Decreased miR-143 and increased miR-21 placental expression levels are associated with macrosomia

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Received May 5, 2015; Accepted January 8, 2016

DOI: 10.3892/mmr.2016.4892

Abstract. Macrosomia, a birth weight ≥4,000 g, is associated with maternal and infant health problems. The dysregulation of microRNAs (miRNAs) in the placenta is associated with adverse birth outcomes, yet whether aberrantly expressed placental miRNAs are associated with macrosomia remains unknown. The aim of the current study was to characterize the expression of three placental miRNAs (miR-6, -21 and -143) and evaluate their association with macrosomia. The miRNA expression in placental tissues from 67 macromesic pregnancies and 64 normal pregnancies were analyzed using reverse transcription-quantitative polymerase chain reaction. The expression of miR-21 was observed to be elevated in macromesic placenta compared with control samples, while miR-143 expression was significantly lower than in control placenta (P<0.05). No significant differences were identified in the miR-16 expression levels between the groups (P=0.955). Following division of miRNA expression levels by quartile, logistic regression models demonstrated that the odds of macrosomia increased with miR-21 expression quartile: Q2, odds ratio (OR)=6.67 [95% confidence interval (CI), 1.39-32.05]; Q3, OR=4.10 (95% CI, 0.88-19.11); Q4, OR=16.19 (95% CI, 2.46-106.68). Conversely, higher levels of miR-143 expression were protective against macrosomia: Q2, OR=0.22 (95% CI, 0.049-0.98); Q3, OR=0.11 (95% CI, 0.024-0.55), and Q4, OR=0.16 (95% CI, 0.032-0.79). Thus, statistical analysis demonstrated that high levels of miR-21 expression and low levels of miR-143 expression predict the risk for macrosomia, indicating an interaction between the two miRNAs. Bioinformatic analysis suggested that they are likely to function in the mitogen-activated protein kinases signaling pathway to influence the risk of macrosomia. The results of the present study provide evidence that placental miR-21 and -143 are important in the formation of macrosomia.

Introduction

Fetal macrosomia has been inconsistently defined as a birth weight of >4,000, 4,500 or 5,000 g, regardless of gestational age (1,2). In China, the most widely used definition of macrosomia is a birth weight ≥4,000 g. Macrosomia can lead to adverse birth outcomes for the newborn, including perinatal asphyxia, neonatal mortality and shoulder dystocia (1,3). In addition, mothers delivering macromesic fetuses are at an increased risk of experiencing prolonged labor, C-section, abnormal hemorrhage and perineal laceration (4,5). Numerous studies have indicated that high birth weight is associated with long-term health risks for the newborn, including an increased risk of obesity, diabetes and certain types of cancer (6-9). In the past two to three decades, the incidence of macrosomia in developed countries has increased by 15-25% (2). In China, the macrosomia occurrence rate increased from 6.5% in 2006 (10) to 7.3% in 2011 (11). However, the mechanisms responsible for fetal macrosomia remains poorly understood.

A large body of research has indicated that epigenetic alterations in placental tissues are associated with adverse pregnancy outcomes and fetal programming (12-14). MicroRNAs (miRNAs) are short noncoding RNA molecules (~22 nucleotides in length) and are important in post-transcriptional gene regulation through their binding with hundreds of different mRNAs (15). A wide range of biological activities are affected by miRNAs, including cellular proliferation, differentiation, apoptosis and the processes of numerous diseases, including cancer, obesity, insulin resistance and diabetes (16,17). Previous investigations have demonstrated that aberrant expression of a number of miRNAs in the placenta was associated with low birth weight and preeclampsia (18,19). Maccani et al (20) reported that decreased levels of placental miR-16 and -21 were associated with poor fetal growth. In addition, miR-143 was implicated as a crucial regulator of adipogenesis (21). Since evidence suggested that miR-16, -21 and -143 are important in...
fetal development, the current study sought to further characterize the association of these miRNAs with macrosomia.

The present study examined the expression of miRs-16, -21 and -143 in the placenta of macromomoc and normal pregnancies and demonstrated miRNA dysregulation in macromomoc placenta.

