Assessment of plasma microRNAs in congenital intestinal malrotation

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Abstract. Intestinal malrotation in newborns often requires urgent surgical treatment, especially in the presence of volvulus. Therefore, early-stage diagnosis is critical. In the present study, differentially expressed plasma microRNAs (miRNAs) were screened for in patients with intestinal malrotation using high-throughput Illumina sequencing, and validated using reverse transcription-quantitative PCR. Receiver operating characteristic curve (ROC) analysis was conducted to evaluate their specificity, sensitivity and assess their diagnostic value for intestinal malrotation. Bioinformatics analysis was performed to investigate the functions associated with the dysregulated miRNAs. A profile consisting of 28 differentially expressed plasma miRNAs was obtained, of which nine were verified to exhibit significantly altered expression. According to a ROC analysis, four of these could represent novel early-stage, non-invasive biomarkers for intestinal malrotation. Bioinformatics analysis demonstrated that the differentially expressed miRNAs were predominantly involved in 'metal ion transmembrane transporter activity' and 'calcium-dependent protein binding', which may be related to the 'endocytosis' pathway. In conclusion, significantly differentially expressed plasma miRNAs were identified in congenital intestinal malrotation and their potential roles were described. These differentially expressed miRNAs may serve as biomarkers of intestinal malrotation and improve early diagnosis for this condition.

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Introduction

Intestinal malrotation is a congenital aberrant positioning of the bowel that is usually accompanied with anomalous bowel fixation through mesenteric bands or a lack of fixation in parts of the bowel (1). Intestinal malrotation was first described by William Ladd in 1936 and later classified as non-rotation, mixed or incomplete rotation, reversed rotation, or mesocolic hernia (2,3). Typically, ≤ one in 200 newborns are estimated to have asymptomatic rotational anomaly (3,4). Symptomatic malrotation affects 1 in 6,000 live births and often occurs in the first few weeks of life, resulting in elevated risk of bowel obstruction, midgut volvulus and necrosis in the bowel (3,5,6). Currently, upper gastrointestinal (UGI) series is the preferred imaging technique for malrotation diagnosis. Nevertheless, both false positive and false negative interpretations occur, with reported false positive rates of ≤15% (1,7,8). When the duodenojejunal junction position is ambiguous based on UGI series, further examination is necessary. A barium enema might prove helpful in such cases; however, the first pass of barium through the duodenum is often overlooked (9). The duodenal anatomy is hard to recognize with inadequate amounts of barium, while excessive barium could cause rapid and uncontrolled passage of barium through the duodenum or vague visualization. Moreover, the use of sonography in malrotation diagnosis often requires the opinion of experienced radiologists, who may not always available at most centers (10,11). Therefore, the diagnosis of intestinal malrotation is often an intricate and complex process, and a more effective and ideally non-invasive diagnostic method is needed (6,11,12).

MicroRNAs (miRNAs) are a group of non-coding RNA molecules, usually 19-25 nucleotides long, which are abundantly and stably expressed in plasma and other bodily fluids (13,14). Differential expression of specific miRNAs has been identified in numerous diseases, and an increasing body of evidence indicates that miRNAs could serve as promising biomarkers for diagnostic use (15-17). For example, previous studies have identified miRNAs associated with various types of cancer, highlighting an opportunity to use these circulating disease-related miRNAs as biomarkers (18,19). In cardiovascular diseases, it was demonstrated that the combined use of multiple miRNAs could improve the predictive efficiency of traditional diagnostic methods (20,21). Thus, understanding the potential role of miRNAs in intestinal malrotation

may provide invaluable insight into improved diagnostic approaches, as demonstrated in other diseases (22-24).

Previously, a mutation in the forkhead box F1 gene was demonstrated to be associated with intestinal malrotation (25). However, to the best of the authors' knowledge, no studies have evaluated epigenetic changes during the pathogenesis of intestinal malrotation (26). In the present study, it was hypothesized that the expression of specific miRNAs might be dysregulated in newborns with intestinal malrotation. Thus, the aim of the present study was to determine whether such differentially expressed miRNAs could be used for improved diagnosis of intestinal malrotation.

Materials and methods

Study design and sample collection. Ten children with intestinal malrotation (age range, 5 days to month) and ten sex- and age-matched controls (age range, 4 days to 1 month) admitted to The Children's Hospital of Nanjing Medical University (Nanjing, China) between February 2018 and July 2019 were enrolled in the present study, and their plasma samples were collected prospectively. Children with immunological disorders, cardiovascular diseases and other congenital digestive tract abnormalities were excluded, as these pathological factors can affect serum miRNA levels. Plasma samples were collected in a plasma separator tube prior to any treatment and stored at -80˚C until use. The study was approved by the Institutional Ethics Committee of The Children's Hospital of Nanjing Medical University (approval no. 201701025). Written informed consent was obtained from a parent of each patient.

RNA extraction. Total RNA was extracted from plasma samples using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. In addition, 5 μl of 200 nM cel-miR-39 (Guangzhou RiboBio Co., Ltd.) was added to each sample to serve as an external control (27). RNA concentration and quality was assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). RNA was stored at -80˚C until use.

