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Research Article

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miRNA Profile in Hippocampus, Prefrontal Cortex and Sperm of Spontaneously Hypertensive Rats (SHR), a Model for Schizophrenia

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Abstract

Environment is crucial for manifestation and development of schizophrenia, bringing to light epigenetic mechanisms involved, such as post-transcriptional control of gene expression by miRNA, in this disorder. MiRNAs participate in physiological processes such as neurogenesis, behavioral manifestations as well as in adverse conditions like psychiatric disorders. Experimental models are essential to investigate the development of schizophrenia from early life, especially its molecular aspects.

Spontaneously hypertensive rats (SHR) are considered a good model for schizophrenia, since they show behavior that can be related to SCZ symptoms in humans and respond to treatment with anti-psychotic drugs. We investigated miRNA profile in prefrontal cortex, hippocampus and sperm of SHR through next generation sequencing (NGS). SHR showed differential expression (DE) of miRNAs related to neurological processes and schizophrenia in sperm, prefrontal cortex and hippocampus. Gene ontology and enrichment analyses showed that the majority of DE miRNA target genes are involved in pathways related to behavior, neurodevelopment and synaptic processes. Among these genes, 12 are dysregulated in schizophrenia patients, what reinforces SHR as a good model for epigenetic and molecular studies of this disorder. Finally, altered miRNA expression in sperm suggests that SHR model may be useful for future studies on epigenetic inheritance of schizophrenia.

Keywords: Schizophrenia, miRNAs, SHR, Prefrontal Cortex, Hippocampus, Sperm.

Introduction

MicroRNA (miRNA) are small non-coding RNA molecules that interact withmRNA to control post-transcriptional gene expression. They function in a vast variety of fundamental biological processes, and many of them have been shown to be very conserved across animal species. Alterations of miRNA levels have been associated with different adverse conditions, including psychiatric disorders [1-6]. In mammals, approximately 70% of all miRNA described in the literature are expressed in the brain, indicating that they are particularly important for neurophysiology and neurodevelopmental processes [7].

Schizophrenia (SCZ) is a severe psychiatric condition that affects around 1% of the world population. People with this disorder has issues related to reality interpretation, to behavior, and to relationships that considerably impair their lives.

Despite the considerable amount of studies about this disorder, its etiopathology is still obscure. There is a substantial amount of studies dedicated to investigate the genetic origins of SCZ and they have been providing relevant data on candidate genes and single nucleotide polymorphisms (SNP).

Although SCZ manifestation generally occurs in the adulthood, its origin is associated to the prenatal life. Therefore, the establishment of animal models is important to enable the investigation of the course of schizophrenia development. The Spontaneously Hypertensive Rat (SHR) is an inbred spontaneous model created at Okamoto Medical School, in 1963. Noteworthy, no significant genetic alteration in these rats has been detected [8, 9]. Although these rats were originally described as a hypertension model, behavioral abnormalities, such as social and attention deficit, impulsivity, hyperactivity and hyperlocomotion have also been reported as important characteristics [10-12]. Increased locomotor activity and reduced social interaction observed in SHR can be related to symptoms of SCZ in humans, such as social.

Withdrawal and disorganized communication [13-15]. In behavioral and pharmacological studies using the SHR, these manifestations are reduced by the administration of antipsychotic drugs used to treat SCZ, such as clozapine and sodium nitroprusside, reinforcing that this model can be used for SCZ studies. Although pharmacological and behavioral characteristics have been addressed in SHR model, to our knowledge studies on the molecular characteristics underlying the schizophrenia-like symptoms have not been studied [16, 17].

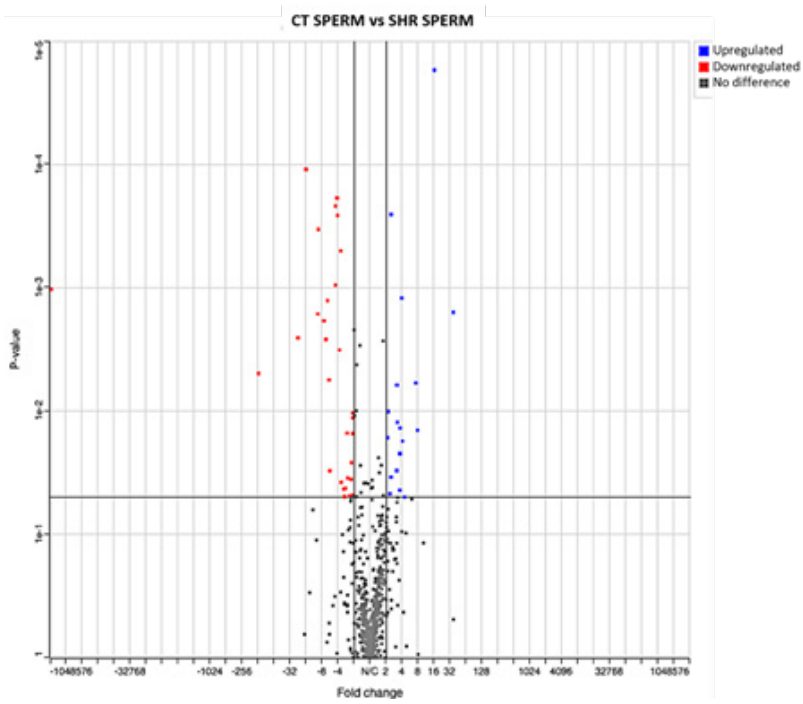
Recent studies reported miRNAs are dysregulated in prefrontal cortex in the hippocampus and in the peripheral blood of SCZ patients. Thus, the role of these molecules as possible SCZ markers or as targets for SCZ treatment has been considered [18-20]. miRNAs are also part of the epigenetic mechanisms involved in diverse biological phenomena and responses to environmental influences. Although the genetic basis of SCZ has been largely studied and the evidences show it plays indeed a crucial role in SCZ etiology and inheritance, environmental factors are very important for its development and manifestation. Trauma and stress for example, have been pointed as catalysts of SCZ [21-24].

As previously mentioned, the description of animal models to study developmental and molecular aspects of SCZ, which cannot be easily addressed in humans, is of great value to understand this disorder. The SHR have been pointed as a useful model to study behavioral and pharmacological aspects of SCZ. In addition, the importance of comprehending its inheritance and, more recently, its epigenetic characteristics reinforces the benefits of animal models to enable long term and extensive studies. Therefore, in the present study, we performed a global miRNA profile analysis of hippocampus, prefrontal cortex and sperm samples from SHR through next generation sequencing (NGS) using well-established databases, performing functional annotations for both rats and humans.

