International Journal of Biochemistry Research & Review

30(6): 31-44, 2021; Article no.IJBCRR.74616
ISSN: 2231-086X, NLM ID: 101654445

mir186 and mir145 *In vivo* Evaluation and Enrichment in Rats Submitted to Treadmill Strenuous Exercise

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RMF, CPSM and SMSF designed the study methodology, conducted the investigation processes, data curation and validation of the results. Authors DSS and PEO performed the text preparation for publication and review. Authors JKRC and CPSM performed the formal analyses. Author VMC administrated, managed, and supervised the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2021/v30i630274
Editor(s):
(1) Prof. Halit Demir, Yil University, Turkey.
Reviewers:
(1) Jean-François Picimbon, Qilu University of Technology, China.
(2) Sonu Mishra, Mewar University, India.
(3) Hani Nasser Abdelhamid, Assuit University, Egypt.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74616

Original Research Article

Received 09 August 2021
Accepted 19 October 2021
Published 25 October 2021

ABSTRACT

Aims: The present study aimed to identify miRNAs differentially expressed in rats submitted to strenuous exercise and *in silico* investigation of the biological implication of the findings.
Place and Duration of Study: The in vivo experiments and analyses were performed in the Laboratory of Biochemistry and Gene Expression – LABIEX of the Superior Institute of Biomedical Science – ISCB from the State University of Ceará. Between 2017-2020.
Methodology: The study was performed using as subjects 2-month-old male wistar rats, which initially were submitted to 2-week adaptation training. Later the animals were separated in two distinct groups, control (C) and trained (T), where only T performed a single session of strenuous exercise, while C were not submitted to this treatment. The applied exercise protocol consisted in a

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running training in treadmill with speed constant increasing until the animal exhaustion which was measured by the animal refusal to keep running. After 24h, soleus muscle was desiccated and submitted to RNAseq sequencing protocols. Obtained data were statistically evaluated in R environment with EBSeq package, to characterize and predict the miRNAs and their targets were used bioinformatics tools Gene Cards, mi RBase enrichR and KEGG.

Results: Two differentially expressed miRNAs were found, mir145 and mir 186, both with downregulated expression pattern in strenuous exercise. These miRNAs have a total of 1201 predicted target genes, 67 were repeated and mostly correlate to cardiovascular disease pathways, between those 5 were differentially expressed as down-regulated.

Conclusion: In conclusion, the findings suggest that mir186 and mir145 down-regulation profile mediated by strenuous exercise implicates in the non-alteration of the target genes expression profile, and consequently did not mediate alterations in the pathways they are evolved, which are mainly related to signaling and disorders.

Keywords: mir145 and mir186; RNAseq; strenuous exercise; data mining.

1. INTRODUCTION

MicroRNAs (miRNAs) are a non-coding RNA class present in a variety of regulation mechanisms, by performing gene expression modulation in multicellular and unicellular organisms [1,2]. miRNAs are recognized for their role in a post-transcriptional silencing mechanism, which consists of a molecular recognition of a mRNA target by the miRNA, based on nucleotide complementarity, this interaction is mediated by a multiprotein complex named RISC (RNA induced silencing complex) that leads to the target inhibition. The inhibition mechanisms consist of mRNA cleavage causing its degradation or translation repression[3]. miRNA editing has become studied to elucidate the mechanisms of RNA modulation[4].

miRNAs are expressed in a variety of tissues having different patterns and regulatory roles according to each organ. Some specific miRNAs families are usually found in particular tissues, an example of this is the expression of the mir-506 family members in abundance in testis tissue [5]. Besides this, the physiologic conditions also can modify miRNA molecule expression profiles, in cancer, there is being reported cases of aberrant changes in miRNA patterns [6]. miRNA presence is commonly described to have a role in the regulation of diseases such as heart disorders, diabetes, and Alzheimer's [7]. Due to this, the miRNA class is useful as a biomarker for studies aiming to provide diagnosis and physiologic process comprehension[8].

Physical activity is well-known as a positive factor in the human biological regulation processes; therefore, exercise can act as a factor that can modify epigenetic factors. The mechanisms related to this alteration are vast, including, methylation, histone modifications, and miRNAs expression. Exercise induces metabolic alterations through changes in the signaling pathways, mainly in skeletal muscle tissue, leading to adaptations that alter the transcriptome profile [9]. One bout exercise activity is related to cellular energy balance mechanisms through AMP-activated protein kinase (AMPK) and Calcium/calmodulin-dependent protein kinase II (CaMKII) signaling. Besides this, there are related increases in oxidative activity and myokines secretion. Consequently, it leads to positive effects in the major systems, such as neurological, immune, and cardiovascular [10].

