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Original Paper

Altered Circular RNA Expression in Patients with Repeated Implantation Failure

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Key Words
Endometrial receptivity • Repeated implantation failure • Circular RNAs microarray

Abstract
Background/Aims: CircRNAs play an important role in regulating gene expression and the specific role of circRNAs in the pathogenesis of repeated implantation failure remains unclear. The aim of this study is to assess the differentially expressed circRNAs in patients with repeated implantation failure. ***Methods:*** We screened circRNA expression profiles in endometrial biopsies taken from six women with repeated implantation failure and control group using circRNA microarray. Bioinformatic analyses were applied to study these differentially expressed circRNAs. Furthermore, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to confirm these results. ***Results:*** The data from circRNA microarrays clearly revealed that 856 unique circRNAs were significantly altered ($p<0.05$). The up-regulated expression of hsa_circRNA_070616, hsa_circRNA_103716, hsa_circRNA_104001, hsa_circRNA_104854 and the down-regulated expression of hsa_circRNA_004183, hsa_circRNA_044353, hsa_circRNA_404686 were further validated by qRT-PCR. ***Conclusion:*** this study demonstrates that a number of circRNAs were differentially expressed in patients with repeated implantation failure compared with normal controls and may offer novel molecular candidates for diagnosis and clinical treatment of embryo implantation failures.

Introduction

Infertility affects approximately 10-15% of couples in their reproductive age worldwide [1]. *In vitro* fertilization (IVF) is a widely used treatment for infertile patients and the success rates of assisted reproductive technologies have improved markedly over the last decades. Nevertheless, there are still a lot of infertile women who has good-quality embryos experiences repeated implantation failure, which may cause by factors related

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to the endometrium [2, 3]. Although endometrial receptivity is assessed by morphologic features currently, exploiting noninvasive diagnostic biomarkers to accompany or replace existing assessment techniques is essential to improve the early diagnostic of implantation failures. Some studies have reported the role of LincRNA and miRNA in the development and progression of endometrial cancer and endometriosis [4-7], however, only a few studies focus on the relationship between non-coding RNA and endometrial receptivity of patients with repeated implantation failure.

CircRNAs, as a special new class of endogenous non-coding RNAs (ncRNAs), are covalently closed continuous loop with higher stability and tissue specific expression, making it more suitable biomarker than other RNA types [8-10]. It has been shown that circRNA regulate gene expression in mammals by sequestering miRNAs as miRNA sponge with miRNA response elements (MREs), and can regulate gene expression at the transcriptional or post-transcriptional level [11]. Growing evidence demonstrated that circRNAs involved in the development of several types of diseases [12-15]. Especially, Qiao and her colleagues [16] have first reported the novel profile of circRNAs in individual human oocyte and pre-implantation embryos, indicating the important role of circRNA during this process. Thus, circRNAs could be considered as potential therapeutic agents or biomarkers with diagnostic abilities in the future. However, little is known about the underlying regulatory mechanisms of circRNAs with repeated implantation failure.

In the present study, the differential expression of circRNAs in endometrial biopsies taken from women with repeated implantation failure and control women with proven fertility was detected using circRNA microarray. Specifically, we identified 856 circRNAs that are up- or down-regulated in repeated implantation failure patients versus normal controls. Among them, 7 circRNAs (hsa_circRNA_070616, hsa_circRNA_103716, hsa_circRNA_104854, hsa_circRNA_104001, hsa_circRNA_004183, hsa_circRNA_044353, hsa_circRNA_404686) were confirmed by realtime qRT-PCR. These circRNAs may exhibit important function in the development and progression of repeated implantation failure. More importantly, the hsa_circRNA_103716 and hsa_circRNA_070616 could be used as potential therapeutic agents and may serve as novel biomarkers with diagnostic abilities in the future.

Materials and Methods

Ethics statement

The project was approved and supervised by the Ethics Committee of the First Hospital of Lanzhou University. All sample were collected from the patient receiving operation in The First Affiliated Hospital of Lanzhou University. All patients included in this study gave their written informed consents prior to surgery.

