Minireview: New advances in microRNAs related to polycystic ovary syndrome (PCOS)

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Abstract: MicroRNAs (miRNAs) encompass a large family of non-coding small RNAs that regulate gene expression at the post-transcriptional level. They are widely involved in many physiological and pathological processes including polycystic ovary syndrome (PCOS). PCOS is a frequent endocrine aberration in women. Currently our understanding of miRNAs in relation to PCOS is at an early stage and is gaining ground rapidly. In this mini review we summarized new advances in recent work on miRNAs with respect to PCOS. The expression profiling of miRNAs has been conducted in whole blood, serum, plasma, follicular fluid, and granulose cells of PCOS patients or animal models. These previous studies have identified differentially expressed miRNAs which influence multiple biological aspects such as insulin resistance, hormone synthesis, and sex-hormone signaling pathway. The diagnostic value of miRNAs has also been evaluated in several studies. Additional studies will provide new insight into the molecular mechanisms of PCOS and offer clues for new treatment strategies.

Keywords: microRNA, polycystic ovary syndrome, biomarker, pathogenesis
Introduction

Polycystic ovary syndrome (PCOS) is a common heterogeneous endocrine disorder in women of productive age. There are mainly three types of diagnostic criteria offered by different groups: the National Institutes of Health/National Institute of Child Health and Human Disease (NIH/NICHD) (1), the European Society of Human Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) (2), and the Androgen Excess and PCOS Society (3). The prevalence of PCOS therefore varied from 6% to 8% and up to 20% depending on the type of criteria used (4-6). No matter which criteria are adopted, diagnosis of PCOS is usually made based on menstrual disturbances, hyperandrogenism, and polycystic ovaries as defined by ultrasound. Typical PCOS symptoms include oligomenorrhea, amenorrhea, hirsutism, acne, overweight or obesity, and infertility. Currently the exact etiology of PCOS is not clear yet. Epidemiological studies revealed that risk profiles of PCOS include family history (7,8), overweight and obesity (9,10), and diabetes (11).

Post-transcriptional regulation by microRNAs (miRNAs) has been a popular and important research field in the past 10 years. miRNAs represent a large family of non-coding small RNAs of 19-25 nucleotides in length (12). The scheme of miRNA biogenesis was shown in Figure 1. The miRNA genes are transcribed by RNA polymerase II into primary miRNAs and then processed by Drosha and Pasha to be pre-miRNAs. Pre-miRNAs are exported into the cytoplasm and cut into mature miRNAs by Dicer. miRNAs recognize and bind to seed sequences in the 3’ untranslated region (3’-UTR) of targeted genes and totally silence or partially inhibit gene transcription (13). The degree of mRNA silencing depends on the degree of complementarity which rarely exits in animals. Therefore the impact of miRNAs on mRNA transcription is mostly repression (14).

The regulation mechanisms of miRNAs in disease development have been studied intensively in cancers, while the possible regulation pattern of miRNAs in PCOS is poorly investigated. Currently only a few miRNA related studies exist as
summarized in Table I. Totally 21 studies were included in this minireview and majority (13 out 21) are published in the last five years, indicating more and more attention has been paid to this research field. The purpose of this minireview is to introduce the current understanding of miRNAs related to PCOS and to provide clues for future studies.

Figure 1. Scheme of miRNA biogenesis (cited from reference 51)

**miRNA profiles in PCOS patients or PCOS animal models**

Totally 28 miRNAs have been reported to be dys-regulated in PCOS. Of these miRNAs, miR-9 (15-17), miR-18b (15-17), mir-21 (16,18-22), miR-27b (19), miR-30c (18,23), miR-103 (16,19), miR-122 (24), miR-135a (15-17), miR-146a (16,23,25), miR-155 (15,16,19), miR-193b (24), miR-194 (24), miR-222 (23,25-27), miR-224 (17,28), miR-383 (17,29,30), miR-638 (31), miR-3665 (31), miR-4463 (31), miR-5706 (31), and let-7i-3pm (31) are up-regulated; miR-19b (32), miR-29a-3p (31), miR-93 (32,33), miR-124-3p (31), miR-128 (31), miR-132 (15,16,25,34), miR-199b (24), miR-324 (35), miR-592 (36), miR-4522 (35), miR-6767-5p (35), and let-7c (31)
are down-regulated. Contradictory results were observed in different studies on the expression of miR-320 (23,25,29,37). These studies have been down in blood samples (whole blood, serum, or plasma), ovaries, as well as in different ovarian components including follicular fluid, granulose cells, blastocysts, and cumulus-oocytes.