The effects of exercise in the regulation pathways from humans and rats are similar, having also a correspondence between the transcriptional profile of these organism [11]. From rodents class, the rat model is widely used in studies with for exercise in view of reproducing similar behavior and physiologic patterns present in humans [12]. Besides this, the cost-benefit of this model is very pleasant when compared to other organism models [13].

miRNAs are present in muscle regeneration and are related to it aging retardation mediated by exercise practice. MyomiRs are an example of miRNA class expressed specifically in skeletal and cardiac muscle and are known as an important key on skeletal muscle exercise adaptation, having each kind of exercise a different role in circulant miRNAs expression [14,15].

Endurance exercise is characterized by aerobic activities such as running, swimming, and cycling

[16]. Different doses of this exercise modality lead to different pathways signatures. Strenuous exercise, which consists in the exercise practice until arrive exhaustion, is linked to the expression of miRNAs in inflammatory alterations, while moderate activity is related to the opposite [17]. Strenuous acute exercise is a modality recognized for promoting changes in the redox balance and is related to up-regulate antioxidant pathways [18].

The miRNAs differentially expressed in aerobic acute strenuous exercise are underexplored, most of the published data have approaches based on circulating blood analyses and there is little information correlating miRNAs in this exercise modality in muscle. Nevertheless, in the present study, we evaluated miRNAs differentially expressed in *Rat* submitted to strenuous exercise by RNAseq, aiming to identify miRNAs pattern and predict by in silico evaluation its possible metabolic implications on the biologic process, and possible interactions with the physical exercise modality practiced.

2. MATERIALS AND METHODS

All exercise protocol, RNAseq experiment, and bioinformatic differential expression evaluation steps were performed as detailed described in the "Transcriptional profile in rat muscle: down-regulation networks in acute strenuous exercise" [19], which is the main study of the project that the present article composes.

2.1 Exercise Protocol

The experiment consisted of animal physical training, through submission to strenuous physical exercise in an adapted treadmill as previously described [19]. The animals choose for the study were healthy wistar male rats, 2-months-old, with average weight of 240-280 g provided by the Superior Institute of Biomedical Sciences bioterium at the State University of Ceará, where were kept in cycles of light/dark (12h/12h), in a controlled temperature (22–25◦C) environment, with water and feed ad libitum.

The animals passed for a two-week adaptation training in rat adapted treadmill in view to promote animal familiarization with the applied environment. Later the rats were randomly separated into two groups, control (C) (n=4) and trained (T) (n=4). The T group were submitted to an only session of strenuous exercise training at 0.5 km/h initial speed, with an addition of 0.2 km/h every three minutes, until animal exhaustion, parameter that was measured by the animal refusal of keep running on the treadmill and loss of the limb coordination.

2.2 Experimental Analysis

All RNAseq steps were performed as described in the "Transcriptional profile in rat muscle: down-regulation networks in acute strenuous exercise" [19], main study of the project that the present article composed.

After 24h of the training exercise all animals were euthanized with Thiopental sodium (150 mg/kg, the soleus muscle was dissected and submitted to biochemistry protocols and total RNA sequencing (RNA-seq). The extraction of total RNA was performed with *TRIzol*® (Thermo Fisher Scientific/Massachusetts, EUA) and *RNeasy Plus Mini Kit*® (QIAGEN) following the recommendations and specifications of manufactures. Samples satisfied RNA quality and integrity requirements for sequencing.

Quality of the extracted RNA was verified by the RNA integrity number (RIN) as previously described. All samples demonstrated integral 18S and 28S bands. RNA concentrations obtained in the samples were of 466.6 ± 21.7 ng/mL and the 28S/18S ratio was of 1.4 ± 0.02. The RIN values obtained were 8.1 ± 0.14. Samples satisfied RNA quality and integrity requirements for sequencing. The cDNA libraries were built with *Ilumina TruSeq Kit* (kits/truseq-rna-v2.html). *Illumina HiSEQ 2500 Platform* was used to perform the sequencing (*Illumina*, San Diego, CA, EUA). Sequencing coverage was 30 million reads per sample, with 100bp paired-end sequence reads.

2.3 Bioinformatic Analysis

Gene differential expression evaluation was using EBSeq v 1.22.1 package in R environment (https://www.bioconductor.org/packages/release/ bioc/html/EBSeq .html) [20], considering RNO 4.0 reference genome with transcriptome version dated from 27/01/2011 obtained in UCSC Genome Browser (https://genome.ucsc.edu/) with the Fold change ≥ 1.4, p ≤ 0:05 and FDR ≤ 0.05 parameters. Differentially expressed miRNAs genes were characterized with GeneCards (https://www.genecards .org/) [21] e miRBase (mirbase.org)[22], in terms of standard expression in muscle (GTEx-RNAseq), nucleotide size, chromosomic location, orthologs

correlation, and related physiological processes reported in the literature.