Subjects and samples

For the control group, endometrial biopsies were obtained from fertile women who had given birth at least once. For the group to investigate, endometrial biopsies were obtained from women who had experienced repeated implantation failure after ovarian stimulation and IVF-ET. Repeated implantation failure was defined as women who had undergone≥3 failed cycles with high-grade embryos were transferred. Three pairs of snap-frozen endometrial tissue with repeated implantation failure patients and control group (3 normal tissues, 3 patient tissues) were collected for circRNA microarray analysis and another 6 samples (3 normal tissues, 3 patient tissues) were collected for qRT-PCR validation.

RNA isolation

Total RNA was isolated from snap-frozen endometrial biopsies of the different groups using the Trizol reagent (Invitrogen, Carlsbad, USA) following the manufacturer's instructions. The quantity and quality of the RNA samples were determined using NanoDrop ND-1000 instrument (Thermo, Wilmington, DE).

CircRNA microarray hybridization

The sample preparation and microarray hybridization were performed according to the Arraystar's standard instructions. Briefly, total RNAs were treated with Rnase R (Epicentre, Inc.), which removed linear

RNAs to enrich circRNAs. The circRNAs were amplified and transcribed into fluorescent cRNA using random primer based on manufacturer's protocol (Arraystar Super RNA Labeling Kit; Arraystar). Next, the labeled cRNAs were hybridized onto the Arraystar Human circRNA Array V2 (8x15K, Arraystar). The hybridized arrays were then washed, fixed and scanned by the Agilent Scanner G2505C (KangChen Bio-tech, Shanghai, China).

CircRNA microarray data analysis

Agilent Feature Extraction software (version 11.0.1.1) was used to analyze acquired array images. Quantile normalization and data processing were executed using R software package. The false discovery rate (FDR) is applied to determine the threshold of p-value. An FDR < 0.05 was recommended. CircRNAs (fold changes≥2.0 and p-values < 0.05) were differentially expressed with statistical significance.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted and reversely transcribed into cDNA using SuperScriptTM III Reverse Transcriptase (Invitrogen). The relative expression of circRNA related with repeated implantation failure that differentially expressed was determined by ViiA 7 Real-time PCR System (Applied Biosystems). Triplicates were performed for each sample in three independent experiments.

Bioinformatics analysis of miRNA response elements

The interactions between circRNA and miRNA were predicted with Arraystar's home-made miRNA target prediction software based on TargetScan and Miranda. The top 5 putative target miRNAs of differential expression circRNAs were identified according to seed match sequences.

Statistical analysis

Data are presented as the mean ± standard error (SE) for triplicate measurements. The significance of the data was estimated by Student's t test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Identification of differentially expressed circRNAs in patients with repeated implantation failure

The concentration and purity of total RNA from different samples were determined using NanoDrop ND-1000 (NanoDrop, Wilmington, USA). All RNA samples showed O.D A260/280 ratio between 1.8 and 2.1 and an O.D A260/230 ratio >1.8.

We identified circRNAs that were differentially expressed with statistical significance between the two groups using Fold Change filtering and Volcano Plot filtering (Fig. 1A and B). Box plots showed the distribution of expression values of circRNAs, which were nearly the same after normalization (Fig. 1C).

Furthermore, we analyzed the distribution of the differential expression of circRNAs on all the human chromosomes (Fig.1D). Also, we analyzed the category of differentially expressed circRNAs. Most of them are transcribed from the protein coding exons, some are from introns and a few other sources of circRNAs (Fig. 1E).

Identification of differentially expressed circRNAs in patients with repeated implantation failure

The hierarchical clustering analysis was performed based on expression pattern of all target circRNAs and circRNAs with the top10 up- and down- regulated circRNAs between the two groups (Fig. 2A and 2B). The combined data suggested that the expression of circRNAs in endometrial biopsies with repeated implantation failure is different from that in control tissues. 856 unique circRNAs were significantly altered in women with repeated implantation failure during the implantation window ($p < 0.05$). Among them, 423 were significantly up-regulated and 433 were down-regulated. The top 10 up- and down-regulated circRNAs between two groups are listed in Table 1 and Table 2, respectively. Among these,