Materials and methods

Study population. Subjects were recruited at Yuying Children's Hospital of Wenzhou Medical University (Wenzhou, China). Macrosomia was defined as a neonate with a birth weight ≥4,000 g. Normal birth weight was defined as 2,500-3,999 g. Samples from macrosomia cases and controls were collected from women between the ages of 18 and 42 years with healthy pregnancies (i.e. pregnancies without hypertension, hepatitis, heart disease, psychological disorders, gestational diabetes or impaired glucose tolerance), whose infants were full-term (≥37 weeks) and viable, with no known genetic disorders. Maternal weight gain during pregnancy was divided into three levels, including low, moderate and high, according to recommendations from the Institute of Medicine (22). A total of 67 macromomoc and 64 control pregnancies were selected. All subjects provided written informed consent to participate in the current study, and the research protocol was approved by the Ethics Committee of Wenzhou Medical University.

Placenta collection. A placental biopsy of ~1 g was excised from the maternal side of each placenta, 2 cm from the umbilical cord insertion site and free of maternal decidua, within 15 min of delivery of the placenta. Biopsies were cut into small sections and immediately placed in RNAlater (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Samples were incubated at 4ºC overnight and stored at -80ºC until RNA extraction was performed.

miRNA extraction and quantitative detection. Total RNA was extracted from placental tissue using a MicroRNA Isolation kit (BioTeck China, Beijing, China), according to the manufacturer's instructions. Extracted RNA was quantified by measuring absorbance at 260 and 280 nm using the Nano-200 Micro-Spectrophotometer (Allsheng Instruments, Co., Ltd., Hangzhou, China). Reverse transcription (RT) of miRNA (200 ng) was performed using the ReverTra Ace® qPCR RT kit (Toyobo Co., Ltd., Osaka, Japan) with Bulge-Loop® stem-loop RT-primers (Toyobo Co., Osaka, Japan) and U6 small nuclear (sn) RNA (Human Control U6 snRNA (MQP-0202) stem-loop RT-primers (Guangzhou RiboBio Co., Ltd., Guangzhou, China), according to the manufacturer's protocol. U6 snRNA was used as an internal control. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assay was performed using Thunderbird SYBR qPCR Mix (Toyobo Co., Ltd.) and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA), in accordance with the manufacturer's instructions. Cycling conditions were as follows: 95ºC for 3 min, followed by 30 cycles of 95ºC for 10 sec, 60ºC for 20 sec, and 70ºC for 10 sec. To check PCR product homogeneity, the temperature was elevated from 65 to 99ºC in 0.1ºC/sec increments to obtain a melting curve. miRNA expression levels were calculated using the 2⁻ΔΔCt method (23).

Bioinformatic prediction of miRNA targets and pathway analysis. miRTarBase (miRTarbase.mbc.nctu.tw, release 4.5) was used to identify target genes (24). DAVID (Database for Annotation, Visualization and Integrated Discovery; david. abcc.ncifcrf.gov) was used to identify pathways involving these target genes (25).

Statistical analysis. Basic characteristics of the macrosomia and control groups were compared using unpaired t-tests for continuous variables and reported as the mean ± standard deviation. Categorical variables were compared using χ² tests. The miRNA expression data were not normally distributed, therefore, relative expression was presented as the median (interquartile range) and the Mann-Whitney U test was used to analyze differences in miRNA expression levels between the macrosomia and control groups. miRNA expression levels were divided by quartile, using the lowest quartile as the reference group. Logistic regression models were used to determine the risk factors of giving birth to a newborn with macrosomia. Spearman's correlation coefficients were used to examine the relationship between miR-21 and -143 expression. All statistical analyses were conducted using SPSS software, version 14.0 (SPSS, Inc., Chicago, IL, USA). All P-values reported were two-tailed, and P<0.05 was considered to indicate a statistically significant difference.

