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VIEWPOINT

The Role of MicroRNAs in the Pathogenesis of Chagas Disease

Cristiane Tefe-Silva,[1] Laura de Oliveira Teixeira,[1] Laura Ramos Durigan,[1] Maria Clara Silva Cardoso,[1] Maria Laura de Castro Davi,[1] Paula Altieri Pin,[1] Stephanie Milanez,[1] Veronica Cestari Lourenço,[1] Thálita Cristina de Sousa Beine,[1] Eloisa Yara Araujo Clemente[1]

Barão de Mauá University Center,[1] Ribeirão Preto, SP – Brazil

Abstract

Chagas disease (CD) is caused by the protozoan *Trypanosoma cruzi* and affects about six to seven million individuals worldwide. The distribution of cases is concentrated mainly throughout Latin America, especially in rural areas. This study aims to evaluate microRNAs (miRNAs) as indicators in CD diagnosis for possible contributions to its management. This is a literature review study, carried out in the PubMed, SciELO, Bireme Library, NCBI, Science Direct, and Embase databases, through which a total of 12 articles were included for qualitative analysis. The discussion of this review was based on the thematic axes regarding the modulation of *T. cruzi* in the immune system and the expression of miRNAs, their production and action, the modulation mechanism of host gene expression, how they act as biomarkers, the importance of miRNAs in the diagnosis of CD, and how their regulation occurs in Chronic Chagas Cardiomyopathy (CCC). Moreover, *T. cruzi* infection is associated with the downregulation of several miRNAs, which directly related to the findings of hypertrophy and fibrosis. When quantified, these could be used as consistent indicators for CD to support the diagnosis of patients with CD complications, as well as a possible therapeutic target. However, the need for clinical studies that evaluate the usefulness of this biomarker in humans is emphasized, considering that in the present study, only experimental *in vitro* studies were evaluated, reflecting a lack of studies with practical applicability.

Keywords

MicroRNAs; Chagas Disease; Immunomodulation.

Introduction

Chagas Disease (CD), caused by *Trypanosoma cruzi*, infects an estimated six to seven million people worldwide. These people are concentrated mainly in Latin America, with major impacts on mortality in these endemic regions. The distribution of CD mainly occurs in rural regions. However, with the increase in population mobility to large centers, a greater spread of the disease to urban areas has been observed.[1] It is important to highlight that many people continue to go undiagnosed and untreated, and around 75 million are at risk of becoming infected.[2]

The diagnosis of CD is based on clinical and epidemiological criteria, as well as on laboratory tests. During the acute phase, with high parasitemia, parasitological methods are carried out to directly identify the etiological agent *T. cruzi* in blood smears and serological tests, the latter of which presents precipitation or precipitin reaction, indirect immunofluorescence reaction (IFAT), and Enzyme-linked-immunosorbent-assay (ELISA).[3] In the chronic stage, the diagnosis is made based on clinical findings and epidemiological history, requiring at least two positive serological tests, including ELISA, immunofluorescence, and/or indirect hemagglutination.[4] In the case of chronic patients, around 30% may develop heart rhythm abnormalities, cardiomyopathy, heart failure, or sudden death due to damage to the myocardium.[4] To identify patients with subsequent cardiac involvement, there are still limited resources that indicate clinical and laboratory markers of prognosis and signs of deterioration of cardiac tissue.[5]

For new diagnostic methods, the use of biomarkers has recently been studied. They predict the progression of the disease in its most severe and asymptomatic or indeterminate form, Chronic Chagasic Cardiomyopathy

Mailing Address: Cristiane Tefe-Silva
Barão de Mauá University Center. Rua Ramos de Azevedo, 423. Postal code: 14090-180. Ribeirão Preto, SP – Brazil.
Email: cristiane.silva@baraodemaua.br

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Central Illustration: **The Role of MicroRNAs in the Pathogenesis of Chagas Disease**