Illumina HiSeq sequencing. A total of six plasma samples (three patients with intestinal malrotation and three controls) were used for sequencing. miRNA-seq library was constructed using NEBNext Small RNA kit (NEB, cat. no. E7300S). DNA integrity was measure using an Agilent 2100 Bioanalyzer with the Agilent DNA 1000 kit (Agilent Technologies, Inc.; cat. no. 5067-1504). Sequencing was performed on the Illumina HiseqX platform (Illumina, Inc.; 150 cycles; paired-end). HiSeq X Ten Reagent kit v2.5 (Illumina, Inc.; cat. no. FC-501-25) was used for sequencing. The concentration of DNA was 30-40 nM, which was measured by real-time PCR. Ligation of 5' and 3' adaptors was carried out following manufacturer's instructions. Small RNAs were amplified by PCR (17 cycles) using adaptor-specific primers according to the manufacturer's protocol The PCR products (fragments of ~90 bp) were purified by 8% PAGE. The size distribution of molecules in each sample were evaluated using an Agilent 2100 Bioanalyzer. Subsequently, amplification of the eligible RNAs was conducted to generate the cluster on the flow cell, which was then sequenced using the HiSeq 2000 System (single end; Illumina, Inc.), following the manufacturer's instructions. miRNAs were considered to be significantly differentially expressed if $P<0.05$ and $\log_2$ (fold-change) ≥ 8 for upregulated miRNAs, or $\log_2$ (fold-change) ≤ -8 for downregulated miRNAs in the intestinal malrotation group, compared with the control group. These criteria applied to the average of the three samples.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO functional enrichment analysis (http://geneontology.org/docs/go-enrichment-analysis/) and KEGG pathway enrichment analysis (http://www.genome.jp/kegg/pathway.html) of DEGs were performed using DIANA TOOLS MirPath version 3 (http://diana.imis.athena-innovation.gr/DianaTools/index.php). GO has three functional categories: Molecular function (MF); biological process (BP); and cellular component (CC). $P<0.05$ was considered as a statistically significant difference.

Quantification of plasma miRNA levels using reverse transcription-quantitative PCR (RT-qPCR). Total RNA extracted from 20 plasma samples (10 patients with intestinal malrotation and 10 controls) was reverse transcribed to cDNA using the PrimeScript™ RT reagent kit (Takara Bio, Inc.), according to the manufacturer's instructions. RT-qPCRs were carried out using SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.) on a Light Cycler 480 (Roche Diagnostics) to measure miRNA expression levels. A total of 18 miRNA candidates (15 upregulated and three downregulated miRNAs in intestinal malrotation) were selected for RT-qPCR analysis. Thermocycling conditions consisted of an initial denaturation at 95˚C for 10 min, followed by 40 cycles at 94˚C for 15 sec and 60˚C for 1 min. Relative expression values were normalized against the spike-in cel-miR-39 and analyzed using the $2^{-\Delta\Delta Cq}$ method (28).

Statistical analysis. Statistical analysis was carried out using GraphPad Prism 8.0 (GraphPad Software, Inc.) and SPSS 23.0 (IBM Corp.). A two-sided unpaired t-test was used to compare differences in miRNA expression between the control and intestinal malrotation groups. Receiver operating curve (ROC) analysis was performed to determine the area under the curve (AUC) of miRNA expression. SPSS 23.0 (IBM Corp.) was used to construct and analyze the curves. The final results were illustrated using GraphPad Prism 8.0 (GraphPad Software, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of differentially expressed serum miRNAs in intestinal malrotation. Serum miRNA levels were measured in six plasma samples (three patients with intestinal malrotation and three controls) using high-throughput sequencing. Overall, ~2,588 miRNAs were identified. The profiles of differentially expressed plasma miRNAs are presented as a heat map and a volcano plot in Fig. 1. Both the heat map and volcano plot show that patients with intestinal malrotation and healthy controls displayed markedly different plasma miRNA profiles. A total of 28 miRNAs exhibited significant differential expression, of which 21 were upregulated and 7 down regulated in patients with IM, compared with controls (Table I).

