The Effect of Acupuncture on MiRNA Expression Profiles in the Ovaries of Rats with PCOS and Insulin Resistance

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The Effect of Acupuncture on MiRNA Expression Profiles in the Ovaries of Rats with PCOS and Insulin Resistance

Running title: Effect of acupuncture on miRNA expression in PCOS with insulin resistance

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Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic diseases of women of reproductive age. The major characteristics of PCOS are anovulation, hyperandrogenism, infertility, and insulin resistance. Previous studies showed that acupuncture could promote menstruation, decrease testosterone levels, increase insulin sensitivity, and improve frequency of ovulation in women with PCOS. However, the mechanism of how acupuncture regulates the reproductive and metabolic abnormalities of PCOS remains unclear. The present study investigated the differential expression of miRNAs in the ovaries of rats with PCOS and insulin resistance (IR) after electroacupuncture (EA) treatment.

Methods: A rat model of PCOS with IR was established by letrozole and a high-fat diet. Rats in the PCOS+EA group received acupuncture treatment five times a week for four weeks. The differentially expressed miRNAs of three rat ovaries of each group (n=3/group) were identified by deep sequencing. The differentially expressed miRNAs are shown by a hierarchical clustering heatmap and volcano plot. Gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted to explore the potential target genes of the differentially expressed miRNAs and identify their putative biological function. Eight of the differentially expressed miRNAs were selected for validation by real-time quantitative PCR (qRT-PCR).

Results: The rats in the PCOS+EA group had significantly decreased body weight and increased insulin sensitivity compared with the rats in the PCOS group. EA tend to reduce testosterone levels in the rats with PCOS and insulin resistance. The rats in the PCOS+EA group displayed fewer large cystic follicles, fewer atretic antral follicles, and more corpora lutea than the rats in the PCOS group. A total of 23 differentially expressed miRNAs were identified in the PCOS with IR group after EA treatment, including 15 miRNAs that were upregulated and 8 miRNAs that were downregulated. GO and KEGG pathway analyses indicated that the predicted target genes were related to cellular processes, and metabolic pathways. Furthermore, qRT-PCR confirmed that miRNA-181 was significantly downregulated in the rat ovaries of the PCOS+EA group.
compared with the PCOS group.

**Conclusion:** Our results indicate that differentially expressed miRNAs may mediate the effect of acupuncture on insulin sensitivity in PCOS with IR. It will be beneficial to study the mechanism of acupuncture in improving insulin resistance in women with PCOS.

**Keywords:** Polycystic ovary syndrome, insulin resistance, acupuncture, miRNAs, high throughput sequencing.

**Introduction**

Polycystic ovary syndrome (PCOS) is one of the most common endocrine and metabolic disorder in women of reproductive age, and the prevalence of PCOS ranges from 6% to 20% worldwide. According to the Rotterdam criteria, PCOS can be diagnosed in any woman presenting with at least two of the three following characteristics: clinical and/or biochemical hyperandrogenism, ovulatory dysfunction and polycystic ovarian morphology. In addition, PCOS is associated with obesity, insulin resistance, hyperinsulinem, type 2 diabetes, oligo-amenorrhoea, infertility, hirsutism, cardiovascular disease and mood disorder. A monozygotic twin study showed that the heritability of PCOS was approximately 70%, but the proportion of heritability accounted for by the PCOS loci identified to date by GWAS is less than 10%. Moreover, mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and other lifestyle issues. Although the pathological of PCOS has been studied for decades, more research is needed to explore the underlying mechanism of PCOS.

