Peer Review File

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Reviewer A

Comment 1:

- The manuscript by Qin et al. entitled, Aberrant miRNA-mRNA regulatory network in polycystic ovary syndrome is associated with markers of insulin sensitivity and inflammation" revealed that the miRNA-mRNA regulatory network in PCOS is associated with markers of insulin sensitivity and inflammation providing a novel epigenetic basis of PCOS pathogenesis. The present paper is very interesting and well-prepared. The hypothesis and experiment design are correct and properly prepared. Furthermore, the newest molecular tool as microRNA-seq and RNA-seq were used. The confirmation of PCOS was also presented. The only question regards "Table 1 Basic information of en bloc procurement surgery" - I suppose it was put into the manuscript by mistake and should be removed.

Reply 1: We highly appreciate your comments and suggestions. We checked the uploaded files including table 1 and found that there was a mistake in the review version generated by the system. The correct table 1 is "Negatively regulated miRNA-gene pairs in PCOS (PNA mice compared with the control mice)". We apologize for the confusion.

Changes in the text:

Table 1. Negatively regulated miRNA-gene pairs in PCOS (PNA mice compared with the control mice)

miRNA	Log_2FC	Expression change	Target Gene	Log_2FC	Expression change
miR-106a-5p	-1.72	Down	*Cd28*	8.39	Up
			Il10rb	2.25	Up
			Stat3	1.36	Up
			Vegfa	1.09	Up
miR-155-5p	-1.14	Down	*Arntl*	1.30	Up
			Gsk3b	2.04	Up
			S1pr1	1.14	Up
			Nr1h3	2.60	Up
			Lpin1	2.22	Up
			Maf	3.74	Up
			Trp53inp1	1.04	Up
miR-184-3p	-1.07	Down	*Fzd4*	1.08	Up

Reviewer B

The paper by Qin et al. examined the connection between altered miRNA expression and its role in PCOS. A strength of the paper is they used both a mouse model of PCOS, as well as cultured cells from women with PCOS. A second strength is that the findings in mice (i.e., altered expression of miR-106-5p and miR-155-5p) was confirmed in human cells, linking PCOS to altered insulin resistance and inflammation pathways. The paper suffers from a number of detractions:

Comment 1:

- grammar throughout the paper is a concern

Reply 1: We are sorry for such insufficient and thanks for your review. We have polished the manuscript by medical writing service (https://www.cwauthors.com.cn/). The language-edited version of our manuscript was re-submitted.

Changes in the text: All edited sentences have been marked in the manuscript.

Comment 2:

- the methods section is overly long

Reply 2: Thank you for your valuable advice. We have streamlined the description of the methods section, including the contents of PCOS mouse model identification, library construction methods, verification experiments, and statistical methods in the revised manuscript.

Changes in the text: We have removed some sentences of the experimental steps that were too detailed "After stood at room temperature for 15-20 min, centrifuged the blood at 1000 g for 10 min. And then, collecting the supernatant and freezing at -80℃. After thawing at 4℃", "After oocyte removal, the surrounding GCs were collected and washed twice with Dulbecco's improved Eagle medium", "Grinding equipment was incubated at 180℃ for more than 12 hours and kept at a low temperature during grounding. Ovarian tissues need fully grounding with liquid nitrogen to make the lysate better penetrate." et al. We have moved the statement "All participants are non-related Han people from the same geographical region with no significant difference in the average age, and agreed to involve in this research" to the results section in lines 241-243 of the revised manuscript.

Comment 3:

- why were the mouse studies conducted in pregnant female mice treated with DHT as they are already pregnant?

Reply 3: Thanks for your review. Women with PCOS have elevated circulating androgens during late gestation, potentially exposing their offspring, who are at increased risk for PCOS. Animal models have exhibited reproductive and metabolic abnormalities similar to PCOS following prenatal androgenization. prenatally androgenized (PNA) mouse is an ideal model for emulating the hyperandrogenic phenotype of PCOS in women. DHT was injected subcutaneously in pregnant mice daily to make the fetal exposure to a high androgen environment. After histological and

serological identification, the offspring of DHT-treated mice were considered to be PNA mice. PNA mice have been used in many studies.

Changes in the text: We described this modelling method in detail in the method section.