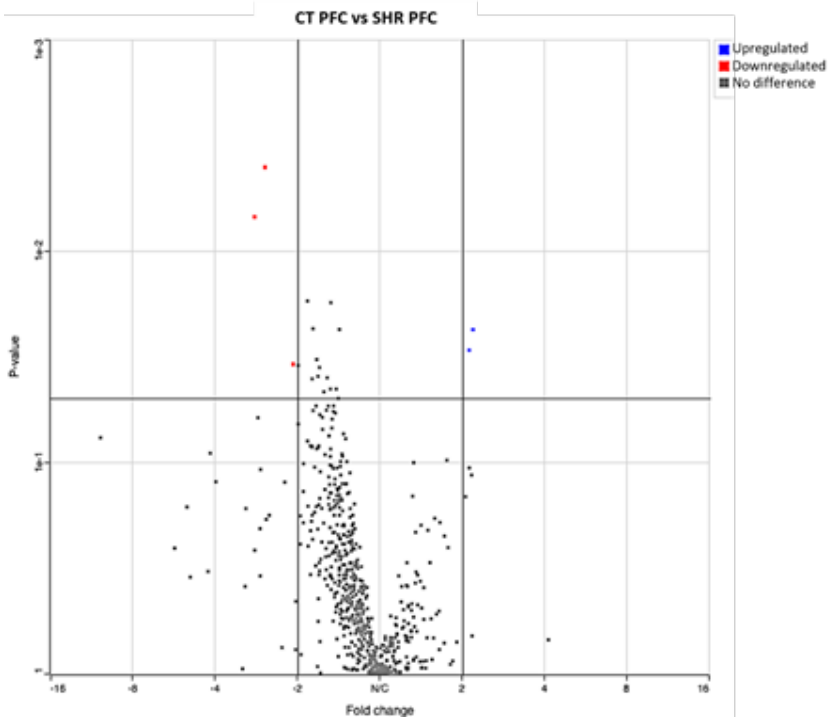
Results

Differentially Expressed (DE) Mirnas in Hippocampus, Prefrontal Cortex and Sperm

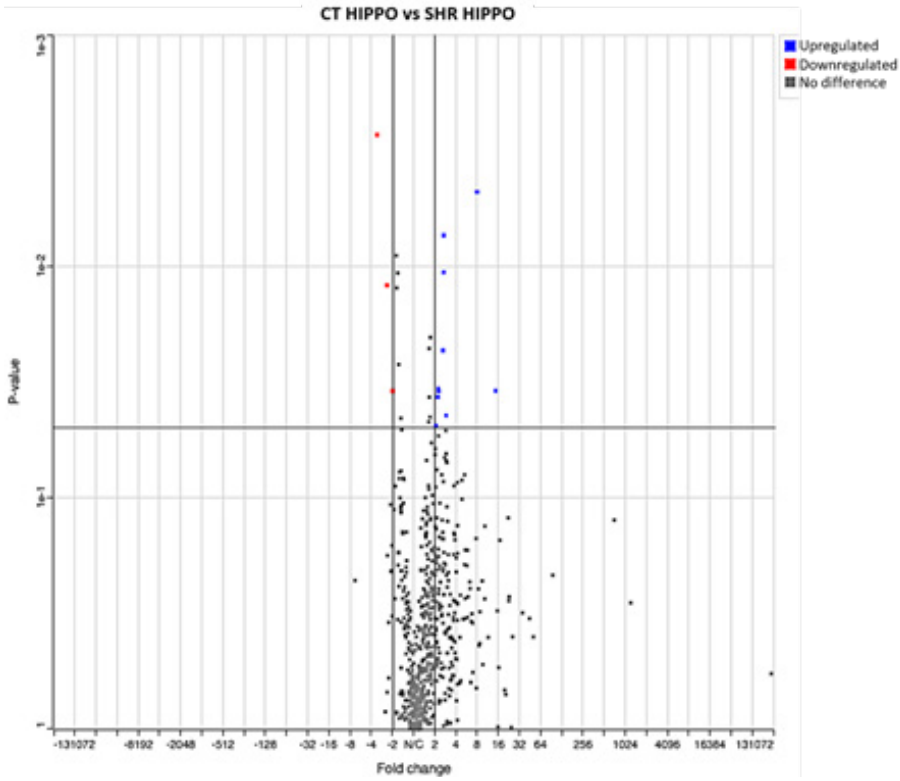
Bioinformatics analysis of HIPPO, PFC and SPERM from Wistar and SHR resulted in the identification of 629 miRNAs. From these 629 miRNAs, 45 miRNAs were differentially expressed (DE) in the SPERM (Suppl. Figure 1), 5 in the PFC (Suppl. Figure 2), and 13 (Suppl. Figure 3) in the HIPPO. Upregulated and downregulated miRNAs observed in the three samples are indicated in Table 1. From the DE miRNAs, miR-130a was downregulated in sperm and PFC (Table 1).



Suppl. Figure 1: Volcano Plot: miRNAs expression in SPERM. Dots above the black horizontal line indicate the miRNAs whose expression analysis showed a value of p <0.05. Gray dots represent miRNAs that showed no significant difference in expression between the two groups. The red and blue dots represent hypo expressed and hyper expressed miRNAs, respectively. Vertical lines: Fold change of ± 2.



Suppl. Figure 2: Volcano Plot: miRNAs expression for CPF. Dots located above the black horizontal line indicate the miRNAs whose expression analysis showed a value of p <0.05. The gray dots represent miRNAs that showed no significant difference in expression between the two groups. The red and blue dots represent hypo expressed and hyper expressed miRNAs, respectively. Vertical lines: Vertical lines: Fold change of ± 2.



Suppl. Figure 3: Volcano Plot: miRNAs expression in HIPO. Dots located above the black horizontal line indicate the miRNAs whose expression analysis showed a value of $p < 0.05$. Gray dots represent miRNAs that showed no significant difference in expression between the two groups. The red and blue dots represent hypo expressed and hyper expressed miRNAs, respectively. Vertical lines: Vertical lines: Fold change of ± 2.

Table 1: Differentially Expressed miRNA in SPERM, PFC and HIPPO of SHR when compared to Control (Wistar) Rats

SPERM		PFC		HIPPO	
Downregulated	**Upregulated**	**Downregulated**	**Upregulated**	**Downregulated**	**Upregulated**
130a	210	130a	465	667	3546
196b-1	541	31a	451	664	30a
93	365	551 b		150	344b-1
223	434-2				17-1
33	423				3589
let-7b	148b				26b
34a	327				499
376a	1843b				3562
218a	1				15b
142	185				16
449c	877				
339	let-7f-1				
320	377				
3558	6320				
106b	1188				
3543	671				

434	341				
375	544				
3072 423					
1249					
675					
674					
365					
let-7e					
322-2 503					
322-1					
708					