MicroRNAs (miRNAs) are small, single-stranded noncoding RNAs consisting of 18–24 nucleotides in length. They regulate post-transcriptional gene expression by binding to the 3’ untranslated region of target RNAs. MiRNAs are involved in many physiological processes including cell growth, differentiation, proliferation, and metabolism. Furthermore, several studies have shown that dysfunctional miRNAs are associated with the pathological mechanism of PCOS. For example, Professor
Stephen R. Hammes discovered that androgen can enhance the expression of miR-125, which in turn suppresses proapoptotic protein expression, ultimately attenuating follicular atresia. Ritu Deswal and Amita suneja Dang conducted a systematic review and meta-analysis to investigate the clinical diagnostic value and role of microRNAs in the pathogenesis of PCOS. They concluded that aberrant expression of various miRNAs plays an important role in the pathogenesis of PCOS and that miRNAs have potential diagnostic value for PCOS. Therefore, miRNAs could be biomarkers that participate in the origin and progression of PCOS.

Currently, the first-line pharmacological treatment for induction of ovulation in women with PCOS is letrozole, clomiphene citrate, and metformin. However, there are some negative side effects, such as headaches, ovarian hyperstimulation syndrome, diarrhoea, and breast tenderness, that limit the use of this treatment. Acupuncture is known to be a safe, inexpensive and effective treatment, that is used worldwide to regulate abnormal reproduction and metabolism. Acupuncture has been demonstrated to ameliorate menstrual frequency and to decrease circulating testosterone in women with PCOS. Moreover, acupuncture could promote ovulation in women with PCOS, and improve follicle development by decreasing AMH expression to regulate FSH and AMH in granulosa cells. In addition, an animal model confirmed the effect of acupuncture was, at least in part, medicated by decreasing sympathetic nerve activity. In our prospective pilot study, we found that 6 months of acupuncture significantly decreased HOMA-IR, indicating that acupuncture can improve insulin sensitivity. In the present study, we conducted high throughput sequencing to identify the differentially expressed miRNAs in the ovaries of rats with PCOS and IR by acupuncture intervention.

Materials and Methods

Rat model of PCOS with insulin resistance

All animal experiments were carried out based on the Guidelines for the Care and Use of Experimental Animals and approved by the Animal Ethics Committee of Guangzhou University of Chinese Medicine (20180715002). Three-week-old female
Sprague Dawley rats were purchased from the Animal Centre of Guangdong Province (Guangdong, China). Animals were fed adaptively for 1 week under conditions of 55%–65% humidity, 21–22 °C temperature, and a 12-h light/12-h dark cycle. Establishment of a rat model with PCOS and IR has been described previously. Briefly, 20 four-week-old female Sprague-Dawley rats were fed a high-fat diet (Research Diet, D12492, 60% fat) for 5 weeks and intragastrically administered 1 mg/kg letrozole in 1% CMC once daily for 3 weeks. The rats were fed a high-fat diet during letrozole treatment.

Acupuncture intervention

Rats with PCOS and IR were randomly divided into two groups, letrozole and high-fat group (n=10, **PCOS Group**) and the letrozole and high-fat and electroacupuncture group (n=10, **PCOS+EA Group**). The rats in the PCOS+EA group were treated daily from Monday to Friday for 4 weeks. First, the rats in the PCOS+EA group were lightly anaesthetized with isoflurane for 2–3 min. Then, the rats were suspended in a fabric harness above the desk and remained conscious during the acupuncture treatment. Acupuncture needles were inserted into the rectus abdominis and triceps surae muscles. The acupuncture needles were in somatic segments corresponding to the ovaries and utereis (i.e., from spinal levels T10 to L2 and at the sacral level), The needles were inserted 0.5–0.8 cm, and were attached to an electric stimulator and stimulated at 2 Hz in 0.1 s, 80 Hz burst pulses. Rats in the PCOS group were handled in the same way as rats in the treatment group, but without insertion of the needles\textsuperscript{24}.

Blood and tissue sampling

All rats were anaesthetized with isoflurane after overnight fasting. Blood samples were collected from the heart and the resulting serum samples were stored at −80 °C. Serum levels of testosterone (T) were measured using ELISA kits (Cloud-Clone Corp., Wuhan, China) according to the manufacturer’s protocol. The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as previously described using the following formula: HOMA-IR = (FBG \times FINS)/ 22.5\textsuperscript{25}.

