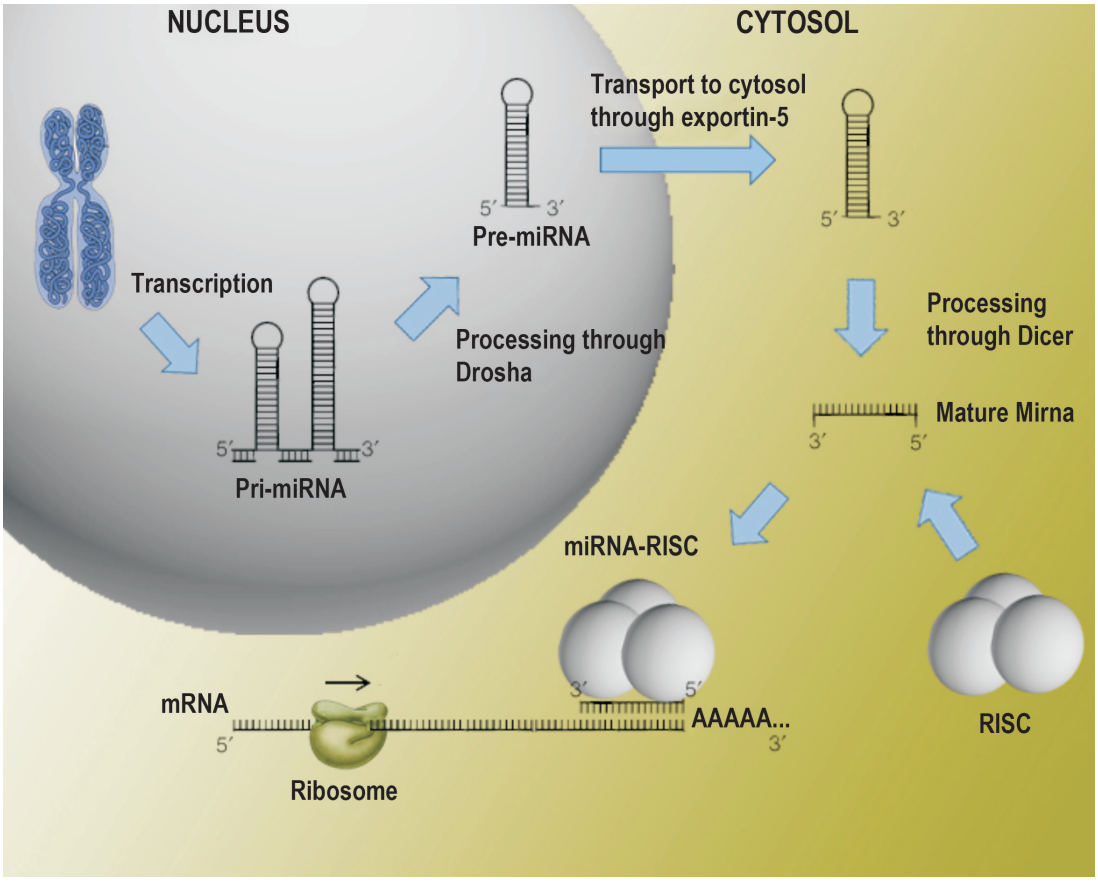

Figure 1 – *Representative scheme of biogenesis and mechanism of action of microRNAs in mammalian cells. mRNA - messenger RNA, miRNA - microRNAs, RISC – RNA - inducing silencing complex. Adapted from Oliveira-Carvalho V et al, Arq Bras Cardiol.2012;98(4):362-70.*

conditions. Specifically, there is a differential expression in hypertrophy, myocardial ischemia and endothelial dysfunction.

Systolic HF is characterized by ventricular dilatation and remodeling and there seems to be an activation of a fetal gene program that triggers pathological changes in the myocardium, associated with progressive ventricular dysfunction[21]. A pattern of miRNA expression very similar to that found in fetal hearts has been identified in the heart of patients with advanced-stage HF[21]. Moreover, in a study evaluating[22] patients with dilated, ischemic and valvular cardiomyopathy (aortic stenosis), the expression profile was different according to the etiology of HF, and the miRNA signature was able to predict diagnosis with an accuracy of 70%.