Results

Subject characteristics. Demographic data are presented in Table I. A total of 131 mothers and their neonates were recruited for the current study. The average birth weight of neonates with macrosomia was 4,303.4±219.5 g, while the average weight of control neonates was 3,398.0±362.2 g. Maternal weight gain during pregnancy was significantly higher in mothers with macrosomia neonates compared with the control group (P<0.001), and neonates with macrosomia were more likely to be male (P=0.004). There were no significant differences in gestational age, maternal weight prior to pregnancy, maternal body mass index (BMI) prior to pregnancy, or maternal age between the macrosomia and control groups.

miRNA expression in macromomoc and control placenta. To determine whether miR-16, -21 or -143 were differentially expressed in macromomoc and control placenta, their expression was measured using RT-qPCR. The Mann-Whitney U-test demonstrated that miR-143 was expressed at lower levels in macromomoc placenta (Fig. 1A; P=0.003), whereas miR-21 expression was significantly higher in macromomoc placenta (Fig. 1B; P=0.043) compared with the control samples. However, no significant difference in miR-16 expression levels was detected between the two groups (Fig. 1C; P=0.955).

Association of miRNAs with macrosomia. The present study examined whether increased miR-21 expression and decreased miR-143 expression were associated with the likelihood of macrosomia using logistic regression models, and adjusted for potential confounders, including maternal age, weight prior to pregnancy, weight gain during pregnancy, gestational age, infant gender and delivery method. miRNA expression levels were divided by quartile (Table II). As presented in Table II,
the likelihood of macrosomia increased with miR-21 expression quartile as follows: Q2, OR=6.67 (95% CI, 1.39-32.05); Q3, OR=4.10 (95% CI, 0.88-19.11); and Q4, OR=16.19 (95% CI, 2.46-106.68). Conversely, higher miR-143 quartiles were associated with protection against macrosomia as follows: Q2, OR=0.22 (95% CI, 0.049-0.98); Q3, OR=0.11 (95% CI, 0.024-0.55); and Q4 OR=0.16 (95% CI, 0.032-0.79). High maternal weight gain during pregnancy and male gender were also demonstrated to be risk factors for macrosomia.

**Interaction between miR-21 and -143.** Low miR-143 and high miR-21 expression levels were associated with an increased risk of developing macrosomia. Therefore, the current study analyzed the interaction between the expression of the two miRNAs and macrosomia risk (Table III). Logistic regression analysis demonstrated no significant difference in the risk of developing macrosomia among samples with high miR-143 and low miR-21, high miR-143 and high miR-21 or low miR-143 and low miR-21 levels. However, low placental miR-143 combined with high miR-21 levels were associated with increased likelihood of macrosomia (OR=26.47, 95% CI, 2.91-240.79). This suggested an interaction between miR-21 and -143 expression in macrosomia risk.

**Correlation between miR-143 and miR-21 expression.** As low miR-143 and high miR-21 levels were demonstrated to be associated with macrosomia risk, the current study analyzed whether miR-143 and -21 levels were inversely correlated in macrosome placenta. However, the microRNAs were positively correlated in macrosomic placenta (Spearman $r_s=0.43$; $P<0.001$; Fig. 2A) Levels of miR-143 and miR-21 were not correlated in the control group ($P>0.05$; Fig. 2B).

**Pathway analysis.** To understand the potential mechanisms by which miR-143 and -21 function in shaping macrosomia risk, the present study identified their potential target genes using miRTarBase, a database that provides experimentally validated miRNA-target interactions. The database suggested

### Table I. Characteristics of the study sample.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n=64)</th>
<th>Macrosomia cases (n=67)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight, g</td>
<td>3,398.0±362.2</td>
<td>4,303.4±219.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>39.1±1.0</td>
<td>39.4±1.0</td>
<td>0.094</td>
</tr>
<tr>
<td>Maternal weight prior to pregnancy, kg</td>
<td>52.3±7.0</td>
<td>54.5±7.2</td>
<td>0.110</td>
</tr>
<tr>
<td>Height, cm</td>
<td>159.9±3.6</td>
<td>160.5±4.5</td>
<td>0.445</td>
</tr>
<tr>
<td>Maternal BMI prior to pregnancy, kg/m²</td>
<td>20.5±2.7</td>
<td>21.2±2.8</td>
<td>0.195</td>
</tr>
<tr>
<td>Maternal weight gain during pregnancy, kg</td>
<td>15.4±5.7</td>
<td>20.1±5.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal age, years</td>
<td>27.8±4.4</td>
<td>29.0±3.8</td>
<td>0.097</td>
</tr>
<tr>
<td>Tobacco during pregnancy, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>64 (100)</td>
<td>67 (100)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Infant gender, n (%)</td>
<td></td>
<td></td>
<td>0.004</td>
</tr>
<tr>
<td>Male</td>
<td>30 (46.9)</td>
<td>48 (71.6)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>34 (53.1)</td>
<td>19 (28.4)</td>
<td></td>
</tr>
<tr>
<td>Delivery method, n (%)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vaginal</td>
<td>55 (85.9)</td>
<td>32 (47.8)</td>
<td></td>
</tr>
<tr>
<td>C-section</td>
<td>9 (14.1)</td>
<td>35 (52.2)</td>
<td></td>
</tr>
</tbody>
</table>