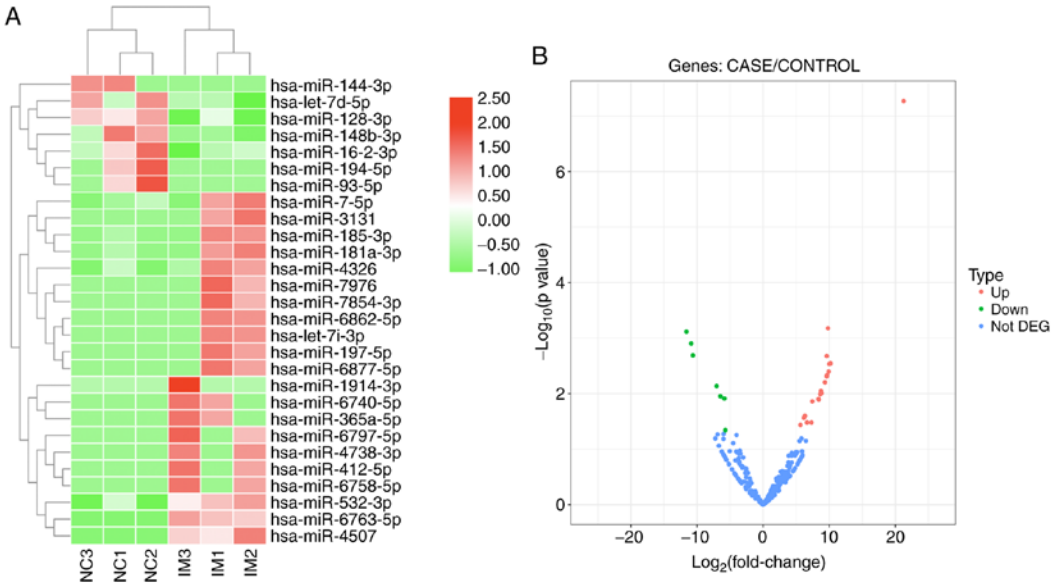

Figure 1. Heat map and volcano plot of significantly dysregulated miRNAs. (A) Heat map and (B) volcano plot of significantly dysregulated miRNAs from the serum of patients with IM. Red indicates upregulated genes and green downregulated genes with P<0.05. miR/miRNA, microRNA; NC, negative control; IM, intestinal malrotation; DEG, differentially expressed gene; Up, upregulated; Down, downregulated.

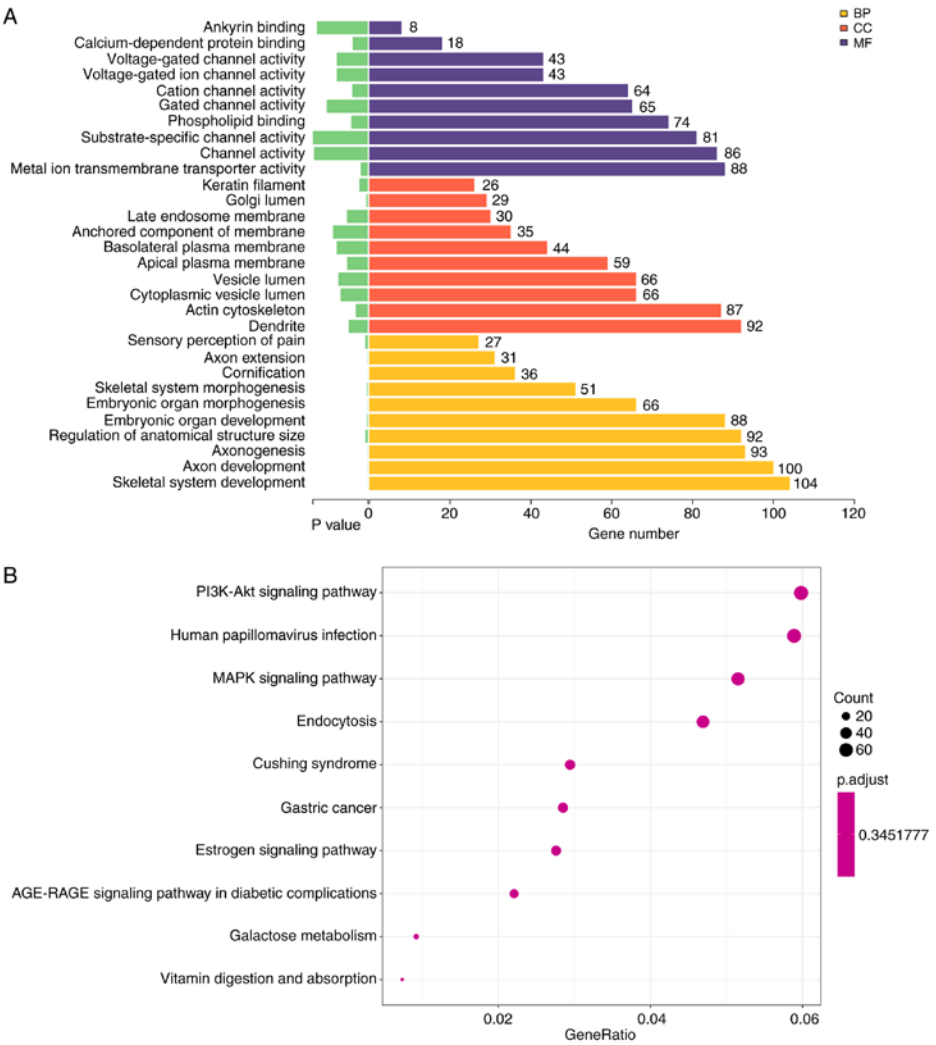

Figure 2. Bioinformatics analysis of the sequencing data. (A) GO enrichment analysis of BPs, CCs and MFs. P-values and the number of genes under each term are shown on the x-axis. GO term descriptions are presented along the y-axis. (B) Bubble graph of Kyoto Encyclopedia of Genes and Genomes enrichment results. The gene ratio of each pathway is indicated on the x-axis. Number of genes involved in each pathway proportional to the size of the bubbles. The color of the bubbles represent the P-values. GO, Gene Ontology; CC, cellular component; BP, biological process; MF, molecular function.