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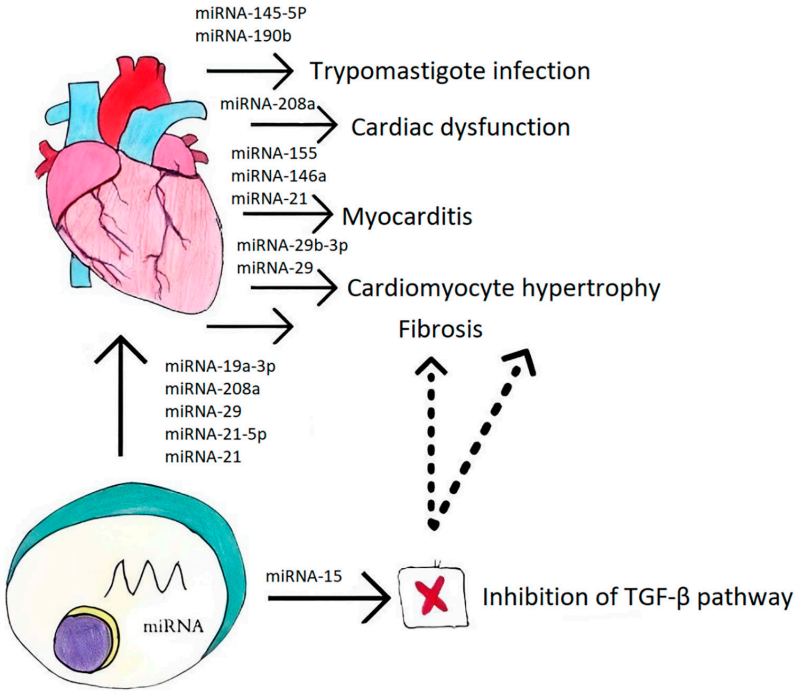

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miRNA: microRNA; TGF-β: Transforming Growth Factor Beta. Source: The author

(CCC), and can guide the implementation of pharmacological therapy aiming to delay the development or progression of cardiac dysfunction by early identification of tissue damage.[6,7]

From this perspective, it is known that microRNAs (miRNAs) - a class of non-coding RNA, which act by regulating the expression of genes and genomes - are not capable of protein-coding, though they do have the capacity to inhibit gene expression, thus acting as regulators. Therefore, they are relevant for understanding the physiological and pathological conditions of human beings.[8,9] Despite the wide existence of cardiovascular tests (electrocardiogram, echocardiogram) and imaging tests to evaluate cardiovascular function in patients who develop CCC, biomarkers that individually predict the risk of progression from the indeterminate form of the disease to CCC are not yet known, and only Seroconversion of serological tests for monitoring treatment effectiveness is considered the gold standard, which generally takes a long time to occur. Therefore, given the prognostic limitations, miRNAs could be possible biomarkers indicative of complications in CD, assisting in therapeutic choice, given that their increase in circulation is associated with cardiac injury and the severity of the disease.[3,6]

The production of miRNAs occurs from precursors called pri-miRNAs, which, under the action of RNA polymerase II, are folded into hairpins (base pairing) and become substrates for the Drosha and the Dicer, which belong to the ribonuclease III family (RNAse). In the first nuclear step, the pri-miRNA is cleaved by the Drosha, forming the pre-miRNA. Then, it is exported to the cytoplasm by Exportin 5 and is subsequently acted upon by the Dicer, forming a double miRNA (miRNA/miRNA). Finally, the Argonaute protein (Ago) forms the RNA-induced silencing complex (miRISC), which, in turn, binds to one of the two dsRNA strands, generating the mature miRNA. The action of miRNAs occurs through binding to the seed sequence of the 3' untranslated region (UTR) of miRNAs. Therefore, these miRNAs silence gene expression by inhibiting protein translation.[10]

Considering, therefore, the high prevalence of CD in the world and its possible negative implications for chagasic patients, especially those affected by the chronic form, for which there is not yet evidence of maximum effective treatment, the search for resources that allow for the evaluation of the progression of CD is necessary to obtain improvements in clinical management and the possibility of early intervention so that major complications are avoided.

Therefore, the objective of this literature review is to provide information about miRNAs and their targets in the pathophysiology of CD, relating to the expression of genes and miRNAs in the heart tissue of people infected with *T. cruzi*. From this data, an approach can be found on the use of miRNAs as a potential diagnostic and prognostic method through their modulation by *T. cruzi*, mainly during the chronic phase of the disease, which is the most common and severe manifestation, and the main cause of morbidity from cardiovascular damage in endemic areas.