Genes candidates for miRNAs target were searched using miRBase through its prediction tool. Each miRNA transcript (5p and 3p) can have innumerous gene targets due to its transcription sense. The gene targets from both miRNAs were mapped according to their chromosomic location with Idiographica (http://rtools.cbrc.jp/idiographica/) [23]. The genes that were recurrent in the results from different miRNAs and transcripts lists were identified over a Venn diagram with InteractiVenn (http://www.interactivenn.net/) [24].

The Enrichr pathway analysis tool (http://amp.pharm.mssm.edu/Enrichr/) [25] and the Kyoto Encyclopedia of Genes and Genomes database (KEGG; http://www.genome.jp/kegg/) [26] was applied to predict pathways involved in the interactions between miRNAs targets. The genome used was human (KEGG: hsa – T01001) due to the absence of the rat genome in the tool. Only pathways with a statistical significance of $p < 0.05$ were considered of interest.

3. RESULTS AND DISCUSSION

3.1 Differentially Expressed miRNAs

All the sequence data showed in the present study are submitted to the National Center for Biotechnology Information's Sequence Read Archive (SRA) [27] through BioProject PRJNA557195. Two miRNAs were found differentially expressed in the RNAseq results evaluations through EBSeq. These miRNAs were mir145 e mi186, both demonstrated a down-regulation pattern (Table 1), indicating that strenuous exercise may have a potential role in decreasing these miRNAs expression when comparing to sedentary individuals.

mir186 is an RNA gene located in the long arm of chromosome 2 in position 45 (2q45) constituted by 21 nucleotides. The mir186 gene from rats is ortholog in humans and mice[22]. Ortholog genes share an evolutionary connection and usually present similar roles in different organisms, so some features of an ortholog gene from a particular species can support the understanding of this feature in another specie[28]. Studies demonstrated a correlation between mir186 expression in regulatory processes involved in the development of human disorders, such as hodgkin lymphoma [29], multiple sclerosis [30], hepatocarcinogen [31], and Head and neck squamous cell carcinoma [32].

mir145 is an RNA gene located in the rat genome at chromosome 18, in the short arm in 12.1 (18p12.1) position and constituted by 23 nucleotides[22]. This miRNA also is an orthologous gene in humans. Mir145 expression is usually related in the literature to play a part in the regulation process from a variety of metabolic pathways, such as miRNAs in cancer [33], heart development, miRNAs in DNA damage response, and smooth muscle differentiation and proliferation[34,35]. This miRNA is also described as a biomarker for cardiovascular diseases [35], B cell lymphoma, Burkitt lymphoma [36], coronary heart disease [34], and diabetes mellitus[37].

3.2 mir145 and mir186 Predicted Targets

Prediction analysis revealed 1201 target genes, distributed throughout the rat genome, being present in all 21 chromosomes, including the X chromosome. Chromosome 20 had the lowest number of targets with 18 genes, while chromosome 1, had the largest, with 112 genes. Gene target distribution is represented below (Fig. 1).

Evaluating the number of genes obtained in prediction analyses from each of the four miRNAs transcripts separately (Table 2) is demonstrated that mir186-5p transcript stood out when compared to the others, in the number of target genes, having the largest number. While mir145-3p gene predicted genes presented the smallest number of targets. Indicating that mir186-5p may be present in a wider variety of processes by regulating these targets in a wider variety of processes by regulating these targets.

Comparative analyses between the target lists are demonstrated below in a Venn diagram in Fig. 2. The results presented 67 repeated genes, mostly present in two different transcripts list. The only gene found three times in the predictions analyses were *Prcki* being present in mir186-5p, 186-3p, and 145-3p transcripts. These findings suggest that *Prcki* has a higher probability of being regulated when mir145 and mir186 are expressed at the same time.

Table 1. EBSeq expression parameters from miRNA

Gene	Log2 FC	P	FDR	GTEx (RNA-seq muscle skeletal human / (100x FPKM)1/2)	Regulation Profile
Mir186	-0.6950	0.0303	0.0303	17	Down
Mir145	-1.2099	0.0295	0.0295	2	Down

Table 2. MiRNAs transcripts gene targets number

mir145		mir186	
5p	3p	5p	3p
321 genes	104 genes	469 genes	307 genes
Total: 427 genes		Total: 778 genes	
mir186 and 145 target genes: 1201			