Ovary samples were quickly collected from the animals and one ovary from each rat
was fixed in 4% paraformaldehyde. The ovary tissue samples were then dehydrated, embedded in paraffin, and sectioned into 4-µm sections. Each section was stained with hematoxylin and eosin (H&E). The other rat ovary was stored at −80 °C and used to establish miRNA expression profiles.

**RNA-sequencing (RNA-seq) and bioinformatics analysis**

RNA extraction and sequencing were conducted as previously described\(^2^6\). Briefly, total ovarian RNA was extracted by TRIzol reagent according to the manufacturer’s instructions. A NanoDrop 2000 spectrophotometer was used to measure the concentration of RNA. An RNA library was generated from total RNA using the NEB Next Ultra Directional RNA Library Prep Kit for Illumina (NEB, MA, USA). The RNA library quality and quantity were assessed with an Agilent 2100 Bioanalyzer and an ABI Step One Plus Real-Time PCR System, respectively. The RNA library was run on a HiSeq 2000 platform (Illumina, CA, USA) for sequence analysis. The clean reads were filtered from raw reads by FastQC and used for further bioinformatics analysis. The differentially expressed miRNAs were selected based on log\(^2\) (PCOS+EA/PCOS)>1 and P value <0.05.

Hierarchical clustering heatmaps and volcano plots generated by R package version 1.0.8 software (https://cran.r-project.org/web/packages/pheatmap/) were used to display the differentially expressed miRNA patterns between the two groups. Gene ontology (GO) analysis was performed to explore the molecular function, cellular components, and biological processes of the differentially expressed miRNAs (http://www.geneontology.org). The biological pathways of the differentially expressed miRNAs were further analysed using the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/) database.

**Real-time quantitative PCR (qRT-PCR)**

To verify the results of RNA sequencing, we measured relative miRNA expression by qRT-PCR analysis using SYBR Premix Ex Taq II (Takara, China). The U6 gene was used as an internal control. The relative expression of miRNAs was calculated using
the 2$^{-\Delta\Delta Ct}$ method. The sequences of the primers used in this study are shown in Table 1.

**Statistical analysis**

All values are presented as the mean ± SD. Comparisons between the experimental and control groups were conducted by a Student’s t-test. Data analyses were performed using SPSS 19.0 software (SPSS Inc., IL, USA). A P value < 0.05 was considered statistically significant.

**Results**

**The baseline characteristics of rats with PCOS and IR rat after acupuncture treatment**

We successfully established a rat model with PCOS and insulin resistance by letrozole and a high-fat diet. Briefly, the letrozole and high-fat diet treated rats showed increased body weight, abnormal estrus, hyperandrogenism, polycystic ovary morphology, and insulin resistance. Then, the rats with PCOS and insulin resistance were treated by electropuncture. After 4 weeks of treatment, the rats in the PCOS+EA group had significantly decreased body weight, and improved insulin sensitivity compared with those in the PCOS group (Figure 1A-B). In addition, electroacupuncture intervention tended to decrease the testosterone levels, which may indicate that electropuncture intervention could promote ovulation in women with PCOS and insulin resistance (Figure 1C). H&E staining showed that the ovaries in the PCOS group exhibited atretic antral follicles, more large cystic follicles with a thinner granulosa cell layer, and fewer corpora lutea. In the PCOS+EA group, the ovary displayed fewer large cystic follicles, fewer atretic antral follicles, and more corpora lutea (Figure 2). Therefore, electroacupuncture could ameliorate the reproductive and metabolic disorders in rats with PCOS and insulin resistance.