P-values were obtained using unpaired t-tests for continuous variables and $\chi^2$ tests for categorical variables. BMI, body mass index.

Figure 1. Relative expression of (A) miR-143, (B) miR-21 and (C) miR-16 in placenta. miRNA expression was measured using reverse transcription-quantitative polymerase chain reaction in placenta from macrosomic (n=67) and control (n=64) pregnancies. U6 RNA was used as an internal control. Data are presented as Tukey Box plots. *$P<0.05$ and **$P<0.01$, comparison shown by brackets.
489 target genes for miR-21 and 20 for miR-143 (Table IV). Potential signaling pathways associated with the target genes, generated by DAVID, are presented in Fig. 3. Identified target genes for miR-21 and -143 included those associated with the mitogen-activated protein kinase (MAPK) signaling pathway, an important pathway in cellular proliferation, growth and apoptosis.

**Discussion**

As the mediator between mother and fetus, the placenta secretes various hormones, delivers nutrients to the fetus, facilitates gas exchange and serves as a barrier to protect the fetus from adverse environmental conditions, thus, is crucial for fetal development (26). Previous studies have demonstrated that aberrant miRNA expression in the placenta was associated with preeclampsia (19), intrauterine growth retardation and large birth size (18). The results of the current study indicated that dysregulation of miR-21 and -143 expression was associated with macrosomia, and that there was an association between the two miRNAs with regards to risk of macrosomia. Collectively, the evidence suggested that aberrant placental miRNA expression serves a prominent role in abnormal fetal development.

miR-21 is established as an oncogene that is important during tumor progression (27). In previous studies, the upregulation of miR-21 in esophageal squamous cell carcinoma induced cell proliferation and invasion (28), and anti-miR-21 inhibited cell growth in breast tumor tissue (29). In addition, aberrant expression of miR-21 has been demonstrated