Table I. Dysregulated miRNAs in plasma from patients with intestinal malrotation and controls.

miR	$\log_2$ (FC)	P-value	Expression
hsa-miR-1914-3p	21.2605	0.00053635	Upregulated
hsa-miR-6763-5p	9.8136	0.00066362	Upregulated
hsa-miR-194-5p	-11.6088	0.00076538	Downregulated
hsa-miR-144-3p	-10.8903	0.00124557	Downregulated
hsa-miR-93-5p	-10.6228	0.00204004	Downregulated
hsa-miR-185-3p	9.6254	0.00209648	Upregulated
hsa-miR-4738-3p	10.1860	0.00284375	Upregulated
hsa-miR-3131	10.0151	0.00290713	Upregulated
hsa-miR-6797-5p	9.9124	0.00399543	Upregulated
hsa-miR-6740-5p	9.6671	0.00463324	Upregulated
hsa-miR-412-5p	9.6234	0.00487532	Upregulated
hsa-miR-6758-5p	9.3378	0.00625270	Upregulated
hsa-miR-16-2-3p	-7.0390	0.00726665	Downregulated
hsa-miR-6862-5p	8.7506	0.00897906	Upregulated
hsa-miR-365a-5p	8.8099	0.00986631	Upregulated
hsa-miR-197-5p	8.6241	0.01019434	Upregulated
hsa-let-7d-5p	-6.4675	0.01114899	Downregulated
hsa-miR-148b-3p	-5.8861	0.01219763	Downregulated
hsa-miR-6877-5p	8.3920	0.01254641	Upregulated
hsa-let-7i-3p	8.3746	0.01255295	Upregulated
hsa-miR-7976	8.4149	0.01268761	Upregulated
hsa-miR-4507	7.4372	0.01375498	Upregulated
hsa-miR-7-5p	6.3143	0.02523047	Upregulated
hsa-miR-4326	6.1695	0.02722018	Upregulated
hsa-miR-181a-3p	6.6420	0.03291677	Upregulated
hsa-miR-7854-3p	7.2962	0.03293265	Upregulated
hsa-miR-532-3p	5.6365	0.03647876	Upregulated
hsa-miR-128-3p	-5.7314	0.04537403	Downregulated

miR, microRNA; FC, fold-change.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO analysis suggested that the differentially expressed miRNAs were mainly involved in involved in 'skeletal system development', 'axon development' (Fig. 2A, Table II). These miRNAs were mainly localized or exerted their functions in dendrites and the actin cytoskeleton (Fig. 2A, Table III). Moreover, these miRNAs were involved in 'metal ion transmembrane transporter activity', 'channel activity' and 'substrate-specific channel activity' and other molecular functions (Fig. 2A; Table IV).

KEGG pathway analysis identified 58 significantly enriched pathways in the sequenced miRNAs. The 10 most enriched pathway terms were 'PI3K-Akt signaling pathway', 'human papillomavirus infection', 'MAPK signaling pathway', 'endocytosis', 'Cushing syndrome', 'gastric cancer', 'estrogen signaling pathway', 'AGE-RAGE signaling pathway in diabetic complications', 'galactose metabolism' and 'vitamin digestion and absorption' (Fig. 2B). The most significant KEGG pathway was 'Endocytosis' (KEGG ID, hsa04144), which involved 51 target genes (P=001; Table V). The network associated was analyzed, which demonstrated TGF-β signaling pathway and endosomal recycling compartment were mainly affected (Fig. 3). Thus, GO and KEGG analysis provided novel insights into the underlying mechanism by which plasma miRNAs might participate in the pathogenesis of intestinal malrotation.

Selection and validation of potential plasma miRNA biomarkers for intestinal malrotation. A total of 18 miRNAs were differentially expressed. Of these, 15 miRNAs were upregulated, including, hsa-miR-1914-3p, miR-6763-5p, miR-185-3p, miR-4738-3p, miR-3131, miR-6797-5p, miR-6740-5p, miR-412-5p, miR-6758-5p, miR-6862-5p, miR-365a-5p, miR-197-5p, miR-6877-5p, let-7 and miR-7976. Moreover, three were downregulated, hsa-miR-194-5p, -144-3p and -93-5p (Table I) and were selected for further validation. The expression levels of all 18 candidates were measured in a larger sample size (10 patients with intestinal malrotation and 10 controls) by RT-qPCR. In total, nine miRNAs were confirmed to be differentially expressed. Of these, six were upregulated, compared with the control group, including hsa-miR-1914-3p, -6763-5p, -185-3p, -3131,

Table II. Top 10 significantly enriched GO biological processes.

GO term	Description	P-value	Count
GO:0070268	Cornification	0.00000056	36
GO:0001501	Skeletal system development	0.00000277	104
GO:0061564	Axon development	0.00000467	100
GO:0007409	Axonogenesis	0.00000507	93
GO:0048562	Embryonic organ morphogenesis	0.00000752	66
GO:0048675	Axon extension	0.00000985	31
GO:0048705	Skeletal system morphogenesis	0.00002872	51
GO:0048568	Embryonic organ development	0.00002935	88
GO:0090066	Regulation of anatomical structure size	0.00009987	92
GO:0019233	Sensory perception of pain	0.00010642	27

GO, Gene Ontology.