**Differentially expressed miRNA profiles in the ovaries of the PCOS+EA and PCOS groups**
We established miRNA expression profiles for three ovarian samples from the PCOS+EA and PCOS groups by high throughput sequencing. A total of 767 miRNAs were detected in the ovarian samples. Among these, 23 differentially expressed miRNAs were found between the two groups (fold change, ≥2; and P value < 0.05). Among the differentially expressed miRNAs, 15 were upregulated while 8 were significantly downregulated in the PCOS+EA group. The differentially expressed miRNAs identified by deep sequencing are shown in Table 2. Furthermore, a hierarchical clustering heatmap (Figure 3A) showed the differentially expressed miRNA profiles between the two groups. Finally, a volcano plot (Figure 3B) also displayed the pattern of upregulated and downregulated miRNAs between the two groups.

**Functional analysis of differentially expressed miRNAs**

PCOS is associated with anovulation, ovarian dysfunction, abnormal follicular development, and poor oocyte quality, which are aggravated by insulin resistance. Acupuncture can decrease body weight, promote ovulation, improve insulin sensitivity, and increase the pregnancy rate in women with PCOS. To gain insight into the underlying mechanism by which acupuncture ameliorates reproductive and metabolic abnormalities in PCOS with insulin resistance, we conducted GO analysis to identify the biological processes, cellular components, and molecular functions of the differentially expressed miRNAs (Figure 4A). We discovered that the most enriched GO term for cellular component was cell (GO:0005623), the most enriched term for molecular function was cellular process (GO:0009987), and the most enriched term for the biological process was metabolic process (GO:0008152). Moreover, the metabolic pathway (pathway: rno01100) was the most enriched pathway in the KEGG database (Figure 4B). The biological functions and pathways for the differentially expressed miRNAs are closely related to the development of insulin resistance in PCOS.

**Validation of candidate miRNAs by qRT-PCR**

According to the literature and the fold-change of the deep sequencing results, four
upregulated miRNAs (miR-742-5p, miR-28-3p, miR-183-5p, and miR-203-5p) and four downregulated miRNAs (miR-135a-3p, miR-181-2b-3p, miR-380-3p, and miR-92b-3p) were selected for validation using qRT-PCR. As shown in Figure 5, qRT-PCR confirmed that miR-181-2b-3p was significantly downregulated in the rat ovaries of the PCOS+EA group compared with the PCOS group. This result was consistent with the sequencing results. However, four upregulated miRNAs (miR-742-5p, miR-28-3p, miR-183-5p, and miR-203-5p) and three downregulated miRNAs (miR-135a-3p, miR-380-3p, and miR-92b-3p) were not significantly expressed or were inconsistently expressed compared with the sequencing results.

Discussion

PCOS is a complex heterogeneous disorder characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. PCOS is also associated with a higher LH pulse frequency, which in turn, stimulates ovarian theca cells to produce excess androgen. In addition, insulin resistance is an independent risk factor for PCOS, which is thought to contribute to the mechanism of anovulation and to increase androgen synthesis by the ovary. In the present study, we successfully established a rat model of PCOS and IR using letrozole and a high-fat diet. Letrozole is an aromatase inhibitor that blocks the conversion of androgen to oestrogen. This results in elevated androgen levels, which is the main pathogenetic mechanism occurring in PCOS. Pubertal female rats were continuously treated with letrozole, inducing increased LH and T levels, an abnormal oestrous cycle, anovulation, increased ovarian weight, cysts, atretic follicles, and no CL in adulthood. A high-fat diet in rats leads to increased body weight, abnormal glucose tolerance, decreased insulin sensitivity, and insulin resistance. Thus, letrozole and a high-fat diet in rats result in both the reproductive and metabolic features associated with PCOS and insulin resistance.