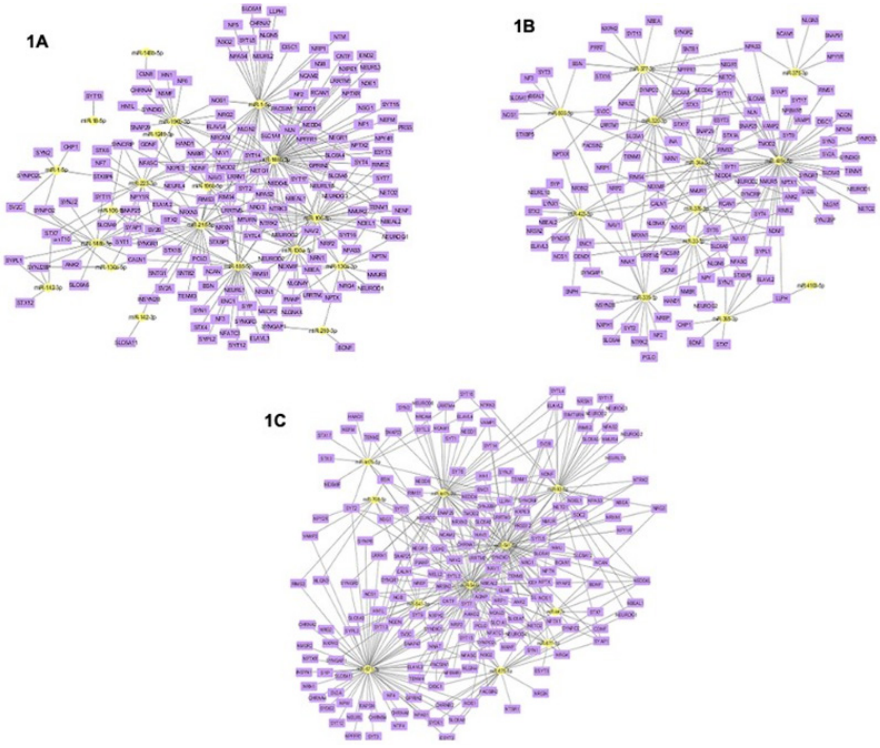
Interactive Analysis of Differentially Expressed miRNAS

Diferentially Expressed miRNA Target Genes

To investigate the possible consequences of changes in expression of the DE miRNAs in SPERM, PFC, and HIPPO, target (miRDB and TargeScan) and pathway interaction (Metascape) prediction tools were used. Hundreds of predicted target genes for the miRNAs differentially expressed in each sample were provided by TargetScan and miRDB platforms, generating more than 9 000 target genes in the PFC, more than 25 000 in the HIPPO and more than 75 000 in the SPERM, what impair the subsequent bioinformatics analyses and graphical representations. Thus, to proceed with the interactive analyses, only the target genes related to nervous system processes were considered. Small differences between the results produced by TargetScan and miRDB platforms were observed; then, only the target genes that appeared in both databases were considered.

The relevant target genes predicted for the differentially expressed miRNAs in SPERM, Cytoscape® networks (Figure 1) represent PFC and HIPPO. These networks also show genes that are targeted by multiple miRNAs.

Because the number of differentially expressed miRNAs was higher in SPERM (Figure 1A – C) than in PFC (Figure 1D) and HIPPO (Figure 1E), a considerably greater number of target genes were found in these cells. Thus, the network representation produced by Cytoscape was separated in three parts (Figure 1A – C) to facilitate the illustration of the data. This separation was based in the number of target genes provided for each miRNA.



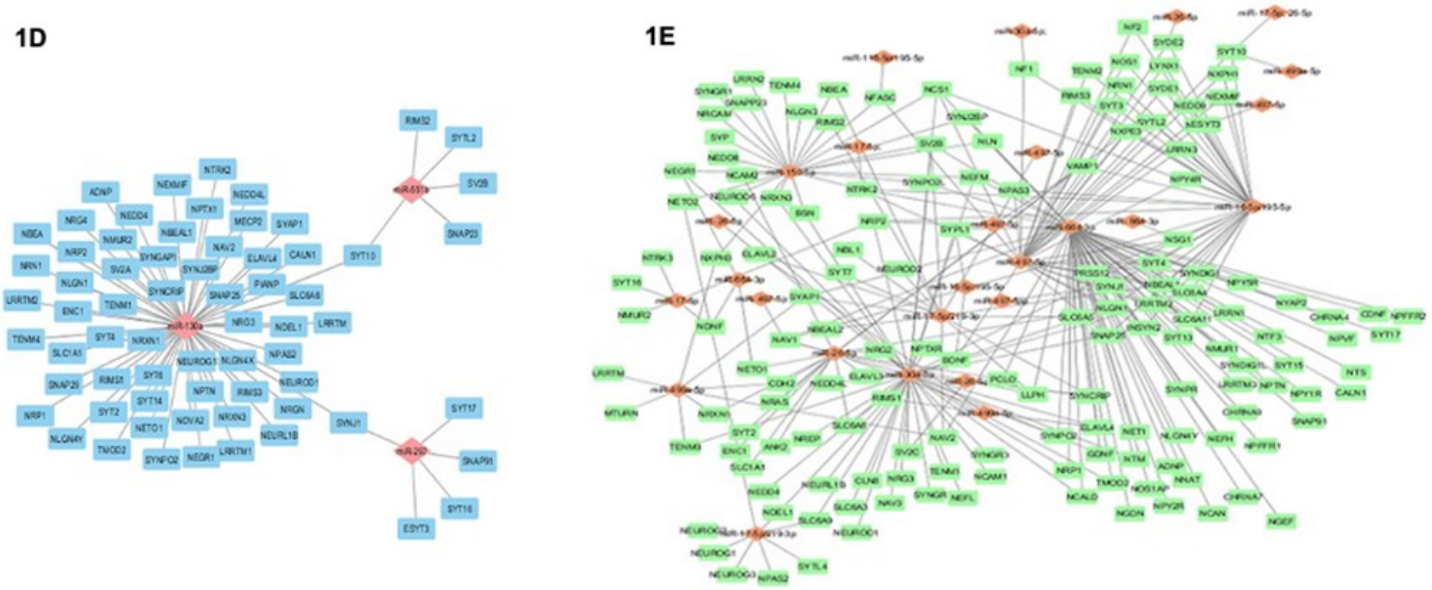


Figure 1: Interaction map of the DE miRNAs (diamonds) and their target genes (rectangles) – in SPERM, part 1 (A), 2 (B) and 3 (C); CPF (D) and HIPPO (E).

Ontology of miRNA Target Genes and Enriched Pathways

The analysis of the target genes of the DE miRNAs using the Metascape platform produced heatmaps of the enriched pathways in SPERM (Fig. 2A), CPF (Fig. 2B), and HIPPO (Fig. 2C). In the three types of samples, the top 20 enriched pathways indicated by Metascape include those related to synaptic processes, behavior, and neurodevelopment.