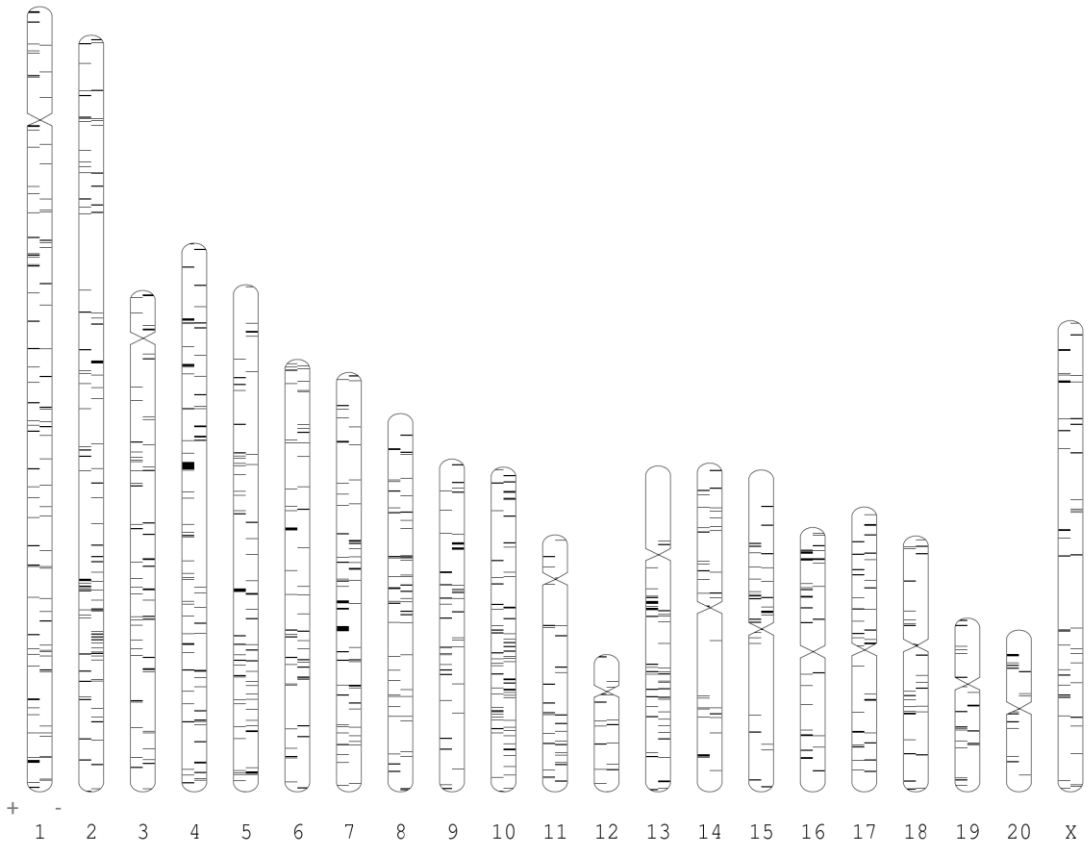

Fig. 1. mir-145 and mir-186 predicted targets gene targets distribution in the rat genome about strenuous exercise

Prcki is a kinase protein-coding gene involved in several biologic processes, playing important roles in metabolic pathways regulation. This gene has a part in cascade regulation events. Some examples of these are the Rap1 signaling pathway (rno04015), Endocytosis (rno04144), Hippo signaling pathway (rno04390), and insulin signaling (rno04910) [21].

mir145 predicted targets presented a total of 425 genes, of which only the gene *Psd3* were repeated both in 5p and 3p transcript. This gene is involved in endocytosis, being part of cellular transport, and catabolism of cellular process. Besides, *Psd3* plays a role in the regulation of membrane trafficking (rno04131) in endocytosis and ARF protein signal in guanyl-

nucleotide exchange in GTPase ARF mechanism [21].

mir186 presented 776 target genes in total, 20 of them present in both 5p and 3p transcripts. Between the duplicated genes were found that all of them are protein-coding genes, mainly related to cellular process regulation. Five of them are related to signal transduction[21]. Suggesting that mir186 can perform important roles in the response of the cellular mechanism.

3.3 Target Genes Expressed in the Study

A total of 12498 genes were found in RNAseq study results wherefrom the predicted target genes 1060 were present, while 142 were not found expressed in any of the conditions[19]. This finding demonstrates that 88.18% of the predicted targets genes are expressed in the study, which agrees with the down-regulation pattern of the miRNAs, since their decreased expression disables its potential action in the post-transcriptional silencing mechanism releasing the targets to express without its regulation interference.

The differentially expressed genes identified in the RNAseq results with EBseq package were compared to the 1201 target predicted gene from mir145 and mir186, which resulted in the finding of six corresponding genes (Table 3) between them. All of these genes presented a down-regulation profile in strenuous exercise experiments considering the values of logFc through EBseq statistics evaluation[19].

These findings suggest that other variants, not these miRNAs, are promoting the decrease of these genes regulation patterns mediated by strenuous exercise, since the sedentary group did not present differential expression. Although the targets differentially expressed in the study were found to represent only 0.5% of the predicted targets. This implicates a low probability of these genes being regulated directly by the miRNAs studied.