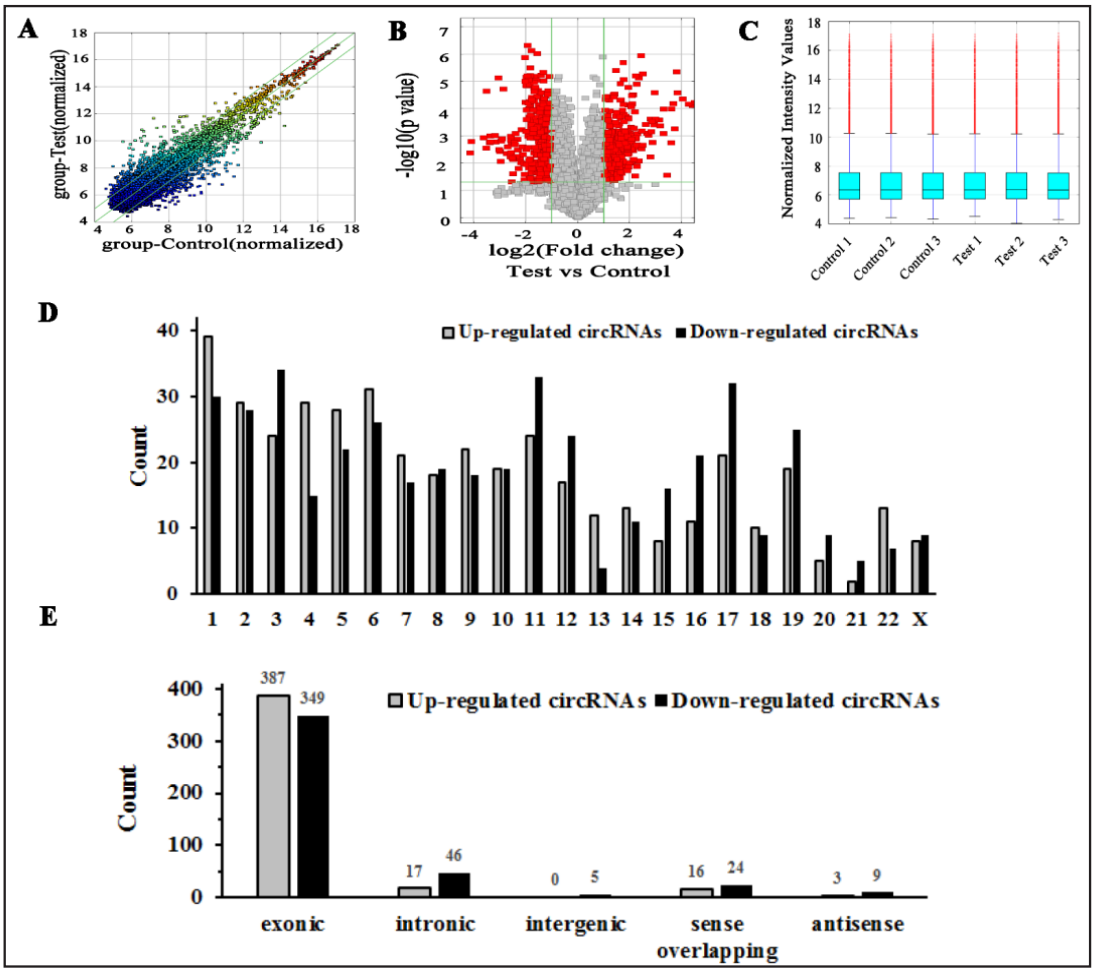

Fig. 1. Bioinformatics analysis of differentially expressed circRNAs in patients with repeated implantation failure. (A) Scatter plot showed the variation of circRNAs in expression in repeated implantation failure (Y-axis) versus the control (X-axis). The values are averaged, normalized signal values (log 2) of circRNA expression in repeated implantation failure. The upper green line to the left indicates a 2.0-fold change of circRNA, while the lower green line indicates a negative fold change of 2.0. After normalization, the distributions of log2 ratios among samples were nearly the same. (B) Volcano plot showed the differential circRNA expression in repeated implantation failure. Red squares mark differentially expressed circRNAs in repeated implantation failure versus the control ($p<0.05$). The vertical lines demark the fold change values. The right vertical line corresponds to 2-fold up and the left vertical line 2-fold down changes, while the horizontal line marks a p-value of 0.05. (C) Box plot showed the distributions of circRNAs in more direct way. (D) The distribution of differentially expressed circRNAs in human chromosomes. (E) The bar diagram shows the circRNA category. Most of differentially expressed circRNAs originate from the exons. Some are from introns, while, a few are other sources.