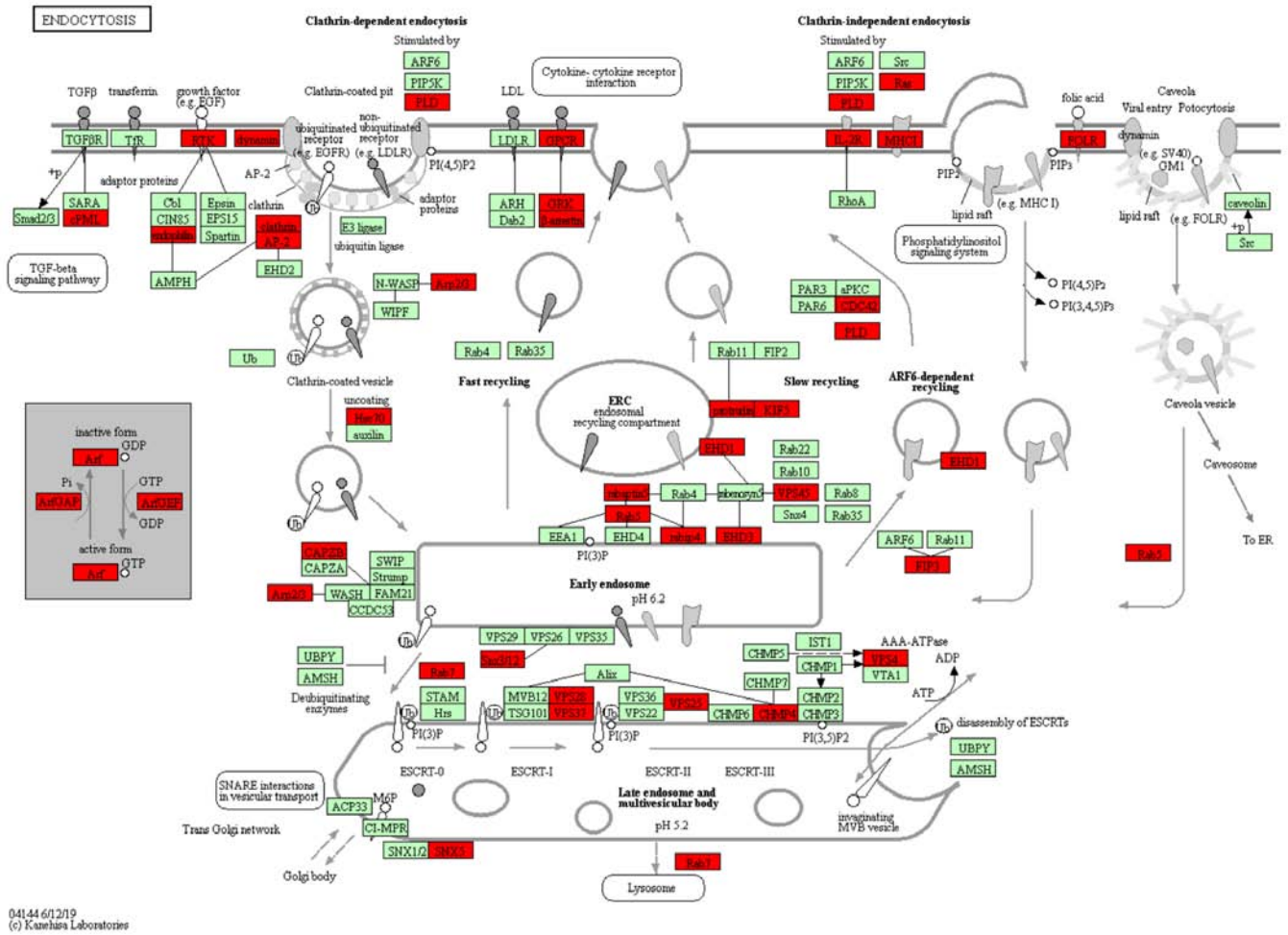

Figure 3. Detailed view of the 'endocytosis' network. Each node represents a key transporter or enzyme. The red nodes indicate miRNA with significantly expression, while the green notes represent miRNAs with no significant change in expression.

-6740-5p and -6758-5p. In addition, three were downregulated, hsa-miR-194-5p, -144-3p and -93-5p (Fig. 4; Table VI).

ROC curve analysis. The sensitivity and specificity of the aforementioned 9 miRNAs as potential diagnostic biomarkers of intestinal malrotation were determined using ROC curve analysis. The AUCs of these miRNAs ranged from 0.727-0.975 (Fig. 5; Table VII). MiR-1914-3p (AUC, 0.975; 95% CI, 0.913-1.000) and miR-3131 (AUC, 0.975; 95% CI, 0.913-1.000) presented the largest AUCs, followed by miR-185-3p (AUC, 0.958; 95% CI, 0.873-1.000) and miR-6763-5p (AUC, 0.950; 95% CI, 0.856-1.000).

Table III. Top 10 significantly enriched GO cellular components.

GO term	Description	P-value	Count
GO:0005796	Golgi lumen	0.00007119	29
GO:0045095	Keratin filament	0.00038711	26
GO:0015629	Actin cytoskeleton	0.00054521	87
GO:0030425	Dendrite	0.00086110	92
GO:0016324	Apical plasma membrane	0.00093914	59
GO:0031902	Late endosome membrane	0.00094567	30
GO:0060205	Cytoplasmic vesicle lumen	0.00123996	66
GO:0031983	Vesicle lumen	0.00134049	66
GO:0016323	Basolateral plasma membrane	0.00140855	44
GO:0031225	Anchored component of membrane	0.00158692	35

GO, Gene Ontology.

