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ARTICLE

miR-483–5p and miR-486–5p are down-regulated in cumulus cells of metaphase II oocytes from women with polycystic ovary syndrome

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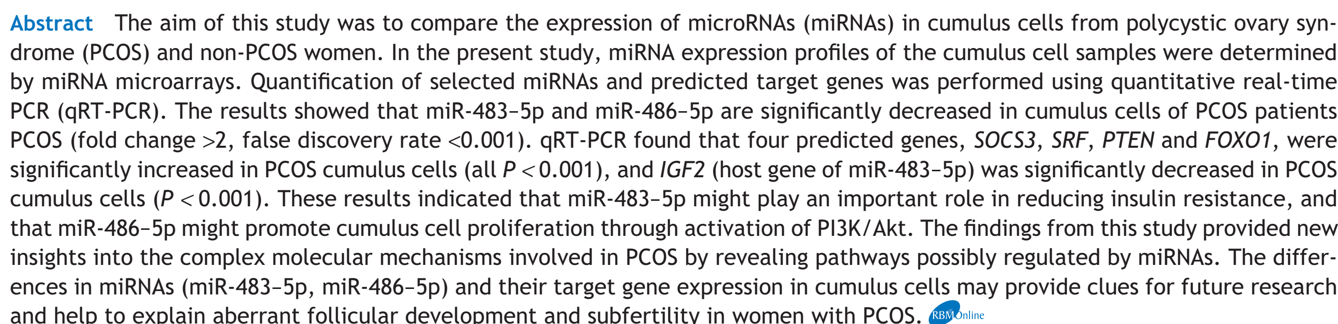

Abstract The aim of this study was to compare the expression of microRNAs (miRNAs) in cumulus cells from polycystic ovary syndrome (PCOS) and non-PCOS women. In the present study, miRNA expression profiles of the cumulus cell samples were determined by miRNA microarrays. Quantification of selected miRNAs and predicted target genes was performed using quantitative real-time PCR (qRT-PCR). The results showed that miR-483–5p and miR-486–5p are significantly decreased in cumulus cells of PCOS patients PCOS (fold change >2, false discovery rate <0.001). qRT-PCR found that four predicted genes, *SOCS3*, *SRF*, *PTEN* and *FOXO1*, were significantly increased in PCOS cumulus cells (all $P < 0.001$), and *IGF2* (host gene of miR-483–5p) was significantly decreased in PCOS cumulus cells ($P < 0.001$). These results indicated that miR-483–5p might play an important role in reducing insulin resistance, and that miR-486–5p might promote cumulus cell proliferation through activation of PI3K/Akt. The findings from this study provided new insights into the complex molecular mechanisms involved in PCOS by revealing pathways possibly regulated by miRNAs. The differences in miRNAs (miR-483–5p, miR-486–5p) and their target gene expression in cumulus cells may provide clues for future research and help to explain aberrant follicular development and subfertility in women with PCOS.

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KEYWORDS: cumulus cell, microRNA, polycystic ovary syndrome

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Introduction

Polycystic ovary syndrome (PCOS) is the most common metabolic dysfunction and endocrine abnormality in women of reproductive age (Franks, 1995; Wood et al., 2007). It is estimated to affect more than 5% of the female population, and is the major cause of menstrual disturbances and anovulatory infertility (Asuncion et al., 2000; Azziz et al., 2004; Ehrmann, 2005; Franks, 1995; Knochenhauer et al., 1998). Although PCOS patients are typically characterized by production of an increased number of oocytes, these oocytes are often of poor quality and the oocyte developmental competence is altered (Dumesic and Abbott, 2008; Ludwig et al., 1999; Mulders et al., 2003; Sahu et al., 2008). Oocyte development and quality depends on the follicular microenvironment and the presence of adequate bidirectional signalling between oocytes and cumulus cells. A bidirectional communication between oocytes and cumulus cells is essential for oocyte and follicular growth and is crucial for fertility (Assou et al., 2010; Eppig, 2001; Ouandaogo et al., 2011, 2012).

MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs that bind to specific mRNAs and repress mRNA translation or promote mRNA degradation (Bartel, 2004). It has been estimated that 30% or more of human mRNAs are regulated by miRNAs (Lewis et al., 2005). Likewise, miRNAs may play an important role in modulating gene expression in the ovary (Fiedler et al., 2008; Ro et al., 2007; Tang et al., 2007; Zhao and Rajkovic, 2008). Studies also indicated that miRNAs are involved in the regulation of steroidogenesis, cell proliferation and apoptosis in human granulosa cells/cumulus cells and may be an underlying aetiology in female infertility (Alford et al., 2007; Jiang et al., 2015; Long et al., 2014; Sirotkin et al., 2009a, 2009b; Sørensen et al., 2014; Toloubeydokhti et al., 2007). However, to date, the miRNA expression profile of cumulus cells from women with PCOS has been investigated rarely. Understanding how miRNAs are regulated in the cumulus cells and the identification of their specific targets and functions may offer novel insights into the aetiology of PCOS and the development of target-specific gene regulation for its treatment.

The aim of this study was to investigate the miRNA expression profile, using miRNA microarray technology, of human cumulus cells isolated from mature metaphase II (MII) oocytes from a homogeneous group (age, body mass index and similar assisted reproductive treatment protocol) of patients with or without PCOS.

Materials and methods

Patients

Patients were scheduled for intracytoplasmic sperm injection (ICSI) at the Assisted Reproduction Centre of Maternal and Child Health Care Hospital of Shaanxi Province (Xi'an, China) from November 2012 to July 2013. The study population included 24 women with PCOS and 24 normal responders without PCOS (non-PCOS, controls). PCOS patients were diagnosed following the Rotterdam 2003 criteria (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004) and on the basis of the presence of oligo- or anovulation and polycystic ovaries (ultrasound imaging). The non-PCOS controls were from women undergoing ICSI only due to male factor and without any other fertility problems. Patients with Cushing's syndrome, congenital adrenal hyperplasia and other systemic diseases were excluded from this study. All patients underwent a routine luteal phase down-regulation protocol with gonadotrophin-releasing hormone (GnRH) agonist (GnRH-a; Decapeptyl, Ipsen, France) and recombinant FSH (Gonal-F Merck Serono, Switzerland or Puregon, NV Organon, The Netherlands) for controlled ovarian hyperstimulation. Each patient gave informed written consent, and the study protocol was approved by the Ethics Review Board of Maternal and Child Health Care Hospital of Shaanxi Province on 18 September 2012.

Cumulus cell isolation

Cumulus–oocyte–complex (COC) retrieval was performed 36 h after human chorionic gonadotrophin (HCG; Profasi Merck Serono, Switzerland) administration by transvaginal ultrasonography-guided needle aspiration. Cumulus cell samples from mature MII oocytes were isolated by mechanical stripping using a syringe needle before ICSI, and were washed in culture medium and stored in liquid nitrogen until use.

RNA extraction

Total RNAs were isolated using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) following the manufacturer's protocol. The RNAs were evaluated for quality and quantity using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA), and the RNA integrity was assessed using agarose gel electrophoresis.

MicroRNA microarrays and data analysis

To avoid possible individual differences, cumulus cell samples were pooled to carry out miRNA microarrays. Each pooled cumulus cell sample comprised three cumulus cell RNA samples from three PCOS or three non-PCOS women. In total, nine PCOS and nine non-PCOS patients' cumulus cell samples were used in the miRNA expression analysis. Six pooled RNAs from cumulus cell samples (three PCOS and three non-PCOS pooled RNAs) were separately analysed on six microarrays. MiRNA expression was surveyed using the GeneChip miRNA 2.0 Array (miRBase V15; Affymetrix, Santa Clara, CA, USA). All procedures were carried out according to the manufacturer's protocol.

Quantitative real-time PCR

The quantitative real-time PCR (qRT-PCR) was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Warrington, UK) using Power SYBR Green PCR Master Mix (Applied Biosystems, Warrington, UK). For miRNA array validation, expression of two target miRNAs (hsa-miR-483–5p and

hsa-miR-486–5p) were analysed relative to the internal human U6 snRNA control in PCOS ($n = 24$) and non-PCOS cumulus cell samples ($n = 24$). For expression of predicted genes, quantification of five target genes (*IGF2*, *SOCS3*, *SRF*, *PTEN* and *FOXO1*) was calculated relative to the internal constant housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) in PCOS ($n = 15$) and non-PCOS cumulus cell samples ($n = 15$). qRT-PCR reactions for each sample were run in triplicate. The data were analysed using the $2^{-\Delta\Delta CT}$ method for relative quantitation (Livak and Schmittgen, 2001).