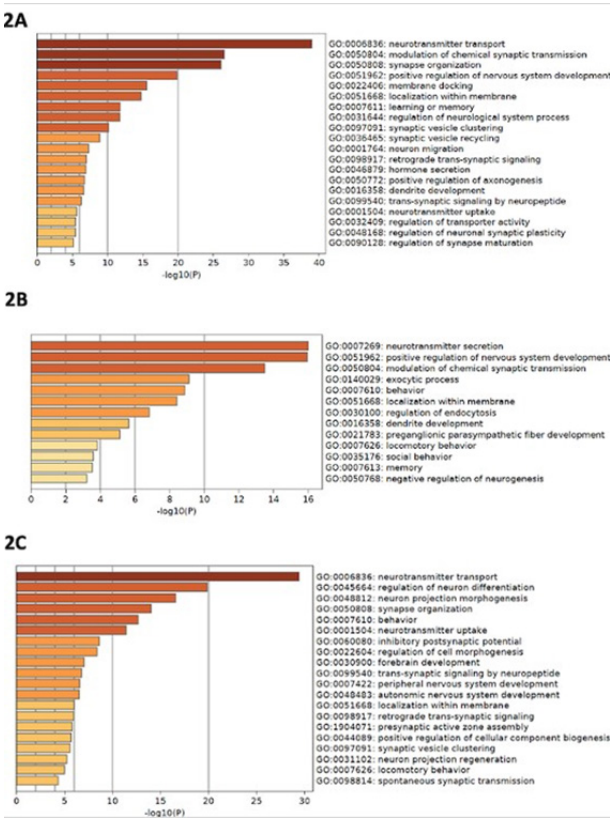


Figure 2: Heatmap Representation of the Biological Phenomena in Which the DE miRNA in SPERM (A), PFC (B) and HIPPO (C) are involved. The colors of the bars indicate the values of P; the darker the colors, the lower the P value. Analysis was performed using rat database.

Metascape analysis also provided the representation of the gene ontology of the target genes in the previously mentioned enriched pathways (Figure 3). The data showed that SPERM (blue fraction) accounts for a large proportion of most enriched pathways (Figure 3A). It was also observed that the pathways related to synapsis organization (Figure 3B, green) and nervous system development (Figure 3B, purple), which are considered to be important for SCZ pathogenesis, are interconnected and concentrate many target genes, as indicated by the size of the nodes. Other processes known to be involved in SCZ such as learning and memory (Figure 3B, brown) also include a large amount of DE miRNA target genes.

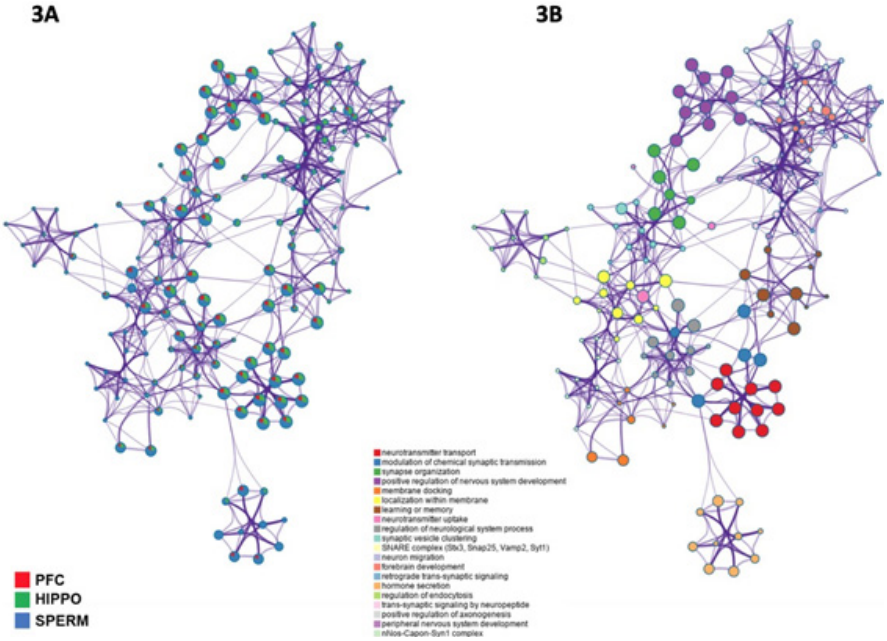


Figure 3: Enrichment Network (Gene Ontology Enrichment) In Which The Nodes Represent The Hits Involved In Each Biological Process. In A, The Nodes Are Represented As Pie Charts. Each Portion of The Pie Represents A Sample, and The Size of Each Portion Represents The Proportion of Each Hit In Each Sample. Each Hit Includes the Genes Involved in Biological Processes, Which Are Identified By The Colors In B. The Size of The Nodes Reproduces The Amount of Genes Involved In The Hits And The Purple Lines Between The Nodes Represent The Interactions. Analysis Using the Rat Database.

A hierarchical heatmap was obtained (Fig. 4), where biological processes wererelated to each other and between the three types of sample. This data illustrates the connection between processes and show which ones are relevant in the SPERM, PFC and HIPPO.

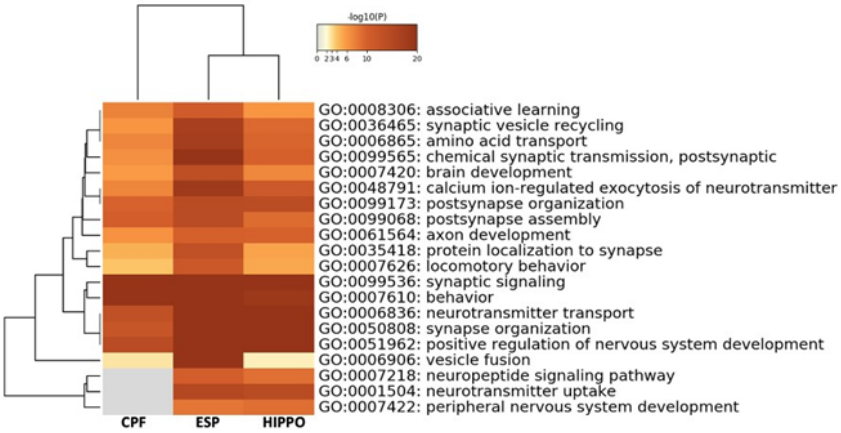


Figure 4: Hierarchical Heatmap Illustrating the Relationship between the Identified Biological Processes and Between Three Types of Sample. The Colors of the Rectangles Indicate the P Values; the Darker the Colors, the Lower the Value of P. It Is Observed That There Is Considerable Similarity Between The Hippocampus And Sperm. Synaptic Organization And Signaling, Learning And Memory, And Neurodevelopment Appear As Processes That Could Be Significantly Affected In The Three Types of Sample. Analysis Was Performed Using The Rat Database.

The Circus Plot (Figure 5) represents the target genes that coincide between the three types of sample and those that appear only one type of sample.