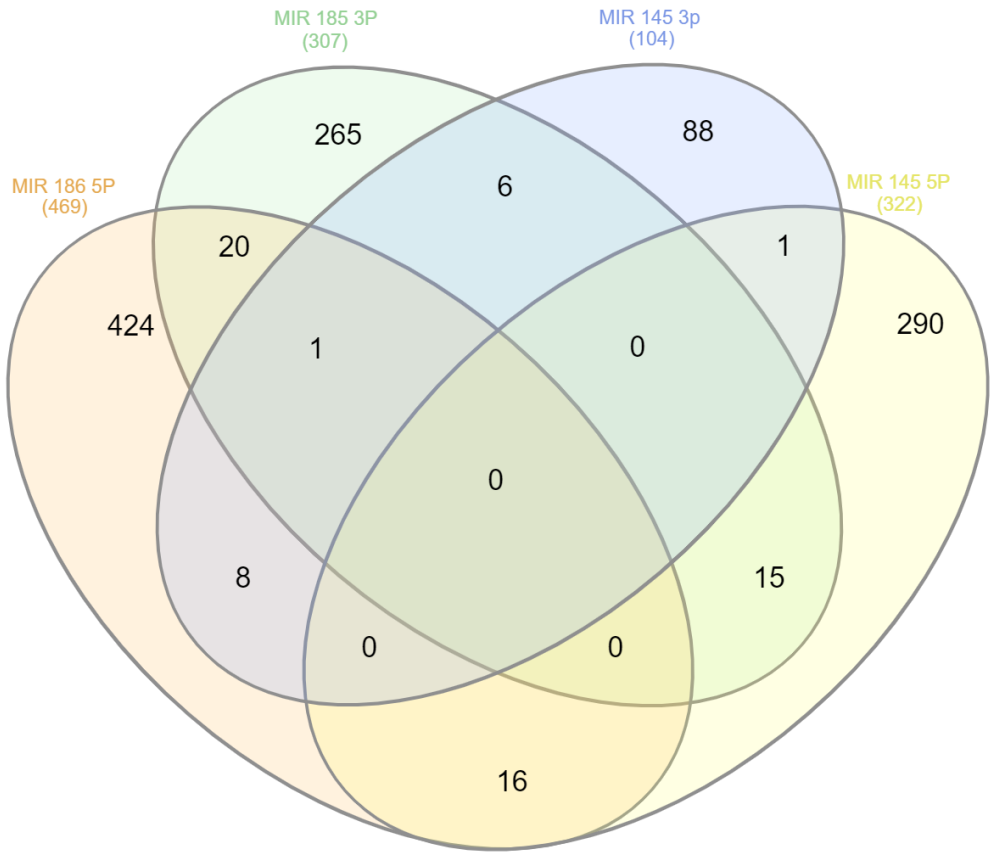

Fig. 2. Venn diagram describing gene shared number between different transcripts

Table 3. Genes in miRNAs target lists and differentially expressed in strenuous exercise.

Gene Symbol	Gene Name	LogFc	Regulation
Cxadr	CXADR, Ig-like cell adhesion molecule	-0.68	Down
Kbtbd8	Kelch repeat and BTB domain containing 8	-0.61	Down
Gria2	Glutamate ionotropic receptor AMPA type subunit 2	-0.70	Down
Pappa	Pappalysin 1	-1.28	Down
Slc7a11	Solute Carrier Family 7 Member 11	-1.08	Down
Unc5c	Unc-5 netrin receptor C	-1.09	Down

3.4 mir186 and mir145 Target Genes Function Evaluation

3.4.1 Gene targets enrichment analyses

Gene enrichment analyses were performed with the miRNAs and the 1201 predicted target genes, based on the human genome in KEGG database. Function characterization revealed that the target genes cluster has a significant statistical probability ($p < 0.05$) to be related to 26 pathways (Table 4) cataloged in KEGG database[16] considering human genome. These data were organized following each pathway hierarchy to demonstrate the whole scenario in which they are involved correlating the possible biologic interactions that can exist in this perspective.

Enrichment results include 10 pathways related to human diseases, 7 to the nervous and endocrine system, and 5 correlated to environmental information processing. The main finds at pathway level according to p value < 0.05 were nervous and endocrine system and human disease, such as cancer, cardiopathies, bacterial and viral diseases, metabolic and endocrine disorders (Table 4).

Despite numerous metabolic pathways founded in our statistical evaluation for miRNAs target genes in enrichment, there is little information exploring miRNAs role in target direct regulation mechanisms and possible changes provoked by them in physiology. However, there is a significant number of studies about these miRNAs correlating them to cardiopathy and cancer development.