Methodology

This is a literature review of national and international scientific articles published in journals from 2005 to 2022, considering articles in English, Portuguese, and Spanish. The search for these articles was carried out in the following bibliographic databases: PUBMED - NCBI (National Library of Medicine), SciELO (Scientific Electronic Library Online), Bireme Library (Biblioteca Virtual em Saúde, LILACS), Science Direct, and Embase through the search function and the Mendeley software, using as descriptors "miRNAs," "CD," and "Immunomodulation." This search resulted in 121 articles. Nine authors examined the titles in experimental studies to determine whether they met the inclusion criteria, which addressed the theme related to the role of miRNAs and their targets in the pathophysiology of CD, as well as the expression of genes and miRNAs in the heart tissue of people infected with *T. cruzi*. During screening, 66 records were excluded: 22 duplicates and 44 that did not address the topic of CD and miRNAs. In the second stage, the authors evaluated the abstracts of the articles that presented sufficient information on the subject of this review, with eight articles excluded for not meeting the inclusion criteria or presenting certain exclusion criteria (articles from review, non-experimental studies, studies that do not address the topic). Finally, in pairs, 25 complete articles were selected for eligibility assessment (each article was read by two reviewers and evaluated according to the inclusion and exclusion criteria), and 16 works were included as shown in Figure 1 and Table 1.

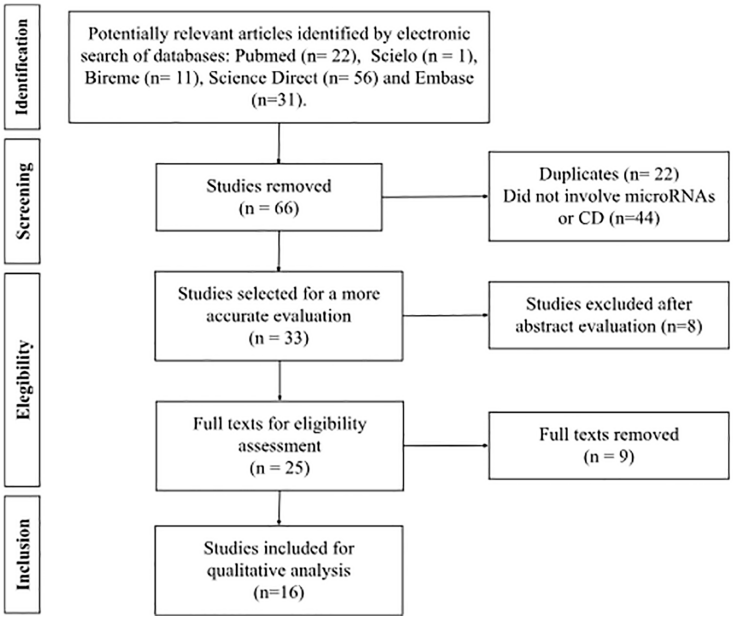

Figure 1 - Flowchart of selection of articles included for analysis.
CD: Chagas Disease. Source: The author

Table 1 - Studies used in the review.