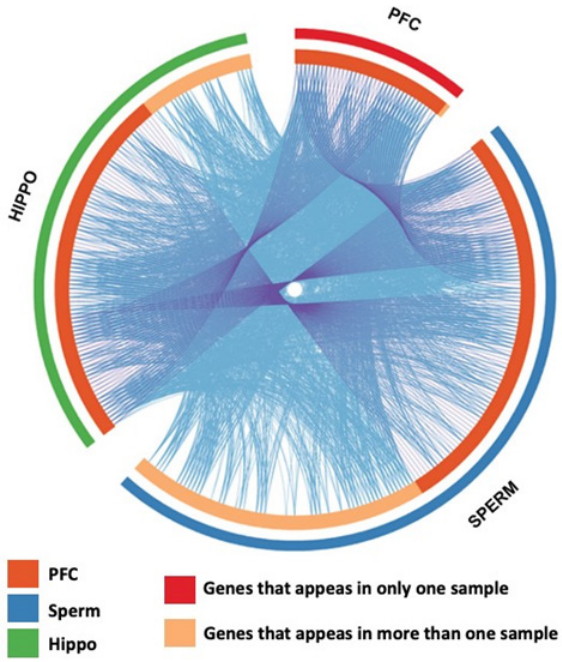
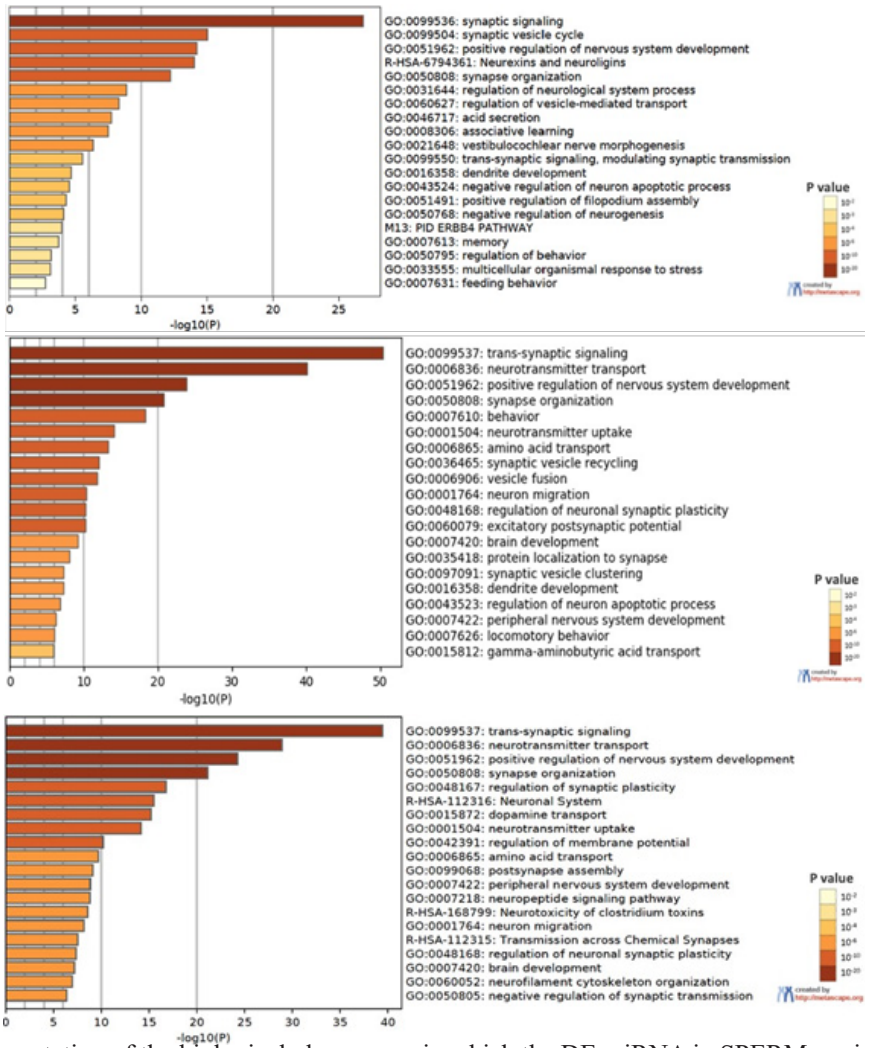


Figure 5: Circos Plot Illustrates How Differently Expressed Mirna Target Genes Coincide Between The Three Types of Sample. The Outermost Arches, Represented In Blue, Red, And Green, Indicate Sperm, Prefrontal Cortex, And Hippocampus, Respectively. The Internal Arcs, Represented By The Colors Dark Orange And Light Orange, Represent The Genes That Appear In More Than One Type of Sample or In A Single Type Of Sample, Respectively. The Purple Lines Indicate Genes That Appear In More Than One Type Of Sample. The blue lines link genes that are in the same "gene ontology term"; in different types of samples. Analysis was performed using rat database.

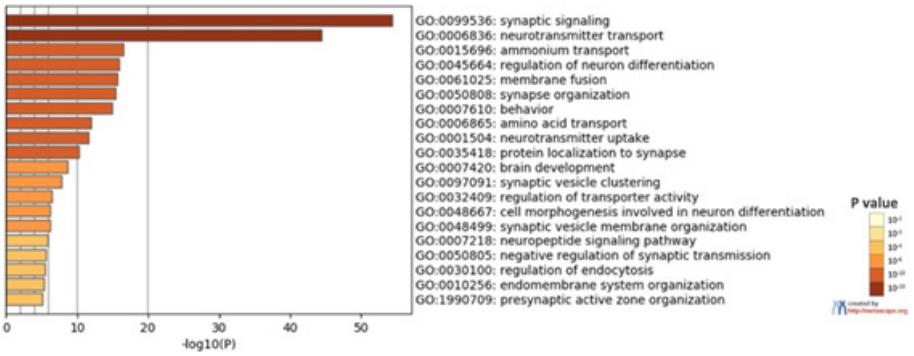
Translational Analysis

Since SCZ is a disorder restricted to humans, and because one of the aims of this study was to corroborate the usefulness of the SHR strain as a model for molecular studies about this disorder, we also analyzed the DE miRNA using human databases available in TargetScan, miRDB and Metascape. These data are presented in supplementary figures 4 to 9 and are very similar to those obtained using the rat database. Interestingly, even more predicted target genes resulted from the TargetScan and miRDB analyses in the human database than in the rat database.