Comment 4:

- determining the impact of changes in miRNA on targets should include protein abundance of a given gene.

Reply 4: We are highly sorry for such insufficient and appreciated your comments. Due to the excessive target genes of the miRNAs, we do not have enough tissue and clinical samples for subsequent protein quantitative detection. We are now actively collecting samples and will explore more completely and rigorously in follow-up research. Once again, thank you very much for your valuable suggestions.

Changes in the text: We have added this limitation "Due to the limitation of the sample size, some extended experiments need supplementing in the following research. The detection of the target protein is necessary for studying the regulation of miRNA on protein abundance intuitively." in lines 364-366 of the discussion section.

Comment 5:

- for a number of the experiments with mice, only a limited number were used; e.g., the transcriptome profiles involved 3 PCOS mice and 2 controls - was this justified statistically? It seems very limited in power. This is also the case for the small RNA sequencing analysis.

Reply 5: We are highly sorry for such insufficient and thanks for your comments. We almost trawled through the database and failed to find other high-throughput sequencing data for the ovaries of PNA mice. However, we found the study that was also based on the ovaries of PNA mice (Lei, Ding et al. 2017). In this study, 2 groups of samples were tested, with each group containing 5 mice ovaries. Over 39,000 transcripts were analyzed with the GeneChip® Mouse Genome 430 2.0 Array by using the selection criteria of fold change ≥1.5. And finally, we found out 1188 differentially expressed genes, including 671 up-regulated genes and 517 down-regulated genes. We intersected these 1188 differentially expressed genes with the 3,432 differentially expressed genes from our own mice RNA-seq data analysis, and found that 362 genes were included in both of them (including *Stat3*, *Stat1* and *Socs3* et al.). Then we did the hypergeometric test of our data and the validation set, and the p-value was less than 10^-10. The results were shown in supplementary materials. In conclusion, our analysis result is reliable indeed.