Author -Year	Reference No.	Sample size	Study design	Main results
Nonaka et al., 2019	6	n = 42	*in vitro* exploratory	Change in the expression of miRNA-19a-3p, miRNA-21-5p, miRNA-29b-3p, and cardiac injury.
Ferreira et al., 2017	14	Unknown	*in vivo* study with mice	Interaction between miRNA and chagasic heart disease in hearts of infected mice.
Navarro et al., 2015	15	n = 36	*in vivo* study with mice	During the acute phase, 17 miRNAs were found to be significantly dysregulated.
WU et al., 2021	16	Not applicable	database	A total of 122 miRNAs and 10 "hub" genes are related to the pathogenesis of CD in different pathways.
Jha et al., 2020	21	n = 5	in vitro	The lack of expression of miRNA-155 means the mice are more prone to *T. cruzi* infection.
Lacerda et al., 2015	22	n = 6	*in vivo* study with mice	In total, 29 out of 85 miRNAs with increased expression pattern.
Medina et al., 2020	24	n = 3, human placenta from pregnant women without complications	*ex vivo* study	DEMs modulate the success of congenital infection and may be therapeutic targets.
Lacerda et al., 2018	25	n = 20	in vitro	Increased expression of miRNA-208a in patients with CCC.
Tijsen et al., 2014	26	n = 36	*in vitro* experimental	The miRNA-15 family acts by inhibiting the TGF-β pathway, which influences the remodeling of hypertrophy and fibrosis processes.
Ferreira et al., 2014	27	n = 20	*in vitro* study with CD patients	MiRNAs play a role in regulating the expression of genes associated with CCC.
Farani et al., 2022	28	Unknown	in vivo	The peak expression of miRNAs-145-5p was related to the release of trypomastigote forms. The importance of miRNAs in signaling pathways during *T. cruzi* infection was highlighted.
Monteiro et al., 2015	29	Unknown	in vitro	miRNA-190b negatively modulates PTEN protein expression.
Sassi et al., 2017	31	n = 6	*in vivo* experimental	Cardiomyocyte miRNA-29 represses Wnt signaling.
Laugier et al., 2020	32	n = 4	biomedical observational	miRNAs in regulating gene expression in myocarditis, fibrosis, and pathophysiological diseases such as CCC.
Ballinas-Verdugo et al., 2021	33	n = 46	in vivo	Presence of miR-21, miR-146a, and miR-155 in cardiac tissue during the acute and indeterminate phase, with miR-146a being a possible biomarker for early detection.
Nonaka et al., 2021	34	n = 2 n = 3	*in vitro* *in vivo*	Decreased fibrosis by reducing the expression of miRNA-21.

CCC: Chronic Chagas Cardiomyopathy; miRNA: microRNA; CD: Chagas Disiase; TGF-β: Transforming Growth Factor Beta; DEM: Differentially Expressed miRNA; PTEN: Phosphatase and Tensin Homolog.
Source: The author

Discussion

Changes in the immune system in *T. cruzi* infection

Several mechanisms of the innate and acquired immune response are mobilized during *T. cruzi* infection, so that the parasite is constantly being fought, with its multiplication reduced. Innate immunity to *T. cruzi* relies on the activation of the complement system via the alternative pathway. Natural Killer (NK) cells also play a fundamental role through the release of the cytokine Interferon-gamma (IFN-γ), which is relevant in the activation of macrophages and destroys parasites by releasing nitric oxide (NO) and superoxide anions. Subsequently, *T. cruzi*, in addition to promoting the nonspecific activation of macrophages and NK cells (innate immunity), activates T and B lymphocytes, producing IgM and IgG immunoglobulins (seven to fifteen days after infection). A few months after infection and after parasitemia has subsided, IgM levels progressively decrease, so much so that cases of positive IgM during the chronic phase are rare. On the other hand, IgG antibodies increase for a few more months and then decrease and stabilize.[3]

The immune response partially controls parasitemia after acute infection. However, low-grade parasites are still present and stimulate the production of IFN-γ, Tumor Necrosis Factor Alpha (TNF-α), and other inflammatory cytokines.[11] It is also worth highlighting that the pro-inflammatory response to *T. cruzi* infection is dependent on the activation of the Toll-like receptor 4 (TLR4) receptor, which leads, as demonstrated *in vitro*, to the activation of NF-κB, an important transcription factor for the expression of genes that lead to the release of cytokines and adhesion molecules.[12] Other important mechanisms for the immunological response to infection are the activation of the alternative pathway of the complement system by the epimastigote form of *T. cruzi*, greater recruitment of NK cells, which promote a cytotoxic effect and are responsible for producing IFN-γ, TNF-α, and opsonization by antibodies.[13]

Experimental studies on mice demonstrated that after 30 days of the disease, around 1,685 genes were considered Differentially Expressed Genes (DEGs), among which some are related to the expression of NK cells and monocytes, activation of dendritic cells, and T and B cells. In addition, 20% of genes related to IFN-γ expression were also altered. Another 113 miRNAs are considered Differentially Expressed miRNAs (DEMs) from 15 to 45 days after infection. Thus, it was observed that different pathways of the immune system were overstimulated, such as pathogen presentation and Th1 response pattern. In this sense, analyses demonstrated that DEMs related to IFN-γ and other cytokines were expressed in large quantities. This response, which involves the exacerbated production of cytokines, may be related to cardiac involvement.[14,15] Furthermore, in another study, 86 homologous genes were selected, including 73 upregulated and 13 downregulated genes that were expressed differently in the two data sets between humans and mice. It was observed that homologous genes are related to the regulation of the immune system. From the analysis of the protein-protein interaction network, it was demonstrated that 122 miRNAs and 10 "hub" genes, namely LAPTM5, LCP1, HCLS1, CORO1A, CD48, TYROBP, RAC2, ARHGDIB, FERMT3, and NCF4, are related in different pathways of CD pathogenesis, such as NK cell-mediated cytotoxicity pathways, cytokine-regulated pathways, and PI3K-Akt signaling pathway.[16]