Acupuncture is a common treatment for various diseases, because it is effective, inexpensive, and has few side effects. Clinical studies have indicated that acupuncture can increase menstrual frequency, promote ovulation, decrease testosterone levels, and
improve insulin sensitivity in women with PCOS\textsuperscript{20, 23, 33}. Moreover, animal studies verified that acupuncture could promote normal reproduction and alleviate metabolic disorder of PCOS, at least in part, mediate by sympathetic nerve activity\textsuperscript{22, 34, 35}. In the present study, acupuncture decreased body weight, reduced testosterone, and increased insulin sensitivity in rats with PCOS and insulin resistance, which was consistent with previous studies. Therefore, it is valuable to study how acupuncture ameliorates reproductive and metabolic abnormalities in women with PCOS.

Increasing evidence indicates that abnormal expression of miRNAs has been detected in follicular fluid, granulosa cells, adipose tissue, serum, cumulus cells, and ovarian tissue of women with PCOS and plays vital role in the origin and progression of PCOS\textsuperscript{12-14}. Moreover, acupuncture treatment could be used in the therapy of diseases such as stoke by restoring abnormal expression of miRNAs\textsuperscript{36, 37}. These results suggest that acupuncture intervention may improve insulin sensitivity by regular miRNA expression in women with PCOS. In the present study, we established miRNA expression profiles from the ovaries of rats in the PCOS+EA group along with rats in the PCOS group. A total of 23 differentially expressed miRNAs in the PCOS+EA ovaries exhibited significant changes compared with those in the PCOS group, including 15 that were upregulated and 8 that were downregulated. The terms biological process, cellular component, and molecular function were predicted by a GO analysis of the differentially expressed miRNAs. Of these, the most significantly enriched term was cellular process, indicating that the mechanism by which acupuncture promotes insulin sensitivity in PCOS may be involved in cellular processes. Likewise, the most enriched pathway from the KEGG pathway analysis was metabolism. This finding is consistent with our result that acupuncture can promote insulin sensitivity in rats with PCOS and insulin resistance. Furthermore, qRT-PCR revealed that miRNA-181 was significantly downregulated in the PCOS+EA rats compared with the PCOS rats. MiRNA-181 is upregulated in rats with PCOS compared with normal rats in our previous study\textsuperscript{31}. Thus, acupuncture may restore abnormal miRNA-181 expression in rats with PCOS, as a result of improving insulin resistance. The mechanism of acupuncture and miRNA-181 in insulin resistance in PCOS needs further investigation.
However, this study has some limitations. In view of the methods and results we have obtained, this paper should be described as a pilot study, and this study is not large enough to account for the heterogeneity of PCOS. However, these results might promote further study of the mechanism of acupuncture on insulin resistance in PCOS. Therefore, we will conduct further experiments to study insulin resistance in PCOS and comprehensive experiments will be performed and reported in the future.

Conclusion

In conclusion, we found 23 differentially expressed miRNAs after acupuncture treatment in ovaries of rats with PCOS and insulin resistance by deep sequencing analysis and verified the differential expression of miRNA-181 by qRT-PCR. Furthermore, miR-181 may represent a target for acupuncture to improve insulin resistance in PCOS. Additional studies are needed to identify the underlying mechanism(s) of miRNA and acupuncture in improving insulin resistance in PCOS.

Abbreviations

PCOS: Polycystic ovary syndrome; IR: Insulin resistance; EA: Electroacupuncture; GO: Gene ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; Real-time quantitative PCR: qRT-PCR; MicroRNAs: MiRNAs; Letrozole and high-fat group: PCOS Group; Letrozole and high-fat and electroacupuncture group: PCOS+EA Group; Testosterone: T; The homeostasis model assessment for insulin resistance: HOMA-IR; Hematoxylin and eosin: HE; RNA-sequencing: RNA-seq.