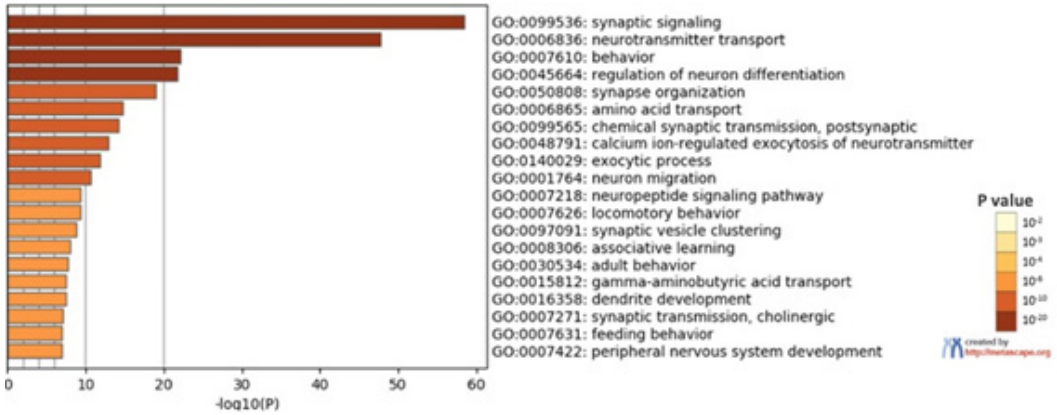
Subsequently, we consulted the SZDB database and found that, from the DE miRNA target genes, 12 show altered expression in SCZ patients. These genes are ADNP, LYNX1, NBEAL, NF1, NLGN1, NRSN1, SLC6A11, STX6, SYT2, SYT14, TENM1 and TENM3. Interestingly, from the 12 SCZ-related genes, 11 are targets for miRNAs differentially expressed in sperm (ADNP, LYNX1, NBEAL, NF1, NLGN1, NRSN1, SLC6A11, STX6, SYT14, TENM1 and TENM3); 5 are targets for miRNAs differentially expressed in PFC (ADNP, NBEAL1, NLGN1, SYT14 and TENM1); 9 are targets for miRNAs differentially expressed in HIPPO (ADNP, LYNX1, NBEAL1, NF1, NLGN1, SLC6A11, SYT2, TENM1 and TENM3).



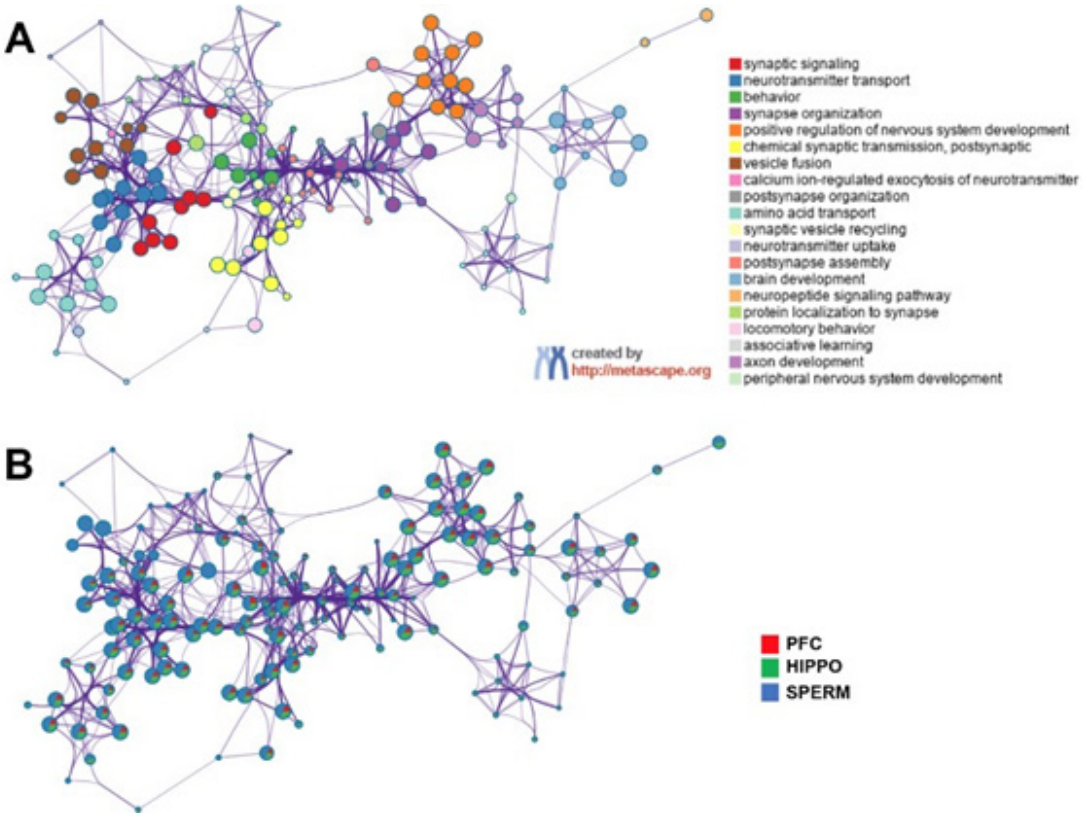
Suppl. Figure 4: Representation of the biological phenomena in which the DE miRNA in SPERM are involved. The colors of the bars indicate the values of P; the darker the colors, the lower the P value. Analysis was performed using the human database. A: Part 1; B: Part 2; C: Part 3.



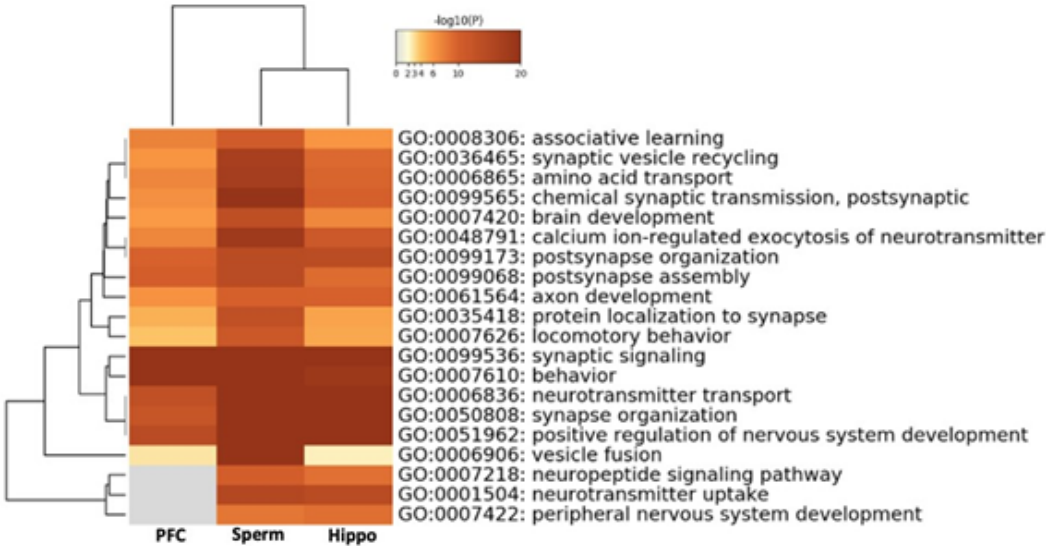
Suppl. Figure 5: Representation of the biological phenomena in which the DE miRNA in PFC are involved. The colors of the bars indicate the values of P; the darker the colors, the lower the P value. Analysis was performed using the human database.