Role of miRNAs as biomarkers

Although most biomarkers are proteins, RNAs that do not have the ability to encode proteins, such as miRNAs, are new and promising candidates for biomarkers, as they present several characteristics of interest.[17] MiRNAs have become a target of interest for studies after an association between their dysregulation and their pathological and phenotypic manifestations was observed, as demonstrated in the central figure, which reveals their potential as a biomarker.[18,19]

Current studies have evaluated the effectiveness of using miRNAs as a potential serum biomarker for the diagnosis of cardiovascular diseases, considering their role in regulating several biological transcriptional processes.[20] Thus, the dysregulation of miRNAs involved in cellular and molecular processes related to cardiovascular diseases can assist in more accurate diagnoses and prognoses of the disease, in addition to guiding new therapeutic lines.[10] In a review, the use of miRNAs was evaluated as a potential biomarker for heart failure since they are involved in the physiological and pathological development of CD. It is worth mentioning

that the use of miRNAs is still a challenge due to methodological difficulties.[19]

In order to understand the effects of miRNA-155 on immune cells during *T. cruzi* infection, they concluded that miRNA-155 deficiency resulted in a significant decrease in CD8+ LT, NK, and NKT cells in infected mice. Furthermore, in the same research, this deficiency caused a decrease in the production of Th1 response pattern cytokines, such as IFN-γ and TNF-α. Thus, it is suggested that miRNA-155 is important for the maintenance of cytotoxic cells and protective cytokines, which control the infection of this parasite.[21]

In acute CD, it is possible for the thymus to be affected and thymic atrophy to develop, according to an analysis carried out on the expression of miRNA in Thymic Epithelial Cells (TEC) of mice with acute *T. cruzi* infection. Eighty-five miRNAs were analyzed, and it was shown that 29 miRNAs were significantly deregulated. Among these, nine miRNAs were modulated by infection depending on the phenotypes exhibited by TEC, which can be cortical or medullary.[22] A probable mechanism involved in severe thymic atrophy is the reduction of thymocytes during acute *T. cruzi* infection with a progressive increase in parasitemia and pro-inflammatory cytokines.[22,23] Therefore, it is suggested that the differentiated expression of miRNAs in TECs caused by acute *T. cruzi* infection in mice may mediate thymic atrophy.[23]

In an *ex vivo* study that evaluated the expression of miRNAs in human placental explants (HPE), 13 DEMS were obtained after inoculation with *T. cruzi*. Downregulation of miRNA-497-5p, miRNA-877-5p, and miRNA-1271-5, and upregulation of miRNA-490-5p, miRNA-146a-5p, miRNA-12135, miRNA-210-5p, miRNA-146b-5p, miRNA-636, miRNA-449a, miRNA-2125-5p, miRNA-561-3p, and miRNA-446-3p were observed, which corroborates the hypothesis of the etiological agent's ability to modulate the regulation of related fundamental cellular processes to placental and embryonic pathologies.[24]

In line with the aforementioned findings, a study observed that the circulating levels of miRNA-208a are significantly increased in the indeterminate phase of chronic CD infection. According to these findings, it is possible that there is mutual regulation between miRNA-208a and its target genes, preceding the fibrosis process characteristic of the chronic phase. Thus, miRNA-208a at increased levels was correlated with early onset events, which are responsible for the initiation of fibrosis processes and cardiac dysfunction, acting, for example, in the regulation of genes related to hypertrophy. In this sense, there is a focus on this miRNA, which behaves as one of the possible candidates to be used as a biomarker during the chronic indeterminate phase of CD, considering its high level of expression and potential for risk predilection in patients with CCC.[25]

miRNA findings in CCC

Table 2 describes the main miRNAs involved in CCC.