Bioinformatic analysis

Putative mRNA target genes were predicted by five algorithms: TargetScanHuman (http://www.targetscan.org), DIANA (http://diana.imis.athena-innovation.gr), miRGen (http://diana.cslab.ece.ntua.gr/mirgen/), MicroCosm Targets Version 5 (http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/), and miRror 2.0 (http://www.proto.cs.huji.ac.il/mirror/search.php). Only the targets identified by at least three of these algorithms were analysed further. KEGG pathway and functional category analysis of the predicted target genes were annotated by the functional annotation tool provided by the Database for Annotation, Visualization, and Integrated Discovery (DAVID), version 6.7 (Huang et al., 2009a, 2009b).

Statistical analysis

Data were reported as mean ± SD for quantitative variables. Two-tailed student's *t*-test was used to assess the differences between groups. A *P*-value <0.05 was considered statistically significant, and a difference in miRNA expression of greater than twofold was considered biologically meaningful. Microarray experiments generate large multiplicity problems in which thousands of hypotheses are tested simultaneously. For large-scale testing problems, such as microarray data, where the goal is to provide definitive results, if a study is viewed as exploratory, control of the false discovery rate (FDR) is often preferred (Storey and Tibshirani, 2003). So the results of microarrays were reported as fold change (FC) and FDR, and the statistical analysis of the microarrays was performed by Significant Analysis of Microarray (SAM) (http://www-stat.stanford.edu/~tibs/SAM/). The other statistical analyses were performed by SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical and medical parameters of study participants

The clinical and medical parameters of patients included in the study are summarized in Table 1. The two groups (PCOS and non-PCOS) did not differ with respect to age and body mass index (BMI). The groups did not differ with respect to baseline oestradiol and progesterone, but LH, testosterone and antral follicle count were each significantly greater in the PCOS group compared with non-PCOS controls (all $P < 0.001$, Table 1).

Table 1 Clinical characteristics of patients.

	Non-PCOS (n = 24)	*PCOS (n = 24)*	*P-value*
Age (years)	28.5 ± 3.6	28.3 ± 3.3	NS
BMI (kg/m^2)	20.7 ± 2.1	21.5 ± 2.5	NS
Oestradiol (pg/ml)	40.6 ± 19.5	42.7 ± 22.3	NS
FSH (mIU/ml)	6.9 ± 1.4	5.6 ± 1.2	0.002
LH (mIU/ml)	5.2 ± 2.0	11.1 ± 5.5	<0.001
LH/FSH	0.76 ± 0.29	2.0 ± 1.11	<0.001
Progesterone (ng/ml)	0.57 ± 0.24	0.73 ± 0.51	NS
Testosterone ((ng/dl)	24.7 ± 13.1	46.9 ± 15.6	<0.001
Endometrial thickness (mm)	11.6 ± 2.1	10.7 ± 2.4	NS
Antral follicle count	14.8 ± 4.2	24.3 ± 9.3	<0.001

Values are expressed as mean ± SD.
BMI = body mass index; NS = not statistically significant; PCOS = polycystic ovary syndrome.

miRNA profiling

miRNA profiling of pooled cumulus cell samples from mature oocytes of non-PCOS and PCOS patients ($n = 9$ per group) revealed that 411 (37.2%) miRNAs were expressed in all samples out of the total 1105 *Homo sapiens* miRNAs on the array. Statistical analysis using the SAM software identified four miRNAs (hsa-miR-483–5p, hsa-miR-486–5p, hsa-miR-675 and hsa-miR-513c) to be differentially expressed between PCOS and non-PCOS samples (FC > 2, FDR < 0.001; Table 2).

miRNA validation

Two miRNAs (hsa-miR-483–5p and hsa-miR-486–5p) were chosen for qRT-PCR validation. Compared with non-PCOS cumulus cell samples ($n = 24$), the relative fold changes of hsa-miR-483–5p and hsa-miR-486–5p in PCOS cumulus cell samples ($n = 24$) were –2.62 ± 0.40 and –2.28 ± 0.41, respectively ($P < 0.01$), which was consistent with the observed down-regulation of the two miRNAs in microarray analysis (–2.73 ± 1.43 and –2.36 ± 0.29, respectively; Figure 1).