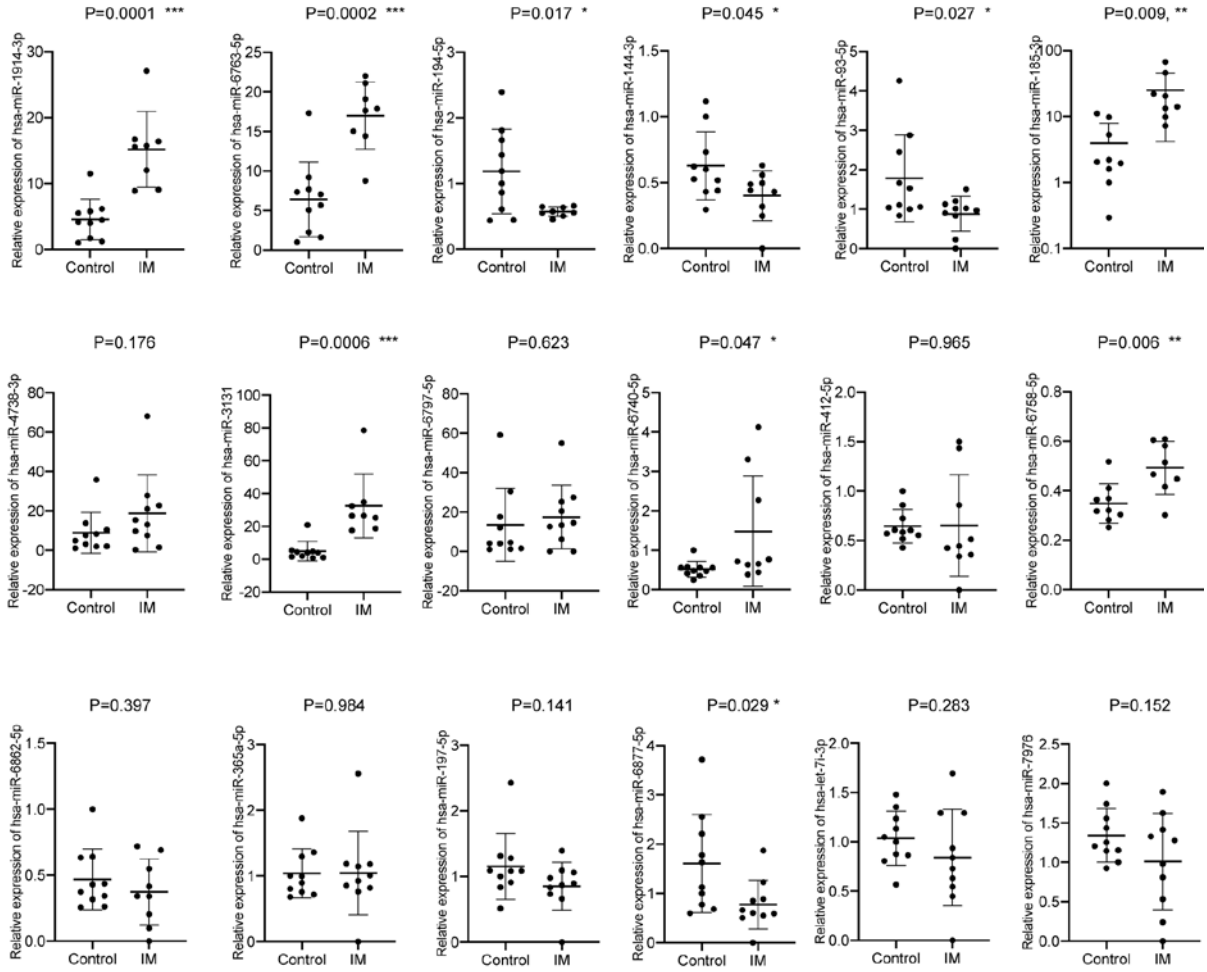

Figure 4. Expression levels of 18 candidate miRNAs in the serum of patients with IM and matched controls. miRNA/miR, microRNA; IM, intestinal malrotation. *P<0.05; **P<0.01, ***P<0.001, vs. control.

Discussion

Malrotation is a congenital abnormal bowel position within the peritoneal cavity that usually involves both the small and the large bowel (29). The term malrotation encompasses a wide range of rotational and fixation abnormalities of the intestines, from readily apparent omphaloceles in newborns to asymptomatic non-rotation in adults (1). Congenital intestinal non-rotation and incomplete rotation usually concur with a narrow base of the mesentery, which might cause duodenal obstruction and midgut volvulus and manifest as acute symptoms (30). Pediatric patients who do not present acute symptoms but are not diagnosed before

Table IV. Top 10 significantly enriched GO molecular functions.

GO term	Description	P-value	Count
GO:0046873	Metal ion transmembrane transporter activity	0.00032224	88
GO:0048306	Calcium-dependent protein binding	0.00068622	18
GO:0005261	Cation channel activity	0.00070680	64
GO:0005543	Phospholipid binding	0.00075808	74
GO:0005244	Voltage-gated ion channel activity	0.00140047	43
GO:0022832	Voltage-gated channel activity	0.00140047	43
GO:0022836	Gated channel activity	0.00187551	65
GO:0030506	Ankyrin binding	0.00233200	8
GO:0015267	Channel activity	0.00245557	86
GO:0022838	Substrate-specific channel activity	0.00251147	81

GO, Gene Ontology.