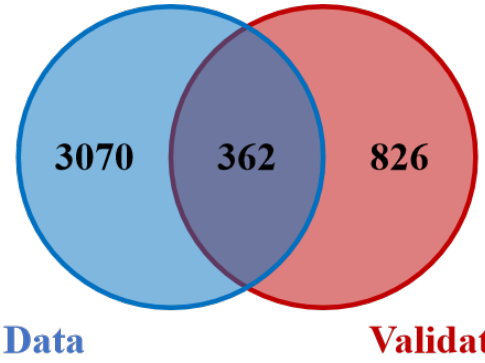

Figure 1: The Venn diagram shows our data and the data in the database

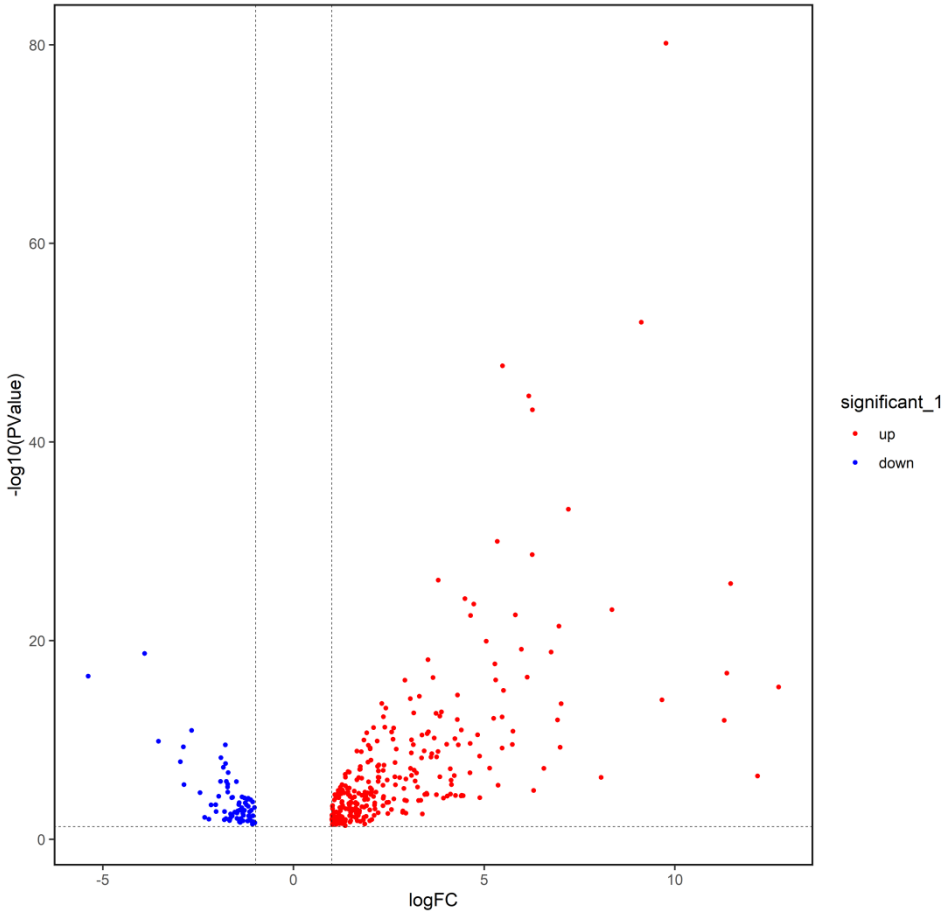

Figure 2: Volcano map shows the expression of common genes

Statement: We declare that Figure 1, Figure 2, and the supplementary table in this review comments are original, have not been published before.

Reference: Lei, L., L. Ding, J. Su, M. Liu, Q. Shi, J. Zhou, H. Sun and G. Yan (2017). "Attenuated expression of MTR in both prenatally androgenized mice and women with the hyperandrogenic phenotype of PCOS." PLoS One 12(12): e0187427.

Comment 6:

- on page 25 is a Table 1 with data on pigs with surgery?????

Reply 6: We highly appreciate your reminder. We checked the uploaded files including table 1 and found that there was a mistake in the review version generated by the system.

The correct table 1 is “Negatively regulated miRNA-gene pairs in PCOS (PNA mice compared with the control mice)”. We apologize for the confusion.

Changes in the text:

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Comment 7:

- were measures of insulin resistance and inflammation performed in the mice to link the results metabolically to the phenotype? The PCOS women do not appear to display any signs of diabetes/ insulin resistance as well.

Reply 7: Thanks for your thoughtful comments and suggestions. We are highly sorry for the lack of detection of insulin resistance and inflammation due to the insufficient PNA mice sample size in the original manuscript. We have trawled through and found some studies on the metabolism of PCOS and PNA mice in the database. As this study, they examined metabolic characteristics of female PNA mice and found that “the PNA mice exhibited increased fasting glucose and impaired glucose tolerance (IGT)” (Roland, A V et al. 2010). In Xie’ s study, they detected the inflammation level in PCOS mice and found the increasing (Xie, Q et al. 2019). We have cited and supplemented the results in our revised manuscript. PCOS is considered a low-grade inflammatory disease and is associated with insulin resistance. In our study, we tested the blood glucose level of PCOS patients during collecting samples, but the p-value is not significant. Insulin tests may require more and complete serological and molecular biology tests to obtain more accurate conclusions. This limitation was illustrated in the discussion. We apologize for the insufficient.

Changes in the text: We have cited and supplemented the results “Previous studies showed that there was an increase in indicators of insulin resistance and inflammation detected in PCOS mice” in lines 349-350 of discussion section. And we have added

“The detection of the target protein is necessary for studying the regulation of miRNA on protein abundance intuitively. Quantitative detection of insulin resistance and inflammation in PNA mice also helps to understand the regulatory mechanism of PCOS.” in lines 365-367 of discussion in our revised manuscript.

Reference: Roland, A V, Nunemaker C S, Keller S R, and Moenter S M. 2010. Prenatal androgen exposure programs metabolic dysfunction in female mice. J Endocrinol 207 (2): 213-23. https://doi.org/10.1677/joe-10-0217.

Xie, Q, Xiong X, Xiao N, He K, Chen M, Peng J, et al. 2019. Mesenchymal Stem Cells Alleviate DHEA-Induced Polycystic Ovary Syndrome (PCOS) by Inhibiting Inflammation in Mice. Stem Cells Int 2019: 9782373. https://doi.org/10.1155/2019/9782373.