Studies demonstrated that mir186 plays a role in the endocrine system being up-regulated prolactin treatment, which promotes a decrease in p21 protein expression. P21 protein plays a role in the cellular regulation process and its inhibition is related to tumorigenesis and drug resistance in cancer treatment[38].

Erfg gene was present in the enrichment of the oxytocin, Hypoxia-inducible factor 1-alpha (HIF-1), forkhead box O (FoxO), proteoglycans in cancer, and pancreatic cancer pathways. In literature, there is data that relate *Egfr* increased expression in several cancers. Mir145 in several cancers is usually found as down-regulated. In this scenario, mir145 has *Ergf* as a target, so when mir145 is up-regulated there is a large probability of *Ergf* gene suppression mediated by mir145, reducing this gene's role in its respective pathological pathway [39].

mTOR (Mammalian/mechanistic target of rapamycin) another pathway found relevant in enrichment analyses, is present in signal transduction, closely related to maintenance of biological processes, such as protein synthesis and cell growth. However, mTOR dysregulation is correlated to pathological disorders, such as diabetes and cancer[40] mTOR participates in the insulin signaling pathway through lipid and glucose metabolism, acting in homeostasis combined with protein kinase B (AKT) and FOXO1. Physical exercise acts in the mTOR pathway in plenty of tissues leading to beneficial results in the heart, muscle, liver, and brain. Studies relate mTOR to endurance exercise[41].

The mir186-5p transcript has expression correlated to cardiac disorders as important elements in cytotoxicity and apoptosis process in diabetic cardiopathy pathological (DCM), which makes its inhibition a great therapeutic proposal for DCM [42]. Up-regulation profile in patients with coronary acute syndrome, is related to glucose metabolism and HIF-1 signaling, making it a promisor biomarker in disease diagnostic[43]. Dawson and collaborator [44] showed that in humans acute strenuous exercise promotes negative effects in cardiac function, provoking cardiac depression, and a decrease in leg vascular function.

miRNAs play essential roles in regulation processes related to nervous system development and functioning in mammals. Bioinformatics analyses also predicted that mir145 and mir186 present target genes in

GABAergic synapse pathway[45]. Although, no further experimental information was found about the mechanisms used by mir145 in this pathway.

mir145 plays an important role in the regulation of plasticity process and smooth muscle cells[35]. These aspects implicate in mir145 presence in vascular system processes, being part of atherosclerosis mechanisms, arterial pulmonary hypertension, vasoconstriction and vasodilatation. Besides that, mir145 also presents an important role in heart protection against cardiomyocyte hypertrophy. mir145 is described with up-regulated profile in induced hypertrophy rats and dilated cardiopathy in the terminal stage, which lead it to be considered a potential biomarker in cardiac disorders[46].

3.4.2 Duplicated targets enrichment analyses

Enrichment analysis was also performed with the 67 repeated genes in the target lists, considering KEGG pathway database with p value < 0.05 (Table 5). Results demonstrate convergency between *Cacnb2, Dag1 e Actg1* genes to heart pathologies pathways, such as Dilated Cardiopathy, Cardiac Hypertrophy, and Right Ventricle Arrhythmogenic Cardiomyopathy.

The correlation between the targets and cardiovascular pathways evidence the likely role of strenuous exercise performed in our study in the expression modulation of mir145 and mir186. The enriched genes *Cacnb2, Actg1, Impad1, Sacm1l,* and *Gria3* were found expressed in the strenuous exercise RNAseq results, however, they did not present differential expression when related to control. In this case, it is supposed that acute strenuous exercise provoked miRNA down-regulation decreasing the possibilities of these genes to be regulated by them, but other mechanisms were responsible for modulating them.

3.4.3 Target gene correlation to exercise and enrichment analyses

Our study exhibits differentially expressed miRNAs in rats' muscle after one session of strenuous exercise in an adapted treadmill. To demonstrate the possible biologic implications of these miRNA regulations through exercise was performed a search for mir145 and mir186 and their target genes in the compendium of physical exercise-related human genes, previously published by our research group also called *The FITNOME Catalogue.* This compendium is a catalog that gathered approximately five thousand genes described in the literature to be related to exercise in studies that used humans as subjects, with an explanation on gene expression context inside exercise study scenario[47].

In *The FITNOME catalog* listed 66 miRNAs, but only one was differentially expressed in our study, mir186, characterized in the catalog to be related to acute endurance exercise in male subjects, in this condition mir186 presented an up-regulation profile [47,48,39,38].

Comparisons between miRNAs targets and the *FITNOME* genes set showed 351 genes in similarity, in which only 4 were both present in mir145 and mir186 target list. In Fig. 3, is describing the interactions between the exercise, miRNAs, miRNAs targets, and results pathway enrichment analyses. Most of these genes had down-regulation profile in the *FITNOME* reports, and its expression was observed in studies involving mainly to chronic endurance exercise [47].