Target gene analysis

miRNA target gene analysis was performed to demonstrate altered mRNA expression in association with aberrant miRNA profiles in PCOS cumulus cell samples. Predicted target genes were chosen for further investigation based on their association with the PCOS phenotype and tissue specificity. There were seven putative target genes for hsa-miR-483–5p and 42 putative target genes for hsa-miR-486–5p, respectively. Furthermore, research showed that miR-483–5p can be co-expressed together with its host gene, insulin-like growth factor 2 (*IGF2*) (Ma et al., 2011). It implied that *IGF2* gene expression was down-regulated in cumulus cells with PCOS.

Table 2 MicroRNAs with more than 1.5 fold-change identified by microRNA microarray between PCOS and non-PCOS cumulus cell samples.

Systematic name	*Fold change*	*FDR*
hsa-miR-483–5p	−2.56	<0.001
hsa-miR-486–5p	−2.40	<0.001
hsa-miR-675	−2.04	<0.001
v11_hsa-miR-768–5p	−1.88	<0.001
hsa-miR-548a-3p	−1.80	<0.001
hsa-miR-1184	−1.67	<0.001
hsa-miR-210	−1.60	<0.001
hsa-miR-212	−1.59	<0.001
hsa-miR-1909	1.54	0.144
hsa-miR-663b	1.56	0.144
hsa-miR-181a-2-star	1.66	0.144
hsa-miR-509–3p	1.80	0.144
hsa-miR-506	1.83	0.144
hsa-miR-508–5p	1.98	0.144
hsa-miR-513c	2.02	<0.001
hsa-miR-514b-5p	2.07	0.144
hsa-miR-509–5p	2.32	0.144
hsa-miR-513a-5p	2.42	0.144
hsa-miR-510	2.46	0.144

Statistical analysis using the SAM software.
FDR = false discovery rate; PCOS = polycystic ovary syndrome.

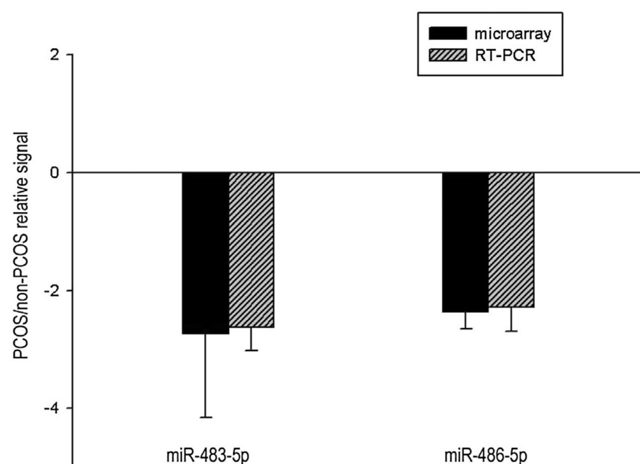

Figure 1 MicroRNA expression validation. Two microRNAs (hsa-miR-483–5p and hsa-miR-486–5p), which were down-regulated in the PCOS cumulus cell group in comparison with the non-PCOS cumulus cell group, were validated by quantitative real-time PCR and in accordance with the miRNA microarray findings.

After target gene prediction, KEGG pathway and functional category analysis was performed on the predicted targets genes using the DAVID tool. After removing specific cancer types (e.g. prostate cancer) pathways, the findings indicated three annotated KEGG pathways for miR-483–5p and three annotated KEGG pathways for miR-486–5p, respectively (**Table 3**). All 49 putative target genes except three genes (*EMP1*, *ITGB5* and *OLFM4*) had been classified into 23 annotated functional categories (**Table 4**).