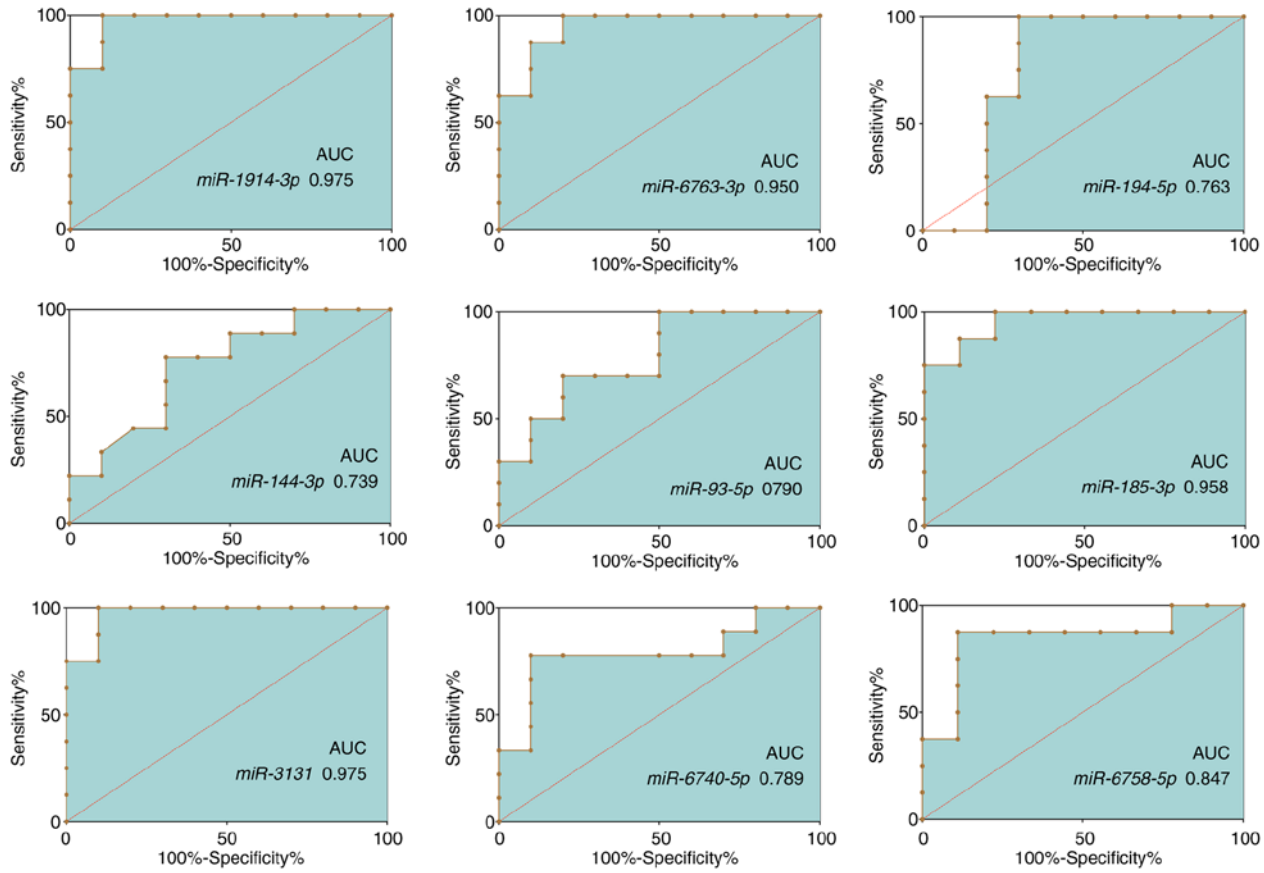

Figure 5. Receiver operating characteristic curves of selected dysregulated miRNAs. Expression of nine candidate miRNAs was assessed using reverse transcription-quantitative PCR. The corresponding AUCs are presented in the diagrams. miRNA/miR, microRNA; AUC, area under the curve.

adulthood are at risk of developing various chronic symptoms later in life, including vomiting, vague abdominal pain, diarrhea, nausea, early satiety and bloating, dyspepsia and other functional or psychiatric disorders (31,32).

Approximately 90% of infants with intestinal malrotation are diagnosed within the first year of life, and ~80% within the first month (33). Common diagnostic tools include UGI, ultrasonography, and barium enema. Although the duodenojejunal junction is usually fixed at the Treitz ligament and functions as a useful landmark, in some cases UGI results may be confusing or hard to interpret, and diagnosis of these cases of malrotation depends on the recognition of anatomic subtleties (8,34). Both false positive and false negative findings have been reported (7,8,35,36). Barium enema can help to some extent in some cases, but challenges exist in terms of the quantity of barium administered to children and the observation of the first pass of barium through the duodenum (5). Moreover, the interpretation of

Table V. Top 10 significantly enriched KEGG pathways.