Several processes in cardiac pathophysiology have been shown to be regulated by miRNAs. An *in vitro* study in cardiac cell culture suggested that the miRNA-15 family, composed of six miRNAs (miR-15a/b, miR-16, miR-195, miR-497, miR-322), possesses targeted genes of the pathway of the Transforming Growth Factor Beta (TGF-β) protein, which acts in the remodeling of processes such as fibrosis and cardiac hypertrophy that occurs in CCC. At another point in the study, they used anti-miRNA based on nucleic acid blockers (NABs) in mice with the aim of inhibiting this family of miRNA, resulting in the worsening of the fibrosis and hypertrophy process when compared to the control group. Therefore, the influence of this family as a potential regulator of the fibrosis and cardiac hypertrophy process was confirmed by how it acts negatively on the TGF-β26 pathway.[26]

It is known that gene expression pattern is modified in CCC, but the molecular mechanisms involved are not well defined. In another study, samples obtained from the left ventricular wall of patients with CCC were evaluated and reduced expression of miRNA-1, miRNA-133a-5, miRNA-133b, miRNA-208a, and miRNA-208b was observed. Therefore, it is suggested that these miRNAs are involved in gene expression and are important factors in the development of cardiovascular diseases. Such findings may contribute to further research on the contribution of miRNAs and their target genes in the development of CCC pathology, as well as its treatment.[27]

Further to the role of miRNAs in the progression of *T. cruzi* infection and cardiovascular disorders, a study carried out with rat hearts found that 17 miRNAs were significantly dysregulated after infection with *T. cruzi*. There was a correlation with clinical patterns of the disease, as well as parasitemia and corrected QT interval (QTc) in the electrocardiogram in six of the 17 miRNAs studied. Furthermore, it was found that genes of these six miRNAs are involved in the signaling

Table 2 - Summary of the main miRNAs covered and their role in the pathogenesis of CCC.

MiRNA	Changes in the myocardium
miRNA-208[a]	Fibrosis and cardiac dysfunction
miRNA-15 family	Inhibition of the TGF-β pathway
miRNA-19a-3p	Fibrosis
miRNA-21-5p	Fibrosis process stimulated by TGF-β
miRNA-29b-3p	Cardiomyocyte hypertrophy
miRNA-29 family	Cardiomyocyte hypertrophy and fibrosis
miRNA-21	Fibrosis and myocarditis
miRNA-146[a]	Myocarditis
miRNA-155	Myocarditis
miRNAs-145-5p, miRNA-190b	Increased at the time of infection

TGF-β: Transforming Growth Factor Beta; miRNA: microRNA.
Source: The author

pathway related to ventricular depolarization and repolarization, which is associated with prolonged QTc. Thus, it was demonstrated that miRNA-142-5p, miRNA-21-5p, miRNA-145-5p, and miRNA-149-5p control the expression of genes related to the length of the QTc interval, because they are involved in both the expression of calcium channels and in gap junctions. That said, the changes that occur in the host's miRNA profile observed here reflect the role of the molecules responsible for conducting the electrical impulse in the acute phase of the infection and can highlight important aspects of pathogenesis, opening up a wide field of possibilities in the study of CD.[15] In line with what was observed in the previous study, another *in vitro* study demonstrated an increase in the expression of miR-145-5p and miR-146b-5p that occurs prior to the release of the promastigote form of the parasite in the cardiomyocytes of mice infected with *T. cruzi*, playing an important role in its interaction and establishment of host infection. Furthermore, by having carried out an evaluation of medications in the modulation of these miRNAs, it is suggested that they may represent potential biomarkers for evaluating the effectiveness of treatments, in addition to the possibility of being used as targets for modulating the infection and decreasing the parasitic load.[28] In regard to the modulating potential of miRNAs in *T. cruzi* infection, through an *in vitro* study with cultured H9c2 cells (a line of rat embryonic ventricular cells), the expression of five miRNAs was evaluated. The results demonstrated a potential association between miRNA-190b and the PTEN protein (Phosphatase Homologous to Tensin - reduced during hypertrophic cardiomyopathy and correlated with various adaptive responses of cardiomyocytes): during immediate and late infection (48 hours later) of the host cell, there was an increase in this miRNA and a reduction in PTEN. Furthermore, when an inhibitor for miRNA-190b was applied, a 40% increase in protein expression was observed. Thus, the reduction in PTEN expression modulated by miRNA suggests a decrease in cell viability, which contributes to *T. cruzi* infection.[29]