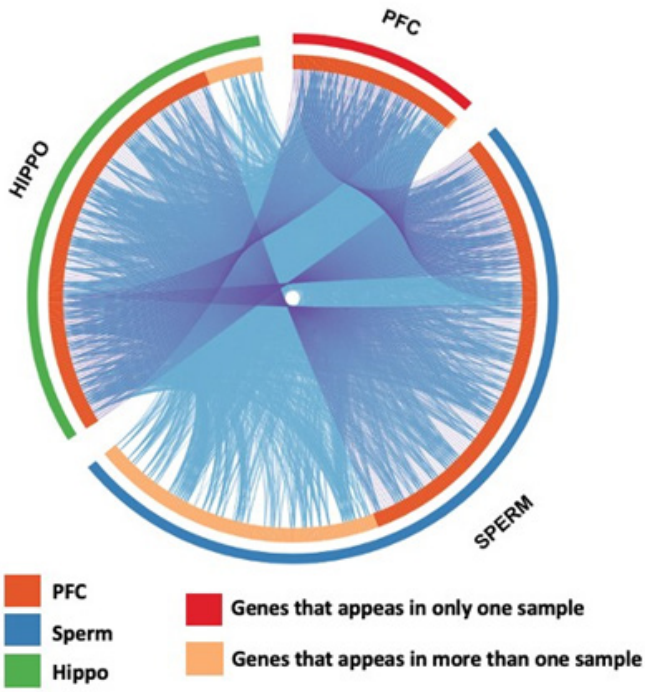
Suppl. Figure 6: Representation of the biological phenomena in which the DE miRNA in HIPPO are involved. The colors of the bars indicate the values of P; the darker the colors, the lower the P value. Analysis was performed using the human database.



Suppl. Figure 7: Enrichment network (Gene Ontology Enrichment) in which the nodes represent the hits involved in each biological process. In A, each process is represented by a color. The size of the nodes indicates the amounts of genes involved in the hits and the purple lines between the nodes represent their interactions. Analysis was performed using the human database. In B the nodes are represented as pie charts. Each portion of the pie represents a type of sample, and the size of each portion represents the proportion of each hit in each type of sample. Each hit includes the genes involved in biological processes, which are identified by the colors in A.



Suppl. Figure 8: Hierarchical heatmap illustrating the relationship between the identified biological processes and between three type of samples. The colors of the rectangles indicate the P values; the darker the colors, the lower the value of P. It is observed that there is considerable similarity between the hippocampus and sperm. Behavior, synaptic organization and synaptic signaling appear as processes that could be significantly affected in the three types of sample. Analysis was performed using the human database.



Suppl. Figure 9: Circus Plot illustrates how differently expressed miRNA target genes coincide between the three types of sample. The outermost arches, represented in blue, red and green, indicate sperm, prefrontal cortex and hippocampus, respectively. The internal arcs represented in dark orange and light orange, represent the genes that appear in more than one type of sample or in a single type of sample, respectively. The purple lines indicate genes that appear in more than one type of sample. The blue lines link genes that are in the same "gene ontology term" in different types of sample. Analysis was performed using human database.

Discussion

The use of experimental models to study SCZ is a challenge, since it is an exclusively human psychiatric disorder with complex symptoms. On the other hand, models are important to investigate SCZ aspects that cannot be addressed in clinical studies. In this study we investigated miRNA profile in the prefrontal cortex and hippocampus of SHR with the aim to contribute to search for SCZ models and to the elucidation of epigenetic mechanisms possibly involved in the pathophysiology of this disorder. We also looked at the miRNA profile in the sperm with the aim to provide preliminary data for future studies on epigenetic inheritance of SCZ.

In the conditions of the present study, five miRNAs showed altered expression in the prefrontal cortex, 13 in the hippocampus and 48 in sperm. Bioinformatic analysis showed the DE miRNA in the SHR are involved in multiple interaction networks with common target genes. The top 20 enriched pathways in which these miRNAs are involved include neurodevelopment, behaviour, memory, learning, and synapsis physiology, all of them known to be affected in SCZ. A recent study also used a rat model of SCZ (methylazoxymethanol acetate model – MAM) to study the expression of possible miRNAs related to schizophrenia and observed changes in the expression of genes related to synaptic chemical transmission, cognition, and inflammatory response in the MAM rats in relation to controls. As observed for MAM rats in the study by Du and collaborators here we also indicate that SHR can be an

interesting model for SCZ since they might be able to reproduce impairment of hippocampus and prefrontal cortex, which is related to SCZ [25]. Additionally, this model contributes to the comprehension of epigenetic aspects of this disorder.

Among the molecular aspects of schizophrenia, genetic aspects have been the focus of most studies. More recently, the epigenetic approach has been also gaining attention. Differential methylation pattern of genes identified as relevant for SCZ, such as DISC1 and BDNF, for example, have already been described in SCZ patients [26-29]. Broader studies have also reported alteration in bulk DNA methylation in patients diagnosed with SCZ [28, 30, and 31]. As previously mentioned, dysregulation of miRNA expression has also been demonstrated in SCZ. Differential expression of miR-137 has been reported in different studies 16, 32, 33, 34 and this miRNA has been suggested as a potential biomarker for this disorder [16, 32, 33, and 34]. Other miRNAs have been reported as differentially expressed in SCZ patients [16, 35, and 39]. However, a considerable discrepancy in the results is observed among the studies. This variability might result from several factors such as the type of sample (blood, different regions of the brain) and the type of techniques applied, what indicates that a wide miRNA analysis providing the profile of altered miRNAs is important.

The function of miRNAs is mostly related to their interaction with target mRNAs, which represent a target gene. Thus, the association of a specific miRNA to a process or disease is given by the role of the target gene in that process or disease. This can be addressed using microRNA Target Prediction databases such as TargetScan and miRDB, followed by gene ontology and gene enrichment analyses. In the "Translational Analysis" of the Results section of this manuscript we mentioned that we found 12 genes related to schizophrenia that were targeted by the miRNA differentially expressed in the prefrontal cortex and in the hippocampus. Therefore, we were able to suggest that the miRNA differentially expressed in these regions can be related to schizophrenia. We found that miR-130a, miR-664, miR-31, miR-150, miR-30a, miR-17, miR-26b, miR-499, miR-15b and miR-16 have been reported as relevant for this disorder, as reviewed by Beveridge and Cairns [1]. We did not find a direct association of miR-551b, miR-465, miR-451, miR-667, miR-3546 and miR-344b, miR-3589 and miR3562 with schizophrenia. However, all these miRNAs are related to neurodevelopmental and/or neurophysiological processes, as shown by our target analysis (miRDB and TargetScan) or by the literature [40-43].