KEGG pathway	Description	P-value	Count
hsa04144	Endocytosis	0.00132301	51
hsa05165	Human papillomavirus infection	0.00253633	64
hsa04933	AGE-RAGE signaling pathway in diabetic complications	0.00411644	24
hsa04915	Estrogen signaling pathway	0.00688242	30
hsa00052	Galactose metabolism	0.00689000	10
hsa04010	MAPK signaling pathway	0.00724133	56
hsa04151	PI3K-Akt signaling pathway	0.00865405	65
hsa05226	Gastric cancer	0.01159650	31
hsa04934	Cushing syndrome	0.01168848	32
hsa04977	Vitamin digestion and absorption	0.01228429	8

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table VI. Relative expression of nine candidate miRNAs validated by reverse transcription-quantitative PCR from patients with IM and controls.

miRNA	Controls	Patients with IM	P-value	Fold change
miR-1914-3p	4.552	15.17	0.0001[c]	3.333
miR-6763-5p	6.422	16.99	0.0002[c]	2.646
miR-185-3p	3.923	25.02	0.0092[b]	6.378
miR-3131	4.868	32.54	0.0006[c]	6.684
miR-194-5p	1.183	0.571	0.0170[a]	0.483
miR-6740-5p	0.522	1.479	0.0470[a]	2.833
miR-144-3p	0.628	0.401	0.0449[a]	0.639
miR-6758-5p	0.349	0.493	0.0061[b]	1.413
miR-93-5p	1.783	0.881	0.0272[a]	0.494

[a]P<0.05; [b]P<0.01, [c]P<0.001 vs. control. miR/miRNA. microRNA; IM, intestinal malrotation. The values in controls and Patients with IM columns represent the relative expression values.

Table VII. The respective AUCs of nine candidate miRNAs.

miRNA	AUC	Standard error	Asymptotic significance	Asymptotic 95% CI
miR-1914-3p	0.975	0.032	<0.001	0.913-1.000
miR-6763-5p	0.950	0.048	0.001	0.856-1.000
miR-185-3p	0.958	0.044	0.002	0.873-1.000
miR-3131	0.975	0.032	<0.001	0.913-1.000
miR-194-5p	0.763	0.128	0.062	0.512-1.000
miR-6740-5p	0.789	0.115	0.034	0.564-1.000
miR-144-3p	0.739	0.116	0.079	0.512-0.966
miR-6758-5p	0.847	0.106	0.016	0.639-1.000
miR-93-5p	0.790	0.102	0.028	0.590-0.991

miR/miRNA, microRNA; AUC, area under the curve.

ultrasonographical findings can be very subjective, and the presence of an experienced radiologist or technician, who may not always be available at every hospital or medical center, is essential (10).

In the present study, potential diagnostic biomarkers were identified by analyzing plasma miRNA levels in pediatric patients with intestinal malrotation. A profile of differentially expressed miRNAs in the plasma of intestinal malrotation patients was obtained by sequencing plasma miRNAs, and a number of differentially expressed miRNAs were identified, including 21 upregulated and seven downregulated miRNAs. Subsequently, bioinformatics analyses were carried out to investigate the potential involvement of differentially expressed miRNAs in intestinal malrotation.

GO enrichment analysis suggested that these miRNAs were mainly involved in 'metal ion transmembrane transporter activity', 'calcium-dependent protein binding', 'cation channel activity' and 'phospholipid binding'. 'Metal ion transmembrane transporter activity' and 'calcium-dependent protein binding' were the most significantly enriched GO terms with respect to molecular function. Interestingly, it was previously reported that calcium channel-blocking drugs could lead to many developmental abnormalities in *Xenopus* embryos, including malrotation of the gut, possibly through dysregulation of calcium ion antagonism (37). However, the potential link between plasma miRNA levels and calcium ion antagonism in intestinal malrotation requires further study to determine where and how plasma miRNAs interfere with calcium ion. 'Cornification' was the most significantly enriched GO term with respect to biological processes, followed by 'skeletal system development cornification'. A previous study reported a higher frequency of malformations in the skeleton system, including the facial skeleton, the small pelvis, and the upper and lower extremities, among pediatric patients with intestinal malrotation (38). The present results indicated that plasma miRNAs might play a role in this association. The most enriched KEGG pathway was 'endocytosis', which is a critical process in the intestine that is responsible for the endocytic uptake of macromolecules from the gut lumen. For instance, but its role in intestinal malrotation has not yet been studied (39,40).

miRNAs have been demonstrated to play a variety of roles in numerous intestinal diseases and several could even function as biomarkers (41-43). Another important finding of the present study was that plasma miRNAs have the potential to serve as circulating biomarkers for intestinal malrotation. Based on the sequencing data and validation by RT-qPCR in a larger number of samples, nine miRNAs were differentially expressed, including six upregulated miRNAs (hsa-miR-1914-3p, -6763-5p, -185-3p, -3131, -6740-5p and -6758-5p) and three downregulated miRNAs (hsa-miR-194-5p, -144-3p and -93-5p). The highest AUCs were obtained for miR-1914-3p, -3131, -185-3p and -6763-5p, suggesting that these miRNAs may serve as diagnostic biomarkers for intestinal malrotation. As a following step, studies with larger sample sizes are necessary to validate the present findings.

In conclusion, a profile of differentially expressed miRNAs in the plasma of patients with intestinal malrotation was identified, and the potential molecular functions associated with these miRNAs were described. Four of the nine confirmed differentially expressed miRNAs have the potential to improve early diagnosis of intestinal malrotation. However, these miRNAs exhibited relatively small 95% CIs, which is a great limitation to our study and requires further confirmation in the future with much larger sample size. These results also suggested that plasma miRNAs play an important role in intestinal malrotation, although this hypothesis requires further investigation.

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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.

Authors' contributions

XL, HL and CL designed the study. HC and XS collected the samples and conducted the sequencing. XL and LZ carried out the PCRs and ROC analysis. XL contributed to writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by The Institutional Ethics Committee of The Children's Hospital of Nanjing Medical University (approval no. 201701025). Written informed consent was obtained from a parent of each enrolled patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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