In another study, a higher expression of miRNA-19a-3p was observed in the serum of individuals with CCC when compared to individuals undergoing the indeterminate phase of the disease. The cardiac tissue of individuals with CCC, compared to the control group of uninfected individuals, showed positive regulation of this miRNA with a possible relationship between the miRNAs and the development of cardiac fibrosis. The results correlated the increased expression of miRNA-21-5p in collagen-producing cells stimulated by TGF-ß, found in greater quantities in individuals with CCC compared to unstimulated cells.[30] Furthermore, there was a correlation between the increased expression of miRNA-29b-3p and the hypertrophic growth of cardiac cells.[6] The miRNA-29 family has a role in pathological myocardial hypertrophy and fibrosis, as this was demonstrated in an experimental mouse model with chronic cardiac pressure overload,

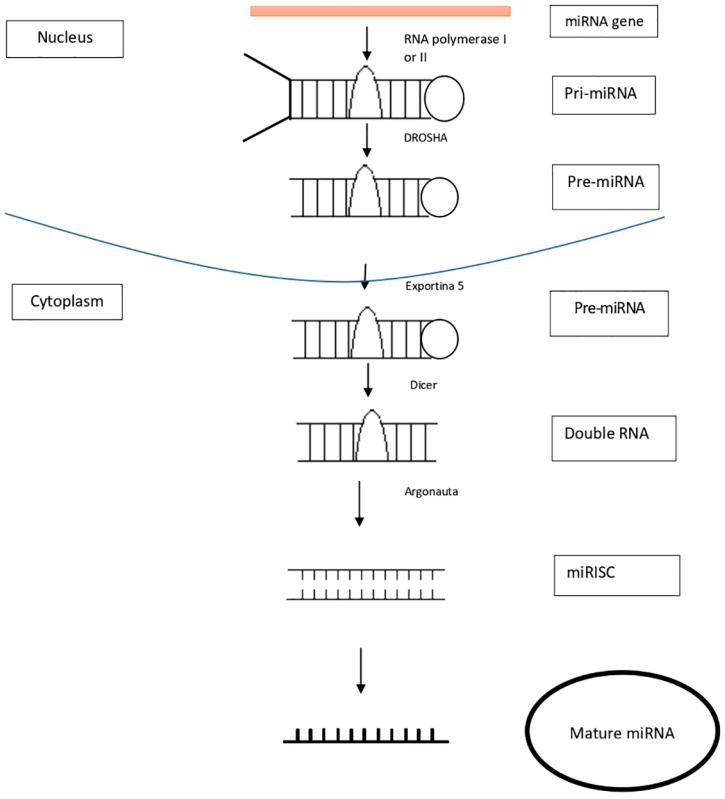

Figure 2 - Simplified scheme of miRNA production.
miRNA: microRNA; RNA: Ribonucleic Acid; MiRISC: microRNA-induced Silencing Complex
Source: The author

in which inhibition (*in vivo* administration of anti-miRNA-29) or genetic deficiency of miRNA-29 protected against cardiac hypertrophy and fibrosis. In relation to fibrosis, the study found an unexpected result: it was found that there was an elevation - instead of inhibition - of miRNA-29 in tissues other than the myocardium, which prevented the development of fibrosis. MiRNA-29 will exert these effects on the cardiomyocyte, directly regulating factors of the Wnt signaling pathway.[31] With these findings, it is clear that innovative studies are fundamental to understanding the functions of miRNAs, as their different expressions reflect specific forms of pathogenesis and related signaling pathways in individuals with CCC.[6]

In CCC, there is an increase in the gene expression of immune cells and a concomitant reduction in the presentation of the individual's cardiac mRNAs. In this sense, 1,535 DEGs and 80 DEMs were verified, and the expression characteristics of such genes and miRNAs allowed for the distinction between samples from patients with CCC and control samples. According to the results of the study, IFN-γ acts on several pathobiological processes, such as modulating transcriptional changes in the myocardium of individuals with CCC, in addition to being an important regulator of gene expression, and it modulates 10% of DEGs. Therefore, it is likely that significant changes in the transcriptome will occur due to the action of miRNAs. There were five DEMs that were suppressed: hsa-miRNA-15a-5p, hsa-miRNA-29c-3p, hsa-miRNA-103a-3p, hsa-miRNA-125b-5p, and hsa-miRNA-296-5p.[32]