Among the DE miRNAs, miR-130a showed differential expression in the PFC and in the sperm. This miRNA is involved in important processes during prenatal neurogenesis, such as neurite growth and dendritic spine density and has also been associated with SCZ [44, 45]. The expression of miR-130a has already been reported in sperm of different species, but it is gametic and the early embryonic function has not yet been well defined. It is known that during the beginning of embryonic development, miR-130a acts as a regulator in physiological processes related to mesodermal specification, angiogenesis and maternal-embryonic transition, which is a crucial moment when the embryo starts to control its own development through the activation of its genome [46, 47]. The importance of miRNAs from sperm for the early development of the embryo has already been indicated [48, 49]. Interestingly, the expression of the same miRNA can be present in different stages of embryonic development, assuming different regulatory functions according to each of these stages. Thus, it would be interesting to investigate whether altering the expression of miR-130a and of the other DE miRNAs found in sperm can affect t embryonic and nervous system development. Studies about paternal epigenetic inheritance of psychiatric disorders are relatively recent and it is not known whether a correspondence between the sperm and the brain is determinant to the transmission of the altered behaviour, since the miRNAs altered in the sperm will be transferred to the embryo and will be soon be degraded. Different studies have shown that changes in miRNA expression in mouse sperm caused by trauma and stress can lead to behaviour alterations in their offspring [50 - 53]. However, no data were found in the literature regarding the possible influence of sperm miRNAs on schizophrenia. The fact that the same SCZ-related miRNA (miR-130a) was altered in the sperm and in the brain reinforces the importance of studying paternal miRNA inheritance in SCZ. Our future aim is to investigate the possible influence of the altered sperm miRNA on the development of the nervous system and in the manifestation of the SCZ-like behaviour in the SHR.

As previously mentioned, one of the great challenges to establish experimental models for psychiatric disorders is the fact that these disorders are human manifestations and their diagnosis is based on consciousness parameters. On the other hand, many biological characteristics are conserved across mammalian species, making possible to carry out translational analyses. Consequently, it is important to obtain information about the biological aspects of proposed models. In our study, the enriched pathways generated by Metascape analysis using human and rat databases were related to neurological processes such as synapse physiology, behaviour, brain development, learning, and memory. These data show that the targets of the DE miRNAs are similar in rats and humans, as are the processes in which they are involved. And more importantly, target genes for these DE miRNAs have been described as altered in SCZ patients, as indicated by the analysis using the SZDB database. These data reinforce results from previous studies suggesting the SHR as a good model for SCZ [54].

In conclusion, the data presented here provide information regarding miRNAs profile in prefrontal cortex, hippocampus, and sperm of SHR aiming to contribute to the characterization of these rats as a model for molecular and epigenetic studies on SCZ. Considering SCZ presents an important environmental component, which is modulated by epigenetic alterations, including miRNA expression, these data can serve as basis for future studies using SHR in this field [55]. Since 70% of the miRNAs described in mammals

are present in the brain , information about miRNA profile related to this disorder can be an important contribution to the comprehension of this disorder and to provide new perspectives on diagnostic strategies and treatment [56]. Finally, our data direct us to future studies about the consequences of SCZ-related miRNA dysregulation in the sperm to the offspring.

Methods

Animals and Sample Collection

Male Wistar rats (Rattus norvegicus albinus) and Spontaneously Hypertensive Rats (SHR) obtained from the Centre for Development of Animal Models for Medicine and Biology (CEDEME) were used for this study. Adult animals (90-100 days old) were kept in plastic cages under a 12–12h light/dark cycle at 23–25 °C. Food and water were allowed ad libitum. The animals were separated into two groups: control (CT), composed by Wistar rats (n=9); and SHR, composed by SHR (n=9). Euthanasia was performed by anaesthesia/analgesia (xylazine/ketamine, 10 mg/Kg and 100 mg/Kg, respectively) followed by cardiac incision. Immediately after euthanasia, the sperm (SPERM), the prefrontal cortex (PFC) and the hippocampus (HIPPO) were collected and submitted to microRNA isolation, as described later.

All animal handling was carried out according to the National Institutes of Health guide for the care and use of Laboratory animals. This study was approved by the Committee for Animal Use (CEUA/UNIFESP - nº 6315260117) of the Universidade Federal de São Paulo (UNIFESP).

miRNA extraction, library construction and sequencing

The extraction of miRNA from sperm, hippocampus and prefrontal cortex was performed using the mirVana miRNA Isolation kit (Cat. AM1561, Thermo Fisher Scientific – Carlsbad, California, USA) according to the manufacturer's instructions. miRNA quantification was performed in Bioanalyzer 2100 (Agilent Technologies) using the Small RNA Kit (Cat. 5067-1548, Agilent – Santa Clara, California, USA). Libraries were constructed using the Ion Total RNA-seq kit v2 for Small RNA Libraries (Cat. 4475936, Thermo Fisher Scientific – Carlsbad, California, USA). Barcoded libraries were quantified in the Bioanalyzer 2100 (Agilent Technologies) using the High Sensitivity DNA Chip (Cat 5067-4626, Agilent Technologies – Santa Clara, California, USA).

Clonal amplification of the libraries was carried out using Ion 540™ kit Chef (Cat. A30011, Thermo Fisher Scientific – Carlsbad, California, USA) and sequenced using the Ion 540™ Chip (Cat. A27766, Thermo Fisher Scientific – Carlsbad, California, USA) in the Ion S5 Semiconductor Sequencer according to the instructions provided by the manufacturer.

Differential Expression Analysis

The analysis of miRNA expression was performed using Partek® Flow® software following the pipeline indicated for miRNA. Torrent Mapping Alignment Program (TMAP) was used for sequence mapping, then it was performed miRNA quantification using annotation model (Partek E/M) followed by a normalization step using counts per million (CPM) method followed by the addition of 1.0E-11. GSA differential analysis algorithm was applied in order to determine differentially expressed (DE) miRNA amongst studied groups. miRNA presenting fold change (FC) < -2 or > 2 and p<0,05 were selected for further analysis.

Target genes and pathway analyses

The DE miRNAs target genes were predicted using TargetScan (version 7.2) and miRDB rat databases and only the ones that appeared in both platforms were considered [57, 58]. Gene-miRNA Interaction networks were obtained through Cytoscape® (version 3.8.0). Metascape enrichment analysis was used to predict the pathways in which the DE miRNA are involved [59].

Translational analyses

To help to validate the information obtained from the SHR model, the DE miRNA were also analysed considering the human databases with the aim to investigate whether the same miRNA plays a conserved role in rats and humans. For this, TargetScan (version 7.2) and miRDB were also used to obtain the predicted target genes, followed by Cytoscape® and Metascape softwares analyses. The relationship of the target genes with schizophrenia was investigated using the Schizophrenia Database (SZDB) version 2.0 [60].

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Competing Interests
Authors declared no competing interests.

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