Determination of predicted target gene expression

Predicted target genes were chosen for further investigation based on their association with the PCOS phenotype. qRT-PCR was performed on PCOS and non-PCOS cumulus cell samples ($n = 15$ per group), generating an mRNA expression profile for the following predicted genes: two predicted target genes of miR-483–5p (*SOCS3* and *SRF*), two predicted target genes of miR-486–5p (*PTEN* and *FOXO1*), and a co-expressed gene of miR-483–5p (*IGF2*). Results showed that four predicted genes, *SOCS3*, *SRF*, *PTEN* and *FOXO1*, had significantly increased expression in the PCOS group compared with non-PCOS controls ($P < 0.001$, **Table 5**). However, *IGF2* had significantly decreased expression in the PCOS group compared with non-PCOS controls ($P < 0.001$, **Table 5**).

Discussion

Beginning with formation of the primordial follicle, oocytes and their surrounding granulosa cells communicate, a dialogue that is essential for fertility (Hawkins and Matzuk, 2010). Granulosa cells have the potential to provide meaningful information regarding their respective oocytes. Therefore, granulosa cells are identified as a valuable area of study of reproductive biology, given the ease of access during assisted reproductive technique procedures (Greenseid et al., 2011). The use of pooled follicular aspirates, which contain a heterogeneous population of granulosa cells and thecal cells from multiple antral follicles, is a recognized limitation of previous studies (Stanek et al., 2007) and get conflicting results (Roth et al., 2014; Sang et al., 2013). Homogeneity of the examined tissue is crucial for a study on differential expression. Cumulus cells are a unique subset of granulose cells that are in direct contact with the oocyte throughout its development and play a major role in the control of oocyte metabolism. It is likely that malfunction of cumulus cells might play an important role in PCOS. In addition, due to the method of separation, the cumulus cells are more homogeneous, with almost no contamination with other cells. Therefore the present investigation focused on cumulus cells isolated from mature MII oocytes from a homogeneous group (age, BMI and similar assisted reproductive technique treatment protocol) of patients with or without PCOS.

Several studies have illustrated that miRNAs are abundant in human ovary (Roth et al., 2014; Sirotkin et al., 2009a; Toloubeydokhti et al., 2007). The current study confirmed this finding, with the detection of 411 miRNAs in the cumulus cells of women undergoing assisted reproductive techniques. Recent evidence suggests that miRNAs play a fundamental role in the outcome of all cellular and tissue activities under both normal and pathologic conditions, but little is known of their role and dysregulation in PCOS. In this study, miRNA microarray technology was used to search for miRNAs with abnormal expression in cumulus cells from PCOS patients compared with non-PCOS subjects. The data revealed only few miRNA to be differentially expressed between PCOS and non-PCOS samples (**Table 2**). Furthermore, qRT-PCR validated that hsa-miR-483–5p and hsa-miR-486–5p are down-regulated in cumulus cells of mature MII oocytes from patients with PCOS.

The miR-483–5p is a conserved sequence encoded in the second intron of the *IGF2* gene (Landgraf et al., 2007). It was

Table 3 KEGG pathway analysis of the over-expressed putative target genes for hsa-miR-483–5p and hsa-miR-486-5p.

miR-483–5p			*miR-486–5p*		
KEGG pathway	*Gene count*	*P-value*	*KEGG pathway*	*Gene count*	*P-value*
Type 2 diabetes mellitus	2	2.7E-2	Focal adhesion	6	2.0E-4
Gap junction	2	5.2E-2	Pathways in cancer	6	1.9E-3
Insulin signalling pathway	2	7.8E-2	p53 signalling pathway	3	1.7E-2

Table 4 Functional category analysis of the over-expressed putative target genes for hsa-miR-483–5p and hsa-miR-486-5p.