<p>| Table II. Results of logistic regression models investigating the association between individual miRNA expression and macrosomia* |
|---------------------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Effect</th>
<th>n (%)</th>
<th>$\chi^2$</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A, miR-21</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-21 relative expression quartiles</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Q1, ≤0.67</td>
<td>28 (25)</td>
<td>1.00</td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td>Q2, 0.68-1.10</td>
<td>27 (24)</td>
<td>5.61</td>
<td>6.67 (1.39-32.05)</td>
<td>0.018</td>
</tr>
<tr>
<td>Q3, 1.11-1.95</td>
<td>33 (30)</td>
<td>3.22</td>
<td>4.10 (0.88-19.11)</td>
<td>0.073</td>
</tr>
<tr>
<td>Q4, &gt;1.95</td>
<td>23 (21)</td>
<td>8.38</td>
<td>16.19 (2.46-106.68)</td>
<td>0.004</td>
</tr>
<tr>
<td>Maternal weight gain during pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>16 (16)</td>
<td>1.00</td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td>Moderate</td>
<td>34 (34)</td>
<td>5.87</td>
<td>3.20 (0.481-21.25)</td>
<td>0.229</td>
</tr>
<tr>
<td>High</td>
<td>55 (50)</td>
<td>3.48</td>
<td>9.78 (1.55-61.90)</td>
<td>0.015</td>
</tr>
<tr>
<td>Infant gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>46 (41)</td>
<td>1.00</td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Male</td>
<td>65 (59)</td>
<td>9.29</td>
<td>6.36 (1.94-20.90)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>B, miR-143</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>miR-143 relative expression quartiles</td>
<td></td>
<td></td>
<td></td>
<td>0.042</td>
</tr>
<tr>
<td>Q1, ≤0.52</td>
<td>27 (24)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q2, 0.53-0.90</td>
<td>29 (26)</td>
<td>3.92</td>
<td>0.22 (0.049-0.98)</td>
<td>0.048</td>
</tr>
<tr>
<td>Q3, 0.91-1.35</td>
<td>29 (26)</td>
<td>7.33</td>
<td>0.11 (0.024-0.55)</td>
<td>0.007</td>
</tr>
<tr>
<td>Q4, &gt;1.35</td>
<td>27 (24)</td>
<td>5.08</td>
<td>0.16 (0.032-0.79)</td>
<td>0.024</td>
</tr>
<tr>
<td>Maternal weight gain during pregnancy</td>
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</tr>
<tr>
<td>Low</td>
<td>18 (16)</td>
<td>1.00</td>
<td></td>
<td>0.010</td>
</tr>
<tr>
<td>Moderate</td>
<td>38 (34)</td>
<td>6.60</td>
<td>3.76 (0.488-28.95)</td>
<td>0.204</td>
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<tr>
<td>High</td>
<td>56 (50)</td>
<td>5.07</td>
<td>14.43 (1.88-110.49)</td>
<td>0.010</td>
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<tr>
<td>Infant gender</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>46 (41)</td>
<td>1.00</td>
<td></td>
<td>0.015</td>
</tr>
<tr>
<td>Male</td>
<td>66 (59)</td>
<td>5.91</td>
<td>4.05 (1.31-12.52)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*Models adjusted for maternal weight prior to pregnancy, gestational age, delivery method and maternal age. Samples lacking values for one or more covariates were excluded from the model. *Maternal weight gain during pregnancy was divided according to Institute of Medicine recommendations (22). OR, odds ratio; CI, confidence interval.
to affect apoptosis and metastasis in glioma and colorectal cancer cells (30,31). Placental and cancer cells share numerous similar features, including proliferative, invasive and migratory capacity, and shared epigenetic mechanisms for regulating trophoblast invasion (32,33). Thus, the present study hypothesized that miR-21 may affect placental function by influencing cellular processes such as proliferation, thereby affecting fetal development. The data demonstrated that placental miR-21 expression was upregulated in macrosomia cases, which is consistent with a previous investigation (34). Maccani et al (20) reported that low placental miR-21 expression was a risk factor for small for gestational age (SGA).

### Table III. Interaction between miR-143 and miR-21 expression and macrosomiaa.

<table>
<thead>
<tr>
<th>Effect</th>
<th>n (%)</th>
<th>$\chi^2$</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-143</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>High</td>
<td>54 (48.6)</td>
<td>7.74</td>
<td>5.87 (1.69-20.44)</td>
<td>0.005</td>
</tr>
<tr>
<td>Low</td>
<td>57 (51.4)</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>miR-21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>57 (51.4)</td>
<td>5.64</td>
<td>4.59 (1.31-16.15)</td>
<td>0.018</td>
</tr>
<tr>
<td>High</td>
<td>54 (48.6)</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>miR-143 and miR-21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High miR-143, low miR-21</td>
<td>17 (15.3)</td>
<td></td>
<td>1.00</td>
<td>0.032a</td>
</tr>
<tr>
<td>High miR-143, high miR-21</td>
<td>37 (33.4)</td>
<td>1.92</td>
<td>3.57 (0.59-21.63)</td>
<td>0.166</td>
</tr>
<tr>
<td>Low miR-143, low miR-21</td>
<td>40 (36.0)</td>
<td>2.65</td>
<td>4.53 (0.74-27.95)</td>
<td>0.103</td>
</tr>
<tr>
<td>Low miR-143, high miR-21</td>
<td>17 (15.3)</td>
<td>8.46</td>
<td>26.47(2.91-240.79)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

aModels adjusted for maternal weight prior to pregnancy, maternal weight gain during pregnancy, gestational age, delivery method, infant gender, and maternal age. Samples lacking one or more covariate values were excluded from the model. Expression levels were cut-off by median. bP-value for trend. OR, odds ratio; CI, confidence interval.