Four families of miRNAs are highly expressed in the heart: miR-1, miR-133, miR-208 and miR-499. The miR-1 family represents 40% of all miRNAs expressed in the heart, whereas the miR-208 is the only one known as cardio-specific. Table 1 contains some miRNAs and their possible biological effects on the adult heart.

The miR-1 is reduced in myocardial hypertrophy, and *in vitro*[23] and *in vivo*[24] studies indicate that there is a causal relationship, in which the reduction of this miRNA is a necessary condition for cell mass increasing[25]. An experiment in rats[26] showed that one of the earliest changes observed after pressure overload to the heart is the reduction in miR-1, even before the increase in cardiac mass. The miR-1 is reduced in the hypertrophic ventricle of patients with acromegaly[26] and aortic stenosis[22] with preserved ejection fraction.

On the other hand, the expression of miR-1 shows different findings in HF. While some authors[22,27,28] observed a reduction in miR-1 in ischemic and non-ischemic dilated cardiomyopathy, others observed an increase[21,29]. Han et al[25] suggest the possibility that miR-1 is reduced in hypertrophy, but returns to normal or above normal when the condition progresses to HF.

The family of miR-208 is restricted to the heart and consists of the miRs-208a and -208b, which are encoded in introns of genes of myosin heavy chains (MHC) α and β, respectively[30,31]. The interactions of these miRNAs with the MHC genes are involved in the development of hypertrophy in adults.

Other miRNAs also show a differentiated expression in hypertrophy and myocardial ischemia. Some examples are shown in Table 2.

Changing in miRNA expression in response to heart functional alterations have also been demonstrated. An interesting study[29] evaluated the profiles of mRNA and miRNA in 10 hearts treated with left ventricular assistance device (LVAD), 17 hearts of individuals with HF not treated with LVAD and 11 controls (no HF). In the hearts of individuals with HF without LVAD, a significant increase in the expression of 28 miRNAs was observed, while in those treated with LVAD, 20 of these miRNAs were completely normalized, and the other eight tended to normalization. Experimental studies[32] have shown that a phenotype can be reversed through the inhibition of a specific miRNA that has an increased expression in this condition. This can be accomplished by administering an anti-miRNA oligonucleotide, which acts as a competitive inhibitor of that miRNA and is called antagomiR. Recently, Montgomery et al[33] demonstrated that administration of an anti-miR-208a antagomiR was able to prevent ventricular hypertrophy, cardiac remodeling and mortality in Dahl rats due to HF (sensitive to salt) subjected to a hypersodic diet.

The study strongly suggests that the effects of antagomiR were due to reduced levels of miR-208a. This result not only establishes a causal correlation of this miRNA with the mechanism of hypertrophy, but also brings a very promising therapeutic use to reverse remodeling. However, in adult rats, the profile miR-208a/α-MHC is predominant, and when pressure overload occurs, there is a shift in the production from α to β-MHC[30,31]. In humans, the role of this mechanism in hypertrophy remains to be clarified,

Table 1 - Examples of microRNAs and their biological effects on the adult heart

microRNA	Biological effect
miR-208a	Regulates production of αMHC[31]/ induces hypertrophy[33]
miR-208b	Regulates production da βMHC[31]
miR-499	Induces hypertrophy[33]
miR-1	Inhibits hypertrophy[60]
miR-133a	Inhibits hypertrophy[24]
miR-126	Role in endothelial function regulation[61]/inhibits VCAM-1 expression[62]
miR-210	Reduces apoptosis in ischemic cell[42]
miR-92a	Inhibits neovascularization after AMI[63]
miR-29	Inhibits fibrosis[61]
miR-21	Increases fibrosis[61]

MHC – myosin heavy chain; VCAM – vascular cell adhesion molecule; AMI – acute myocardial infarction.

Table 2 - Expression of some microRNAs in cardiac hypertrophy and myocardial ischemia[25]

Clinical condition	increased miRNA expression	reduced miRNA expression
Cardiac hypertrophy	miR-208b	miR-133
	miR-21	miR-1
	miR-23a	miR-30c
Myocardial ischemia	miR-1	miR-92a
	miR-133	miR-199a
	miR-21 (fibrosis)	miR-21 (cell viability)
	miR-210	miR-29
	miR-126	miR-320
	-	miR-494

as this species is not able to reverse the MHC isoforms, and the β-MHC is the predominant form, together with the miR-208b[31]. In addition, experimental models must be viewed with caution when extrapolating data, as they are artificial and may not accurately mimic the clinical conditions observed in humans[10].