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Not applicable

Author contributions

CZ: Project development, Validated the sequencing data, Data collection, Data analysis, Manuscript writing. CY: A rat model establishment, Acupuncture treatment. SH: Acupuncture treatment. ZL: Data collection, Data analysis, Prepare the figures,
Manuscript writing. RZ: Data analysis. YS: Manuscript revision. HL: Sample collection. JL: Manuscript revision. KL: Design the study, Data analysis. HM: Project development, Funding acquisition, Data analysis, Manuscript editing. All authors read and approved the final version of the manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

All animal experiments were conducted under the principles of the Guidelines to the Care and Use of Experimental Animals and approved by the animal Ethics Committee of Guangzhou University of Chinese Medicine.

**Consent for publication**

Not applicable

**Competing interests**

The authors declare no potential conflicts of interest to declare.

**References:**


3. Boyle JA, Teede HJ. PCOS: Refining diagnostic features in PCOS to optimize


15. Sørensen AE, Wissing ML, Englund ALM, Dalgaard LT. MicroRNA Species in...


**Figure legend**

**Fig.1** Changes in body weight, HOMA-IR, and serum hormone levels between the PCOS+EA and PCOS groups. A) Comparison of weight between the two groups. B) The values of HOMA-IR were determined for the two groups. C) The T levels were determined for the two groups. *P < 0.05; **P < 0.01. HOMA-IR: homeostasis model assessment of insulin resistance; T: testosterone.

**Fig.2** Morphological changes in rat ovarian tissues as detected by H&E.

**Fig.3** Differentially expressed miRNAs were identified by deep sequencing. A hierarchical clustering heatmap (A) and a volcano plot (B) were used to display the differentially expressed miRNA patterns between the two groups.

**Fig.4** Gene Ontology (GO) and KEGG pathway analysis for predicted targets of the differentially expressed miRNAs.

**Fig.5** Validation of the miRNAs in the rat ovaries between the two groups by qRT-PCR. The expression of four upregulated miRNAs (miR-742-5p, miR-28-3p, miR-183-5p, and miR-203-5p) and four downregulated miRNAs (miR-135a-3p, miR-181-2b-3p, miR-380-3p, and miR-92b-3p) identified by deep sequencing were determined. *P < 0.05; **P < 0.01.
Figures

Figure 1

Changes in body weight, HOMA-IR, and serum hormone levels between the PCOS+EA and PCOS groups. A) Comparison of weight between the two groups. B) The values of HOMA-IR were determined for the two groups. C) The T levels were determined for the two groups. *P < 0.05; **P < 0.01. HOMA-IR: homeostasis model assessment of insulin resistance; T: testosterone.

Figure 2

Morphological changes in rat ovarian tissues as detected by H&E.
Figure 3

Differentially expressed miRNAs were identified by deep sequencing. A hierarchical clustering heatmap (A) and a volcano plot (B) were used to display the differentially expressed miRNA patterns between the two groups.

Figure 4

Top 20 Pathway Enrichment

- Wet signaling pathway
- Regulation of actin cytoskeleton
- Ras signaling pathway
- ERK signaling pathway
- Proteoglycans in cancer
- Protein processing in endoplasmic reticulum
- PI3K-Akt signaling pathway
- mTOR signaling pathway
- Metabolic pathways
- MAPK signaling pathway
- HTLV-1 infection
- Hypo signaling pathway
- FoxO signaling pathway
- Focal adhesion
- Endocytosis
- Chemokine signaling pathway
- cGMP-PKG signaling pathway
- Calcium signaling pathway
- Axin guidance

Percentage of genes

Input number

Cellular Component Molecular Function Biological Process

P-Value

0.56
0.64

0.66

0.88

0.88
Gene Ontology (GO) and KEGG pathway analysis for predicted targets of the differentially expressed miRNAs.

Figure 5

Validation of the miRNAs in the rat ovaries between the two groups by qRT-PCR. The expression of four upregulated miRNAs (miR-742-5p, miR-28-3p, miR-183-5p, and miR-203-5p) and four downregulated miRNAs (miR-135a-3p, miR-181-2b-3p, miR-380-3p, and miR-92b-3p) identified by deep sequencing were determined. *P < 0.05; **P < 0.01.