Figure 2. (A) Positive correlation between miR-21 and miR-143 expression in placenta from macrosomic pregnancies (P<0.0001). (B) No correlation was observed in the control group (P>0.05).

Figure 3. Possible pathways regulated by (A) miR-21 and (B) miR-143. The number of enriched genes for each pathway is indicated in parentheses on the vertical axis. The horizontal axis denotes enrichment score $\log_{10}(P\text{-value})$. For each pathway shown, $P<0.05$. TGF-β, transforming growth factor-β; Jak-STAT, Janus kinase-signal transducer and activator of transcription; MAPK, mitogen-activated protein kinase; GnRH, gonadotropin releasing hormone; VEGF, vascular endothelial growth factor.
Similarly, a study in mice demonstrated that miR-21 inhibition reduced obesity through the reduction of adipocyte size (35). Other reports have indicated that miR-21 regulated adipogenesis in human primary adipocytes and that its expression was positively correlated with BMI (36). miR-21 was also reported to regulate adipogenic differentiation and adipose proliferation (37,38). Therefore, the present study hypothesized that the upregulation of placental miR-21 leads to macrosomia by promoting cell differentiation and proliferation. However, further experimental evidence is required to evaluate this.

To date, the role of miR-143 in the placenta has remained unknown. To the best of our knowledge, the current study was the first to demonstrate that placental miR-143 was downregulated in macrosomia. Among the miRNAs associated with human obesity, miR-143 was the first reported to regulate adipogenic differentiation and adipose proliferation (37,38). Therefore, the present study hypothesized that the upregulation of placental miR-21 leads to macrosomia by promoting cell differentiation and proliferation. However, further experimental evidence is required to evaluate this.

According to DAVID analysis (Fig. 3), miR-21 and -143 were associated with MAPK signaling, which is a pivotal pathway in apoptosis. For example, overexpression of miR-143 repressed cell proliferation in melanoma and gastric cancer by targeting syndecan-1 (41) and cyclooxygenase-2 (42), which are also expressed in the placenta (43,44). This may partially explain the downregulation of miR-143 in macrosomic placenta demonstrated in the current study. Consistent with the present results, another investigation demonstrated that miR-143 was downregulated in maternal plasma in macrosomic pregnancies (45). However, an association between miR-143 in maternal plasma and placenta remains unclear.

Another notable observation of the current study was the opposing changes to the miR-21 and -143 levels in association with macrosomia. Similarly, Keller et al (36) reported that the expression of miR-21 was positively correlated with BMI. Expression of miR-21 was higher in the adipocyte tissue of adults with BMI>30 than in that of adults with lower BMIs, whereas miR-143 expression was reduced in adults with lower BMIs. The same trend was also been demonstrated in colorectal carcinoma and colorectal liver metastasis tissues, which exhibit overexpression of miR-21 and undereexpression of miR-143 relative to control tissues (46). These results suggest a functional correlation between miR-21 and -143 expression, as does the relationship identified by the logistic regression analysis in the current study.

According to DAVID analysis (Fig. 3), miR-21 and -143 were associated with MAPK signaling, which is a pivotal pathway in