miRNAs are stable in serum, which makes miRNAs suitable as non-invasive biomarkers for diagnosis and prognosis. Therefore, many studies chose blood samples as a source of miRNA profiling. A case-control study investigated miRNA expression in 12 PCOS patients, 12 healthy females and 12 males (19). Four miRNAs (miR-21, miR-27b, miR-103, and miR-155) were reduced in whole blood and bioinformatics analysis suggested that these miRNAs could be involved in hormone metabolism. Another microarray assay compared miRNA expression in age-matched PCOS patients and controls (23). After initial stage of miRNA profiling and second stage of quantitative PCR validation, miR-222, miR-146a, and miR-30c were found increased in the PCOS patients. Correlation analysis showed that miR-222 was positively correlated with serum level and miR-146a was negatively correlated with serum testosterone level. Note worthily, this study demonstrated that the miRNAs differentially expressed in ovarian tissues were not consistent with the difference in blood. Microarray and quantitative PCR are useful tools for large-scale screening of differentially expressed miRNAs. In addition to the study by Long et al, there were three other studies used this tool combination. The study by Ding et al identified 5 up-regulated circulating miRNAs (let-7i-3pm, miR-5706, miR-4463, miR-3665, and miR-368) and 4 down-regulated miRNAs (miR-124-3p, miR-128, miR-29a-3p, and let-7c) in PCOS patients (31). Joint prediction by different statistical methods revealed that these miRNAs were widely involved in immune system, ATP binding, MAPK signaling, apoptosis, angiogenesis, oxidative stress, and p53 signaling pathways. The study by Jiang et al identified that miR-122, miR-193b, and miR-194 were up-regulated and miR-199b-5p was down-regulated in PCOS patients (24). Multiple linear regression analyses showed that miR-193b and body mass index (BMI) were independent predictors of impaired glucose metabolism in PCOS patients. The
predicted targets of these miRNAs mainly involved glycometabolism, insulin signaling pathway, neurotrophin signaling pathway, PI3K-AKT signaling pathway, and regulation of actin cytoskeleton. The study by Song et al identified that miR-4522, miR-324-3p, and miR-6767-5p were down-regulated in PCOS patients compared with age- and BMI-matched controls (35). Further experiments showed that miR-6767-5p was negatively associated with fasting glucose and positively associated with the number of menses.

**Regulation targets of miRNAs in PCOS**

PCOS is usually associated with higher prevalence of impaired insulin tolerance. In type 1 diabetic patients, the risk of PCOS was increased to 15.4 times compared with the control group (95% confidence interval [CI]: 2.2-110.2, \( P<0.0001 \)) (38). A meta-analysis showed that the prevalence of PCOS in type 1 diabetes was 24% (95% CI: 15%-34%) (39). The prevalence of PCOS in type 2 diabetes differs from 8.3% (95% CI: 4.5%-13.4%) in Amini’s study (40) to 26.7% in Peppard’s study (41). Several studies discovered that dys-regulated miRNAs could target key molecules of insulin signaling pathway. Glucose transporter 4 (GLUT4) is the major insulin-dependent glucose transporter. miR-93 was down-regulated in adipocytes of PCOS patients and GLUT4 was a potential target of miR-93 (33). Insulin receptor substrate 2 (IRS2) is a cytoplasmic signaling molecule that mediates effects of insulin. The study by Roth et al indicated that IRS2 was significantly repressed by dys-regulated miRNAs (miR-9, miR-18b, and miR-135a) (17).

In PCOS patients, ovarian androgen synthesis is usually up-regulated. The ovarian cycle is also altered due to abnormal hormone secretion of hypothalamic-pituitary-ovarian axis. In addition, insulin inhibits hepatic synthesis of sex-hormone-binding globulin and leads to increased free testosterone concentrations. Many studies have investigated the correlation between miRNAs and sex-hormone levels. What’s more, miRNAs have been demonstrated to play a role in modulating homeostasis of steroid hormones by targeting steroid receptors or steroid synthesis.
enzymes. miR-222, which was highly expressed in follicular fluid and serum of PCOS patients, was a regulator of estrogen receptor 1 (27). miR-320 was suggested to target steroidogenic factor 1 (SF-1), although the expression of this miRNA was inconsistent in different studies (29). miR-592 was down-regulated in serum of PCOS patients and could directly target luteinizing hormone/chronic gonadotropin receptor (36).