An experimental study with the goal of evaluating the expression of miRNA-146a, miRNA-155, and miRNA-21 was carried out with samples from mice infected with the Mexican TcI Ninoa strain. The group of infected mice had a maximum peak of parasitemia observed on the 27th day post-inoculation. During the acute phase, compared to the control group, a slight presence of lymphocytes and the appearance of amastigote nests in the cardiac tissue were observed. During the indeterminate

phase, the inflammatory process intensified with the presence of mature lymphocytes and an increase in the number of amastigote nests, establishing the diagnosis of myocarditis. To verify the expression level of the selected miRNAs (miRNA-21, miRNA-155, and miRNA-146a), an RT-qPCR test was performed, with the following results: during the acute and indeterminate phase, compared with the control group, the levels of these miRNAs increased, especially miR-21 in the heart and miR-146a in plasma during the indeterminate phase. Extracellular vesicles (EVs) play an important role as mediators of communication between host cells and parasites through the secretion of lipoproteins, virulence factors, cytokines, nucleic acids, and growth factors. Therefore, they can be used to screen molecules such as miRNAs. EV particle counts in plasma were performed during the acute and indeterminate phase of the disease. The number of particles was higher during the acute phase compared to the control group and the expression of miR-21 and miR-146a in this phase was also high. However, during the indeterminate phase, the number of EV particles dropped slightly, and only miR-146a was upregulated in this phase compared to the control. In conclusion, miR-146a can be used as a potential tool for early detection of CD, considering that in both stages of the disease – acute and indeterminate phase – there was positive regulation in plasma, cardiac tissue, and EV particles.[33]

Finally, in an experiment carried out in another study, miRNA-21 was the only miRNA found that was deregulated at all times of data analysis, both for *in vitro* and *in vivo* samples. Furthermore, there was an increase in this miRNA during the first 24 hours in rat cardiac fibroblasts. In this same analysis, it was observed that the treatment of animals with LNA-anti-miR-21, which is in the chronic phase of CD, not only reduced the cardiac expression of miRNA-21, but also resulted in a reduction in fibrosis in relation to those that were treated with a saline solution.[34] Such results, together with those demonstrated by other studies, corroborate the hypothesis that, in addition to their role as biomarkers, miRNAs can be used as therapeutic targets to control the parasite cycle and the damage it causes.[28]

Conclusions

In view of the above, *T. cruzi* infection is associated with the dysregulation of several miRNAs evaluated in *in vivo* and *in vitro* experimental studies. These are mediators of inflammatory processes that affect several organs, specifically the myocardium in CCC. According to the expression profile of specific miRNAs and genes, it was possible to associate this regulation pattern with findings of hypertrophy, fibrosis, and myocardial dysfunction observed in the studies, which are associated with the pathophysiology of CCC. However, none of the previously mentioned miRNAs were identified as highly specific for the pathology, as they are present in other diseases and inflammatory processes that present the same findings as CCC. From another perspective, prognostic markers for CCC are currently limited, making the quantification and characterization of miRNAs innovative resources that could be used as consistent indicators of CD progression in the future, and could also be applied to support the diagnosis of patients with complications from the disease. However, more clinical and longitudinal studies are needed to determine the applicability of specific miRNAs in humans that are abnormally expressed at each stage of the disease in order to detect, at an early stage, any level of tissue involvement that may occur and ensure reliability as a prognostic indicator.

Author Contributions

Conception and design of the research, writing of the manuscript and critical revision of the manuscript for intellectual content: Tefe-Silva C, Teixeira LO, Durigan LR, Cardoso MCS, Davi MLC, Pin PA, Milanez S, Beine TCS, Lourenço VC, Clemente EYA.

Potential Conflict of Interest

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

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Study Association

This study is not associated with any thesis or dissertation work.

Ethics Approval and Consent to Participate

This article does not contain any studies with human participants or animals performed by any of the authors.

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