### Table IV. Representative target genes of miR-21 and miR-143.

<table>
<thead>
<tr>
<th>miR-21 target gene</th>
<th>miR-143 target gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene ID</td>
<td>Official full name</td>
</tr>
<tr>
<td>PLPP1</td>
<td>Phospholipid phosphatase 1</td>
</tr>
<tr>
<td>PLD2</td>
<td>Phospholipase D2</td>
</tr>
<tr>
<td>BCAT1</td>
<td>Branched chain amino-acid transaminase 1</td>
</tr>
<tr>
<td>LARS</td>
<td>Leucyl-tRNA synthetase</td>
</tr>
<tr>
<td>PDHA2</td>
<td>Pyruvate dehydrogenase (lipoamide) α2</td>
</tr>
<tr>
<td>TGFBI</td>
<td>Transforming growth factor β1</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Transforming growth factor β receptor II</td>
</tr>
<tr>
<td>SP1</td>
<td>Sp1 transcription factor</td>
</tr>
<tr>
<td>MYC</td>
<td>v-myc avian myelocytomatosis viral oncogene homolog</td>
</tr>
<tr>
<td>FAS</td>
<td>Fas cell surface death receptor</td>
</tr>
<tr>
<td>IL1B</td>
<td>Interleukin 1β</td>
</tr>
<tr>
<td>BCL2</td>
<td>B-cell CLL/lymphoma 2</td>
</tr>
<tr>
<td>STAT3</td>
<td>Signal transducer and activator of transcription 3</td>
</tr>
<tr>
<td>PIAS3</td>
<td>Protein inhibitor of activated STAT3</td>
</tr>
<tr>
<td>AKT2</td>
<td>v-akt murine thymoma viral oncogene homolog 2</td>
</tr>
<tr>
<td>PTEN</td>
<td>Phosphatase and tensin homolog</td>
</tr>
<tr>
<td>CDK6</td>
<td>Cyclin-dependent kinase 6</td>
</tr>
<tr>
<td>MAPK3</td>
<td>Mitogen-activated protein kinase 3</td>
</tr>
<tr>
<td>WNT5A</td>
<td>Wingless-type MMTV integration site family member 5A</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal growth factor receptor</td>
</tr>
</tbody>
</table>
cell proliferation and differentiation. miR-143 has been previ-
ously reported to target extracellular signal-regulated kinase 5
(ERK5), a member of the MAPK family that promotes cell
proliferation and growth. Overexpression of miR-143 reduced
ERK5 expression and activation, thus inhibiting cell prolifera-
tion and growth in human colon carcinoma (47). By contrast,
mir-21 promoted proliferation of Eca109 cells and inhibited
apoptosis by activating the ERK1/2/MAPK pathway (48).
Together, these results predict an inverse correlation between
levels of miR-143 and -21. Unexpectedly, the current study
demonstrated a positive correlation between the two miRNAs
in macromomia but not in control placenta, suggesting that a
more complex mechanism co-regulates mir-21 and -143. One
such plausible mechanism is proposed as follows: A restric-
tive correlation between mir-21 and -143 in the regulation of
fetal weight. Essentially, the two miRNAs independently and
inversely regulate growth in fetuses of normal weight, but in
macromomia the two have a mutually restraining relationship,
that is, with the risk factor mir-21 increased, the protective
factor miR-143 was also increased, to protect the fetus from
overgrowth. This may be a homeostatic mechanism to maintain
normal growth in the fetus.

Previous studies have demonstrated that miR-16
targets genes in the pathways regulating numerous cellular
processes (49,50). Low levels of placental miR-16 expression
are associated with SGA (20). However, the current study
did not identify significant differences in placental miR-16
expression between macromomia and control placent.
In addition, the present study demonstrated that male infants were
at a greater risk of developing macromomia than females. This
result is consistent with a number previous reports (11,51). One
result is that male embryos have a higher
rate of cell division and metabolism than female embryos (52).

It is noted that this was a hospital-based case-control
study. All subjects were recruited from a large, comprehensive
hospital that serves all people in the Wenzhou region. Thus,
our data are likely to be representative of the population in the
Wenzhou region. Regardless, due to the small sample size, the
confidence intervals were relatively large.

In summary, the present study demonstrated that low
miR-143 expression and high miR-21 expression are risk
factors for macromomia. Furthermore, there is an association
between the two miRNAs and macromomia. These results
provide evidence for the effect of aberrant placental miRNA
expression in macromomia and offer valuable insight into
the mechanisms underlying macromomia. Future studies are
required to determine the functional mechanism linking
placental miRNA expression to macromomia and may provide
novel information on the potential use of miRNAs as a marker
of abnormal fetal growth.

Acknowledgements

The National Natural Science Foundation of China (no. 81072378), and the Natural Science Funds of Zhejiang
(no. Y2101185). We gratefully acknowledge the placent
samples provided by the Department of Obstetrics, Yogaing
Children's Hospital of Wenzhou Medical University. We also
thank the residents of the Wenzhou region for their support for
our epidemiological study.

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