However, 17 genes, present in both *FITNOME* genes set and miRNAs targets were related to acute resistance exercise. Our study also was with acute exercise, but on the other hand, contemplated another exercise modality, strenuous resistance exercise. However, our prediction findings suggest that both kinds of exercise affect these genes expression modulations.

The presence of some of the target genes in the *FITNOME* gene set confirms the relation between them and exercise, even in different modalities, which implicates selectivity in expression regulation mediated by the exercise type. Data found in our study demonstrated strenuous exercise acts as a negative regulator in mir145 and mir186 expression in rat skeletal muscle, which implies the decrease of miRNAs modulation in the target genes regulation, which allows target genes to have regular expression without miRNAs intervention.

Corresponding genes between miRNAs targets and *FITNOME* catalog, in enrichment analysis, presented a similar metabolic profile. Revealing the correlation between predicted genes and exercise expression patterns, being possible that the specific exercise factor has a direct effect on miRNAs expression, as demonstrated in our study with strenuous exercise, and consequently in its targets.

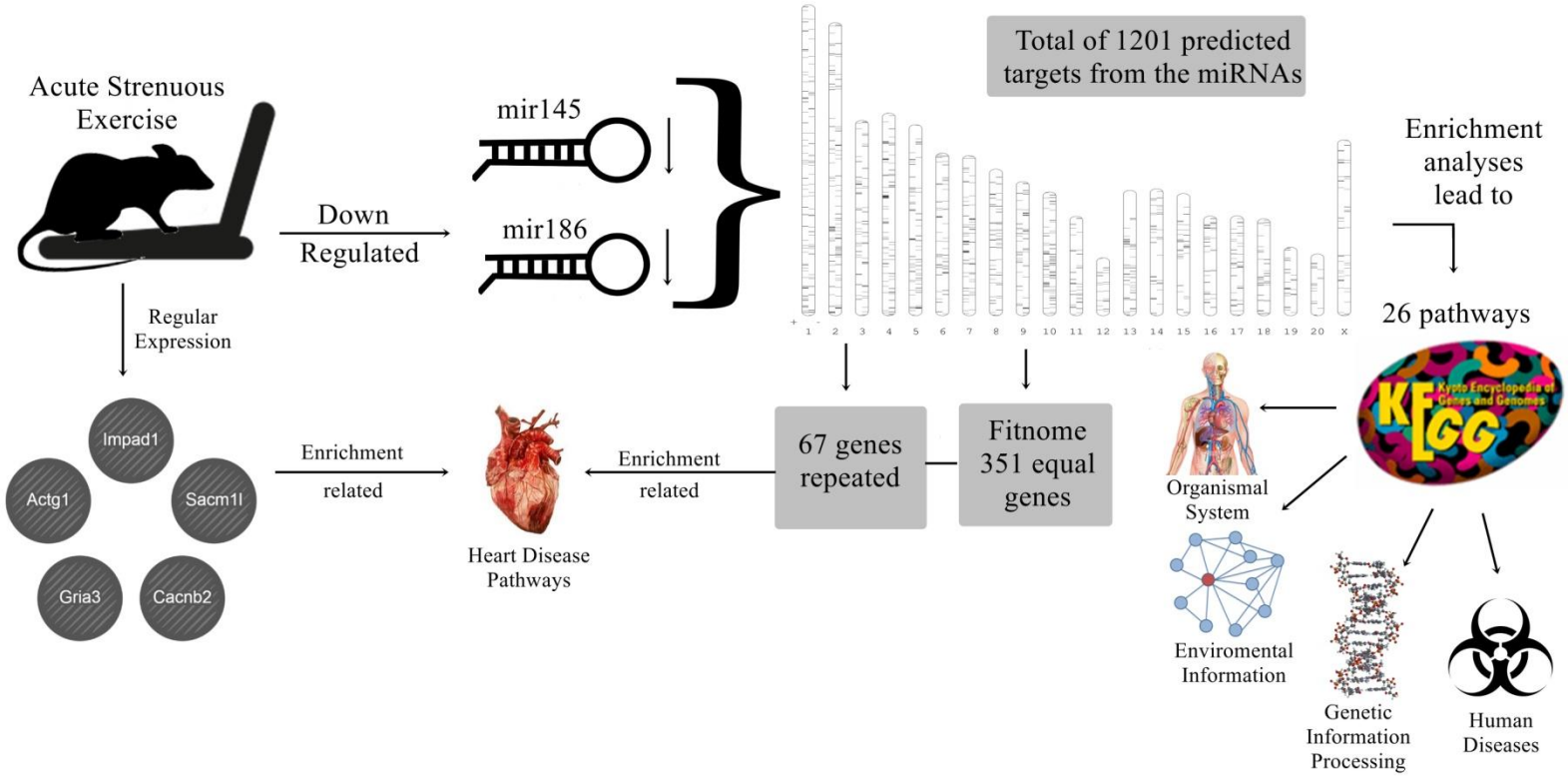

Fig. 3. The relation between the profile of miRNAs expression in exercise and possible main pathways related to the targets enrichment analyses considering the human KEGG database