Functional category	*Genes*	*Genes count*	*P-value*
Phosphoprotein	*ARHGAP5, BTAF1, CYLD, DOCK3, DYRK1A, FBN1, FOXO1, HAT1, LMTK2, MAPK3, MARK1, MECP2, MTCH1, NCOA6, NDEL1, NEK2, NFE2L1, PGRMC1, PIK3R1, PIM1, PPP2R5D, PTEN, RASSF3, RCOR3, RUSC1, SHB, SIRT1 SOCS3, SPAST, SRF, ST5, STK4, TOB1*	33	4.6E-5
Nucleus	*BTAF1, DYRK1A, FOXO1, HAT1, MECP2, MXI1, NCOA6, NEK2, NFE2L1, NR2F1, PARP2, PDGFC, PIM1, PLAGL2, PPP2R5D, RCOR3, RUSC1, RXRB, SIAH1, SIRT1, SPAST, SRF, STK4*	23	2.5E-4
Serine/threonine-protein kinase	*DYRK1A, LMTK2, MAPK3, MARK1, NEK2, PIM1, STK4,*	7	3.5E-4
Cytoplasm	*ARHGAP5, CYLD, DOCK3, FOXO1, HAT1, MARK1, NDEL1, NEK2, PDGFC, PIM1, PPP2R5D, PTEN, RASSF3, RUSC1, SHB, SIAH1, SPAST,STK4*	18	2.0E-3
Apoptosis	*MTCH1, PTEN, SHB, SIAH1, SIRT1, STK4*	6	2.5E-3
DNA-binding	*BTAF1, FOXO1, MECP2, MXI1, NFE2L1, PARP2, PLAGL2, RXRB, SRF*	9	5.0E-3
Cell cycle	*CYLD, MAPK3, NEK2, PIM1, SIAH1, SPAST*	6	5.7E-3
Transferase	*CRLS1, DYRK1A, HAT1, LMTK2, MAPK3, MARK1, NEK2, PARP2, PIM1, STK4*	10	6.9E-3
Kinase	*DYRK1A, LMTK2, MAPK3, MARK1, NEK2, PIM1, STK4*	7	7.0E-3
ATP	*DYRK1A, MAPK3, NEK2, PIM1*	4	2.1E-2
Nucleotide-binding	*BTAF1, CLCN3, DYRK1A, LMTK2, MAPK3, MARK1, NEK2, PIM1, SPAST, STK4*	10	2.2E-2
Transcription regulation	*FOXO1, MECP2, MXI1, NCOA6, NFE2L1, NR2F1, PLAGL2, RCOR3, RXRB, SIRT1, SRF*	11	2.6E-2
Differentiation	*NDEL1, SHB, SIAH1, SIRT1, SPAST*	5	2.7E-2
Transcription	*FOXO1, MECP2, MXI1, NCOA6, NFE2L1, NR2F1, PLAGL2, RCOR3, RXRB, SIRT1, SRF*	11	3.0E-2
SH2 domain	*PIK3R1, SHB, SOCS3,*	3	3.1E-2
ATP-binding	*BTAF1, FOXO1, MECP2, MXI1, NFE2L1, NR2F1, PARP2, PLAGL2, RXRB, SRF*	10	3.9E-2
Activator	*NCOA6, NFE2L1, NR2F1, PLAGL2, SRF*	5	4.0E-2
Growth factor	*IGF1, PDGFC, PDGFD,*	3	4.2E-2
Developmental protein	*NDEL1, SHB, SIAH1, SIRT1, SPAST, SRF*	6	4.4E-2
NAD	*CYB5R4, PARP2, SIRT1*	3	8.1E-2
Phosphotransferase	*MAPK3, PIK3R1, PIM1,*	3	9.0E-2
Mitogen	*PDGFC, PDGFD,*	2	9.3E-2
Sh3 domain	*DOCK3,PIK3R1, RUSC1*	3	9.7E-2

previously revealed that miR-483–5p can be co-expressed together with its host gene. Therefore miR-483–5p plays a biological role as a functional partner of *IGF2* and was identified as an oncogenic factor in cancer cells (Ma et al., 2011, 2012). However, Wang *et al.* reported that the over-expression of miR-483–5p suppressed glioma cell proliferation and induced a G0/G1 arrest, suggesting that miR-483–5p can serves as a tumour suppressor (Wang et al., 2012). It also reported that miR-483–5p might be involved in fat metabolism (Yankun et al., 2008; Zhang et al., 2009), and regulated the progesterone concentration in a steroidogenic human granulosa-like tumour cell line (Sang et al., 2013).

The miR-486–5p is generated by processing intronic RNA from the *ANK1* gene, and may function as cell-specific tumour suppressor or oncogene (Narducci et al., 2011; Oh et al., 2011; Peng et al., 2013). miR-486–5p could enhance PI3K/Akt signalling by repressing expression of *PTEN* and *DOCK3* (Alexander et al., 2014; Small et al., 2010). However, it also could reduce

Table 5 Predicted target genes expression between PCOS and non-PCOS cumulus cell samples ($n = 15$).