MicroRNA as biomarker in heart failure

For a widespread use of miRNA as a biomarker in heart failure, its determination must be simple and reliable, preferably dispensing with the need to perform an invasive procedure such as a biopsy. Although the research of miRNAs in tissues and cells have been carried out for some time, only recently it was found the existence of a series of these miRNAs in circulating blood. Mitchell et al[34] demonstrated in patients with prostate cancer that tissue-specific circulating miRNAs could be detected in plasma. The mechanisms by which they are released are uncertain, but it has been postulated to be through the secretion within microvesicles called exosomes[35]. It is unknown whether this occurs with other miRNAs, but characteristics such as specificity and stability in plasma make it very promising as a marker of tissue injury[36].

The miRs-208a, -208b and -499 miRNAs are specific to cardiomyocytes and were evaluated in the diagnosis of conditions that cause myocardial injury. There was an increase of more than 1,000-fold in acute myocardial infarction[6,37-39] of miRs-208b and-499. In this condition, they reflect tissue injury and cell death. In addition, miR-208a showed diagnostic accuracy that was similar to troponin, with the advantage of being detected earlier in plasma[6].

In order to determine which could be used as biomarkers in HF, Tijsen et al[40] compared the expression profile of miRNAs in the plasma of 12 healthy subjects with 12 patients admitted for acute HF. Among 108 miRNAs that showed significant difference between the groups, 16 were selected and validated in a larger group, including 30 patients with HF, 20 who had dyspnea due to other causes rather than the HF, and a third group of 39 healthy individuals. In this validation cohort, an elevated level of mir-423-5p was strongly associated with the diagnosis of HF in a logistic regression model including age and sex. The mechanisms by which there is an increase in this marker in patients with HF remains unknown. Whether this is due to cell damage and subsequent release of that in plasma, or is related to a specific secretory pathway of a given cell, it remains to be clarified[41]. In another study[7], plasma concentrations of miR-126 showed an inverse correlation with the levels of brain natriuretic peptide (BNP). However, the patient sample was heterogeneous and the criteria for HF were not well established. More studies are needed to define the role of these miRNAs as biomarkers in HF.

MicroRNA and exercise

Physical exercise, pregnancy and the individual's growth are stimuli for physiological increase of the heart. In the mid-nineteenth century, descriptions of the so-called "athlete's heart" had deleterious connotations[8]. More recently, the multiple benefits of physical training on survival and the harmful effects of a sedentary lifestyle made this therapeutic modality required to treat patients with heart disease, including those with systolic ventricular dysfunction, who have already shown a pathological increasing in ventricular volumes and masses. Its benefits regarding functional capacity can be explained by effects on endothelial function, peripheral vascular resistance and changes in the structure of skeletal muscle, although significant improvement in left ventricular ejection fraction have not been demonstrated.

The study of microRNAs may generate hypotheses about the mechanisms by which exercise affects the pathophysiology of HF. Interestingly, the signaling pathways leading to hypertrophy due to physiological stimuli such as exercise, are different from those that cause pathological hypertrophy and thus may trigger different expressions in miRNAs[8]. Ischemic preconditioning and its relationship with the miRNA is already being evaluated in experimental studies[42].

When analyzing individual responses to physical training, doubts are raised concerning the reasons for the variability of their results. There is evidence that physiological adaptability to exercise has a parallel with gene expression[43]. However, the complexity of the genetic interaction limits the identification of individual genes that may explain this variability[44]. Moreover,

its expression changes according to the stimulus that the body receives and it is in this aspect that the study of miRNAs has a promising role.

The skeletal muscle is also an organ with high plasticity, capable of altering the phenotype in response to mechanical overload[45]. Experimental studies have identified changes in the skeletal muscle profile of specific miRNAs in aerobic and resistance exercises[46] (Table 3).