**Further perspectives**

Currently the exact etiology of PCOS remains unclear and a rising interest in omics studies has been developing quickly. So far there are studies of genetics (42-44), proteomics (45,46), and metabolomics (47,48). Transcriptomics study of miRNA expression has also added new insights into this research field. There are several reviews summarizing the microRNAs that are differentially expressed either in PCOS patients or animal model (49,50).

One advance in these studies is the shift of sample types. Earlier studies usually reveal specific miRNAs in rat models or in human tissue samples such as granulose cells and follicular fluid; while recent studies focus more on serum samples because serum miRNA profiles might provide potential non-invasive biomarkers for PCOS diagnosis. Study by Jiang et al. evaluated diagnostic values of several miRNAs and found that combination of miR-193b and BMI could offer a moderate diagnostic value with area under the curve of 0.752 (24). The change of research object also brings about the problem of poor reproducibility. With exception of a few miRNAs, many expression variations of miRNA were reported in single study. Therefore, more repeated studies are needed to confirm which miRNAs are indeed universally differentially expressed, and which miRNAs are the results of single study.

Another progress in the studies is that more and more research begun to investigate the molecular mechanism of miRNAs. In early studies, a common research pattern was to compare the expression of miRNAs in cases and controls, and then select differentially expressed ones. In recent studies, a number of studies have begun to explore the specific functions of miRNAs. Bioinformatics databases are used
to predict potential targets of dys-regulated miRNAs and dual-luciferase reporter system is used to verify the direct binding between miRNAs and their targets.

Although the list of miRNAs and their targeting genes discussed above is by no means exhaustive, it is plausible to infer that miRNA regulation is an important mechanism driving PCOS progression. It remains to be seen whether miRNAs can be successfully used as diagnostic or prognostic biomarkers. It is also worthwhile to explore the possibilities of anti-miRNA therapy in PCOS treatment. Currently our knowledge of miRNAs related to PCOS seems to be inadequate and additional research efforts are definitely required to fill the blank.

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References


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Table I. List of microRNAs differentially expressed in polycystic ovary syndrome (PCOS)