Target genes	*Fold change*	P-*value*
SOCS3	1.63 ± 0.29	<0.001
SRF	1.51 ± 0.22	<0.001
PTEN	1.67 ± 0.21	<0.001
FOXO1	1.39 ± 0.25	<0.001
IGF2	−2.94 ± 0.53	<0.001

Values are expressed as mean ± SD.

PI3K/Akt signalling by repressing expression of *IGF1* and *PIK3R1* (Peng et al., 2013). Despite the above observations, the functions of miR-483–5p and miR-486–5p in cumulus cells remain unknown.

Computational predictions of miRNA target genes may help to analyse the function of miRNA and provide a global view of the gene regulation network by miRNAs. In the present study, KEGG-pathway enrichment analysis indicated that miR-483–5p was significantly enriched in the type 2 diabetes mellitus pathway, whereas miR-486–5p was significantly enriched in focal adhesion, pathways in cancer and the p53 signalling pathway (**Table 3**). These findings revealed a possible role of miR-483–5p in the regulation of insulin resistance, and a possible role of miR-486–5p in the regulation of cell proliferation in cumulus cells.

PCOS and insulin resistance are interlinked, as approximately 40% of women with PCOS have been found to be insulin resistant (Sawathiparnich et al., 2005; Vigouroux, 2010; Wijeyaratne et al., 2002), and the risk for impaired glucose tolerance and type 2 diabetes is increased in PCOS (Moran et al., 2010). In addition, insulin resistance is a common feature in both PCOS and non-insulin-dependent (type 1) diabetes mellitus. However, persistent reproductive disturbances were limited to the PCOS, suggesting that insulin resistance in the ovary itself may be responsible for this susceptibility (Wu et al., 2003). In support of this view, gene expression analysis in the present study found that four predicted target genes of miR-483–5p and miR-485–5p, *SOCS3*, *SRF*, *FOXO1* and *PTEN*, are over-expressed in PCOS cumulus cells, and all of them are associated with insulin resistance and glucose intolerance (Dey et al., 2000; Jin et al., 2011; Kousteni, 2012; Lo et al., 2004; Wijesekara et al., 2005). This supported the possible role of miR-483–5p and miRNA-486–5p in the negative regulation of insulin resistance.

Previous studies have shown that PI3K activity is significantly decreased in PCOS (Corbould et al., 2005; Dunaif et al., 2001). PTEN, a negative modulator of the PI3K/Akt signalling pathway (Goto et al., 2009; Myers et al., 1998), have been shown to be over-expressed in PCOS granulosa cells (Iwase et al., 2009), and the loss of *Pten* in granulosa cells promotes follicle growth and enhances fertility (Fan et al., 2008). In the present study, the over-expression of *PTEN* and low expression of miR-483–5p in PCOS cumulus cells suggested that miR-486–5p can be a positive regulator of PI3K/Akt signalling by repressing expression of *PTEN*, and miR-486–5p deficiency-induced over-expression of *PTEN* in cumulus cells may be a pathogenic factor for PCOS.

It is reported that follicular fluid *IGF2* concentrations decrease in women with PCOS, compared with normally ovulating women (Artini et al., 2007); *IGF2* expression was significantly down-regulated in both cumulus cells and mural granulosa cells in women with diminished ovarian reserve (Greenseid et al., 2011); Furthermore, high *IGF2* concentrations in follicular fluid at the time of oocyte retrieval suggested better oocyte maturation (Wang et al., 2006). These observations implied that *IGF2* is positively correlated with follicular development. In the present study, as an miR-483–5p co-expressed gene (Ma et al., 2011), *IGF2* gene expression was significantly down-regulated in cumulus cells with PCOS, which may be also one of pathogenic factors for PCOS.

In this study, the expression profile of miRNAs was investigated and it was observed that miR-483–5p and miR-486–5p were significantly down-regulated in the cumulus cells of patients with PCOS. Although the expression of predicted target genes and their regulation by these miRNAs need to be further clarified by more experiments, the findings from this study provide new insights into the complex molecular mechanisms involved in PCOS by revealing pathways that are possibly regulated by miRNAs. The differences in miRNAs (miR-483–5p, miR-486–5p) and their target gene expression in cumulus cells may provide clues for future research and help to explain the aberrant follicular development and subfertility seen in women with PCOS.

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