There are several miRNAs present in skeletal muscle, and miRs-1, -133a, -133b and -206 comprise 25% of them, often being referred to as "myomirs"[47]. In healthy subjects, these four myomirs decrease significantly after 12 weeks of aerobic training[48], indicating that they adjust quickly to the level of physical activity. Interestingly, these levels return to baseline after 14 days of its discontinuation. When evaluating their response to a single exercise session, an increased expression of miR-1 and 133a was observed only before the training period.

With respect to resistance exercise, the gain in muscle mass is also highly variable among individuals. This variability is also accompanied by differences in the behavior of miRNAs. A study[47] that analyzed biopsies of the vastus lateralis muscle of 56 men undergoing resistance training for 12 weeks found that the expression of miR-378 decreased and that of miR-478 increased, both significantly, only in those who responded little to the training. In addition, there was a strong correlation between the variation in the expression of miR-378 with the gain in lean body mass. With respect to myomirs, resistance exercise did not change miRs-1 and -133 in this study.

With respect to the levels of miRNAs in circulating blood, Baggish et al[49] studied the behavior of miRNAs that were previously implicated in angiogenesis (miR-20a, miR-210, miR-221, miR-222, miR-328), inflammation (miR-21, miR-146a), cardiac and skeletal muscle contractility (miR-21, miR-133a) and muscular adaptation to hypoxia and ischemia (miR-21, miR-146a, and miR-210). The results of this and other studies involving the behavior of miRNAs in exercise in humans are summarized in Table 4.

The study of miRNAs can also help to understand the effects of exercise on immune response, of which mechanisms remain unclear. For instance, based on the analysis of cytokines, it was observed that both pro-inflammatory and anti-inflammatory pathways are activated after strenuous exercise[50]. Studying the behavior of neutrophils in peripheral blood[51] after a workout, it was possible to observe changes in the expression profile of miRNAs, suggesting that these contribute to changes in gene expression of immunocompetent cells[52].

By the time of publication of this review, we are not aware of studies that have evaluated the behavior of miRNAs in response to physical training specifically in patients with HF.

Perspectives

Much has been discovered on the effects of exercise training on HF. In addition to improving functional capacity and survival, other evidence has shown positive results of this therapy, such as an increasing in circulating endothelial progenitor cells[53], anti-inflammatory effects and effects on the endothelium, and changing in levels of interleukin-1β[54], -6[54,55], -10[56], tumor necrosis factor-α and its receptors 1 and 2 and C-reactive protein[56], inducible nitric oxide synthase (iNOS)[54], soluble intracellular adhesion molecule (sICAM)[55], adiponectin[57,58], brain natriuretic peptide (BNP)[59] and others (Fig. 2). Therefore, a promising tool emerges for diagnostic and prognostic evaluation of treatment in HF, which will increase the knowledge of the effects of physical exercise in these patients, going deeper into the molecular mechanisms, with the advantage of reflecting more directly the genetic signaling pathways, linking environmental stimuli to the hereditary aspects of the individual. MicroRNAs represent a new era in knowledge of heart failure and the possible effects that exercise has on it.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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Tabela 3 - Effects of physical exercise on expression of several microRNAs in experimental studies

microRNA	Correlation with physical exercise	Studied population	Type of sample	Reference
miR-1, -107, -161	Increased after 90 minutes of exhaustive aerobic exercise (acute)	C57BL/6J Rats	Skeletal muscle	Safdar et al[64]
miR-23	Decreased after 90 minutes of exhaustive aerobic exercise (acute)	C57BL/6J Rats	Skeletal muscle	Safdar et al[64]
miR-1 e -133a	Decreased by 50% after functional muscular overload	C57BL/6J Rats	Skeletal muscle	McCarthy et al[65]
miR-696	Decreased after 4 weeks of aerobic training and increased after 5 days of immobilization	C57BL/6 Rats	Skeletal muscle	Aoi et al[66]
miR-27a e -27b	Increased after aerobic training	Wistar Rats	Heart tissue	Fernandes et al[67]
miR-143	Decreased after aerobic training	Wistar Rats	Heart tissue	Fernandes et al[67]