<table>
<thead>
<tr>
<th>microRNA</th>
<th>Species</th>
<th>Detected in Tissue/Cell</th>
<th>Major findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-9↑</td>
<td>Human</td>
<td>Follicular fluid granulosa cells</td>
<td>Inhibits testosterone release; Increases expression of PCNA; Targets IL-8, SYT1, IRS2.</td>
<td>15-17</td>
</tr>
<tr>
<td>miR-18b↑</td>
<td>Human</td>
<td>Follicular fluid granulosa cells</td>
<td>Promotes progesterone release; Inhibits testosterone and estradiol release; Suppresses PCNA expression; Promotes Bax expression; Targets IL-8, SYT1, IRS2.</td>
<td>15-17</td>
</tr>
<tr>
<td>miR-19b↓</td>
<td>Human</td>
<td>Blastocysts</td>
<td>N/A</td>
<td>32</td>
</tr>
<tr>
<td>miR-21↑</td>
<td>Human</td>
<td>Whole blood</td>
<td>Blocks apoptosis in mouse periovulatory granulosa cells; Decreased in obese individuals or type 2 diabetic patients; Increased to FSH exposure; Targets LATS1.</td>
<td>16,18-22</td>
</tr>
<tr>
<td>miR-27b↑</td>
<td>Human</td>
<td>Whole blood</td>
<td>Decreased in obese individuals; Positively correlated with testosterone.</td>
<td>19</td>
</tr>
<tr>
<td>miR-29a-3p↓</td>
<td>Human</td>
<td>Serum</td>
<td>N/A</td>
<td>31</td>
</tr>
<tr>
<td>miR-30c↑</td>
<td>Human</td>
<td>Serum granulosa cells</td>
<td>Increased to FSH exposure.</td>
<td>18,23</td>
</tr>
<tr>
<td>miR-93↓</td>
<td>Human</td>
<td>Blastocysts</td>
<td>Targets SIRT1 and GLUT4.</td>
<td>32,33</td>
</tr>
<tr>
<td>miR-103↑</td>
<td>Human</td>
<td>Whole blood granulosa cells</td>
<td>Promotes progesterone release; Inhibit estradiol release; Reduced in obese individuals.</td>
<td>16,19</td>
</tr>
<tr>
<td>miR</td>
<td>Species</td>
<td>Source</td>
<td>Effect</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>-------</td>
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<td>---------------</td>
</tr>
<tr>
<td>miR-122 ↑</td>
<td>Human</td>
<td>Serum</td>
<td>Increased in PCOS patients with impaired glucose metabolism than in PCOS patients with normal glucose tolerance.</td>
<td>24</td>
</tr>
<tr>
<td>miR-124-3p ↓</td>
<td>Human</td>
<td>Serum</td>
<td>N/A</td>
<td>31</td>
</tr>
<tr>
<td>miR-128 ↓</td>
<td>Human</td>
<td>Serum</td>
<td>N/A</td>
<td>31</td>
</tr>
<tr>
<td>miR-132 ↓</td>
<td>Human/Rat</td>
<td>Follicular fluid, Granulosa cells</td>
<td>Increases estradiol secretion; Inhibits progesterone and testosterone release; Increases PCNA exposure; Increased after hCG-induced ovulation and FSH exposure; Inhibits Bax expression; Targets HMGA2 and Ctbp1</td>
<td>15,16,25,34</td>
</tr>
<tr>
<td>miR-135a↑</td>
<td>Human</td>
<td>Follicular fluid, Granulosa cells</td>
<td>Reduces progesterone and testosterone release; Inhibits Bax exression; Targets IL-8, SYT1, IRS2</td>
<td>15-17</td>
</tr>
<tr>
<td>miR-146a ↑</td>
<td>Human</td>
<td>Serum, Follicular fluid, Granulosa cells</td>
<td>Suppresses release of progesterone, estradiol, and testosterone</td>
<td>16,23,25</td>
</tr>
<tr>
<td>miR-155 ↑</td>
<td>Human</td>
<td>Serum, Granulosa cells, Follicular fluid</td>
<td>Inhibits testosterone release; Decreases PCNA expression; Inhibits Bax expression.</td>
<td>15,16,19</td>
</tr>
<tr>
<td>miR-193b ↑</td>
<td>Human</td>
<td>Serum</td>
<td>Increased in PCOS patients with impaired glucose metabolism than in PCOS patients with normal glucose tolerance.</td>
<td>24</td>
</tr>
<tr>
<td>miR-194 ↑</td>
<td>Human</td>
<td>Serum</td>
<td>Increased in PCOS patients with impaired glucose metabolism than in PCOS patients with normal glucose tolerance.</td>
<td>24</td>
</tr>
<tr>
<td>miR-199b ↓</td>
<td>Human</td>
<td>Serum</td>
<td>N/A</td>
<td>24</td>
</tr>
<tr>
<td>miR-222 ↑</td>
<td>Human/Rat</td>
<td>Ovary, Granulosa cells</td>
<td>Increased in type 2 diabete patients; increases estradiol release; Targets estrogen receptor 1.</td>
<td>23,25-27</td>
</tr>
<tr>
<td>miR-224↑</td>
<td>Human/Mouse</td>
<td>Follicular fluid, Cumulus-oocyte</td>
<td>Promotes granulose cell proliferation; Increases estrogen release; Targets PTX3 and Smad4</td>
<td>17,28</td>
</tr>
</tbody>
</table>
Symbols: ↑, up-regulation; ↓, down-regulation; -, inconsistent reports

Abbreviations: Ctbp1, carboxyl-terminal-binding protein-1; FAI, free androgen index; FSH, follicle stimulating hormone; GLUT4, glucose transporter 4; HMGA2,
high-mobility group AT-hook 2; IL, interleukin; IRS, insulin receptor substrate; LHCGR, luteinizing hormone/chronic gonadotropin receptor; PCNA, proliferating cell nuclear antigen; PTX3, pentraxin 3; SF-1, steroidogenic factor 1; SHBG, sex hormone-binding globulin; SIRT1, sirtuin 1; SYT1, synaptotagmin 1; TGF-β1, transforming growth factor-β1.