Table 4. Enrichment analyses from target genes with KEGG database [16]

Super pathway	Sub pathway	KEGG ID	Pathway	P-value
Organismal System	Endocrine System	hsa04917	Prolactin signaling pathway	0.0071
		hsa04921	Oxytocin signaling pathway	0.0175
	Nervous System	hsa04727	GABAergic synapse	0.0004
		hsa04723	Retrograde endocannabinoid signaling	0.0045
		hsa04725	Cholinergic synapse	0.0100
		hsa04724	Glutamatergic synapse	0.0124
Environmental information	Signal transduction	hsa04066	HIF-1 signaling pathway	0.0006
		hsa04150	mTOR signaling pathway	0.0018
		hsa04630	Jak-STAT signaling pathway	0.0175
		hsa04390	Hippo signaling pathway	0.0132
		hsa04070	Phosphatidylinositol signaling system	0.0227
		hsa04068	FoxO signaling pathway	0.0386
Human Diseases	Cancer	hsa05205	Proteoglycans in cancer	0.0012
		hsa05202	Transcriptional misregulation in cancer	0.0269
		hsa05212	Pancreatic cancer	0.0323
	Infectious Disease: Bacteria	hsa05100	Bacterial invasion of epithelial cells	0.0043
	Infectious Diseases: Virus	hsa05132	Salmonella Infection	0.0233
	Cardiovascular Disease	hsa05410	Hypertrophic cardiomyopathy	0.0186
		hsa05414	Dilated cardiomyopathy	0.0308
	Metabolic and endocrine diseases	hsa04931	Insulin resistance	0.0447
		hsa04933	AGE-RAGE signaling pathway in diabetic complications	0.0277
Genetic Information Processing	Folding, sorting and degradation	hsa04120	Ubiquitin mediated proteolysis	0.0186
		hsa03018	RNA degradation	0.0296

Table 5. Top 5 metabolic pathways related to repeated gene targets

KEGG ID	Metabolic pathways enrichment based on Human KEGG database	Genes	*p* value
hsa05412	Arrhythmogenic right ventricular cardiomyopathy (ARVC)	CACNB2; DAG1; ACTG1	0.0015
hsa05410	Hypertrophic cardiomyopathy (HCM)	CACNB2; DAG1; ACTG1	0.002
hsa05414	Dilated cardiomyopathy (DCM)	CACNB2; DAG1; ACTG1	0.003
hsa04070	Phosphatidylinositol signaling system	IMPAD1; SACM1L; ITPR2	0.003
hsa04724	Glutamatergic synapse	SLC1A2; ITPR2; GRIA3	0.007

Cardiovascular pathways were also the main results in the enrichment analysis with the target genes present in the *FITNOME* gene set, also considering with $p<0.05$ value. The evaluation presented enrichment based in *Dag1* and *Actg1* genes, which also were found duplicated in the miRNAs target lists. These genes are described in the FITNOME gene set as related to chronic endurance exercise with up-regulated profile [47]. Proteins coded by these genes when associated with each other have a large probability to be involved in cardiopathies development [49].

4. CONCLUSION

Our findings in the present study demonstrate that mir186 and mir145 are down-regulated by strenuous exercise. These miRNAs present a relevant number of gene targets when simultaneously expressed. The down-regulated profile of the miRNAs found in strenuous exercise exhibit a low probability of the target genes being potentially regulated by them. Data about the expression in both studied conditions confirm the presence of those gene targets, without differential expression.

Changes in the expression of these miRNAs presents a high probability to alter several essential regulation processes, mainly involved in signaling transduction and human disorders. In this scenario, metabolic pathways related to signaling transduction are responsible for helping biologic process occurrence, as a cascade reaction, mTOR pathway is an example of this. Another relevant finding is presence of the miRNA predicted targets in FITNOME catalog, which correlates the targets with exercise annotation and its altered expression is related to cardiac pathways modulation.

Nevertheless, the findings suggest that the down-regulation of mir145 and mir186 prevent the mediation of alteration of the gene expression and its pathways.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All procedures of this study described were reviewed and approved by the Ethics for Animal Use Committee of the State University of Ceará – UECE, with the protocol number: 1592060/2014.

ACKNOWLEDGEMENTS

We cordially thank the Coordination for Personal Improvement of Higher Education Personal (CAPES) for the provided fellowship.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74616