Table 4 - Effects of physical exercise on the expression of several microRNAs in humans

microRNA	Correlation with physical exercise	Population studied	Type of sample	Reference
miR-133a	Decreased after 7 days of rest	12 healthy young men	Biopsy of vastus lateral	Ringholm et al[68]
miR-21, -146a, -221, 222,	Increased after 90 days of aerobic training	10 male athletes	Plasma	Baggish et al[49]
miR-146, -222	Increased in acute exercise before and after aerobic training for 90 days	10 male athletes	Plasma	Baggish et al[49]
miR-21, -221	Increased in acute exercise before, but not after aerobic training for 90 days	10 male athletes	Plasma	Baggish et al[49]
miR-20a	Increased with aerobic training, but does not modify in acute exercise; it variation has linear correlation with change in VO2 peak	10 male athletes	Plasma	Baggish et al[49]
miR-146	Linear correlation with VO2 peak*	10 male athletes	Plasma	Baggish et al[49]
miR-1	Acute reduction after single resistance exercise (8 series, 10 repetitions) in young individuals	12 healthy men	Biopsy of vastus lateral	Drummond et al[69]
miR-378	Decrease only in weak responders after 12 weeks of resistance exercise	56 healthy men	Biopsy of vastus lateral	Davidsen et al[47]
miR-451	Increase only in weak responders after 12 weeks of resistance exercise	56 healthy men	Biopsy of vastus lateral	Davidsen et al[47]
miR-1, -133a, -133b, -206	Decrease only after aerobic exercise for 12 weeks	10 healthy men	Biopsy of vastus lateral	Nielsen et al[48]

**VO2 peak - peak oxygen uptake.*

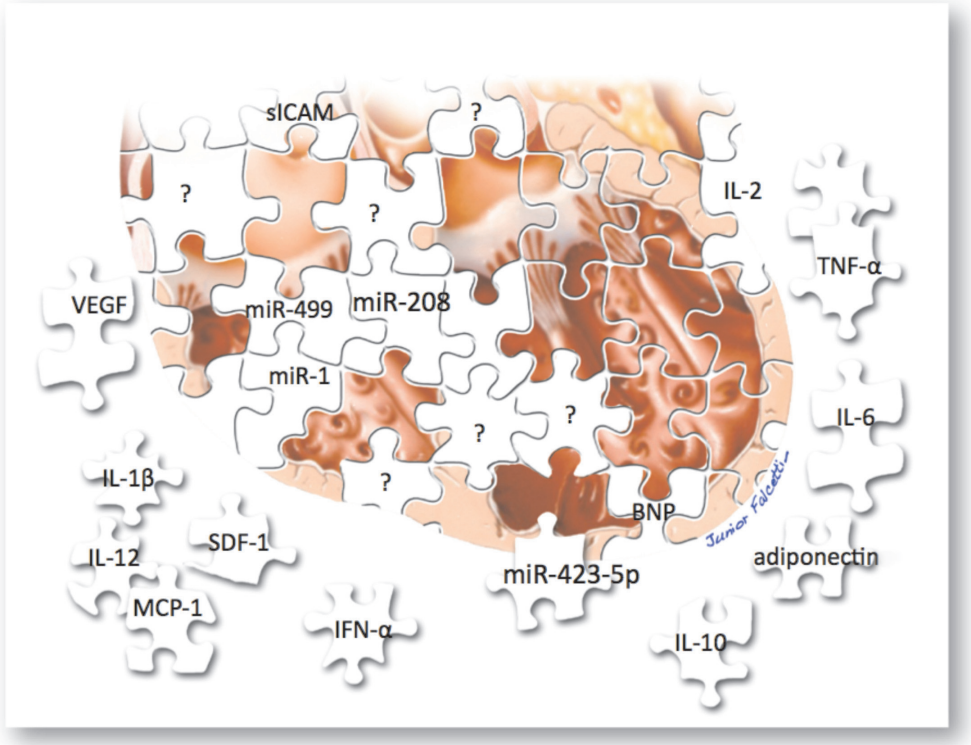

Figure 2 – *Schematic illustration of several markers and molecules involved in heart failure. VEGF - vascular endothelial growth factor, sICAM - soluble intracellular adhesion molecule, SDF-1 - stromal cell-derived factor 1, IL - interleukin, MCP-1 - monocyte chemoattractant protein 1, IFN - interferon, TNF - tumor necrosis factor; BNP – natriuretic brain peptide. Figure provided by Edmar Bocchi.*

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