the expression levels of hsa_circRNA_103717, hsa_circRNA_103828, hsa_circRNA_100834, hsa_circRNA_070616, hsa_circRNA_100833 were up-regulated and hsa_circRNA_405443, hsa_circRNA_023461, hsa_circRNA_104121, hsa_circRNA_403556, hsa_circRNA_000367 were down-regulated as top 5, respectively.

Validation of the differential expression level of circRNAs using qRT-PCR

To confirm the circRNA microarray profiling expression data, 7 typically differential expression circRNAs (hsa_circRNA_070616, hsa_circRNA_103716, hsa_circRNA_104854, hsa_circRNA_104001, hsa_circRNA_004183, hsa_circRNA_044353, and hsa_circRNA_404686)

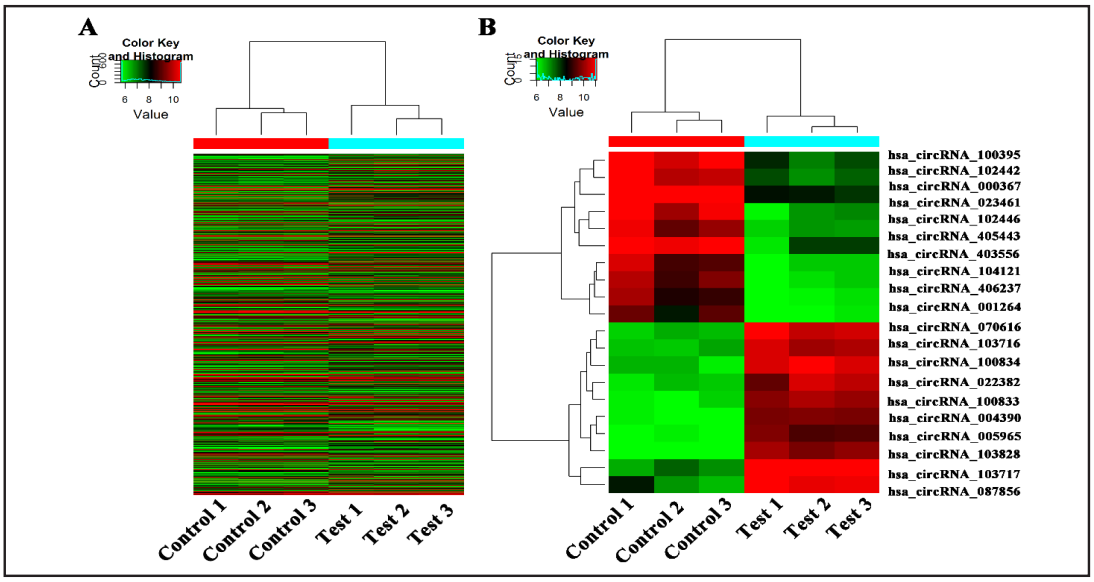

Fig. 2. Heat map and hierarchical clustering showing expression values of all target circRNAs and the most up and down regulated circRNAs. Each circRNA was represented by a single row of colored boxes and each sample was represented by a single column. Red strip represents high relative expression and green strip represents low relative expression. (A) Hierarchical cluster analysis of all target circRNAs. (B) Hierarchical cluster analysis of the most 10 up- and down- regulated circRNAs.

Table 1. Comparison of repeated implantation failure (RIF) versus the control for the top 10 up-regulated expression of circRNAs (fold change > 2 and p < 0.05) sorted by their fold change (FC)

circRNA	circRNA alias	Symbol	FC	p-value	MRE1	MRE2	MRE3	MRE4	MRE5
hsa_circRNA_103717	hsa_circ_0070617	PAPSS1	22.611582	6.004E-05	miR-520a-5p	miR-338-3p	miR-628-5p	miR-525-5p	miR-370-3p
hsa_circRNA_103828	hsa_circ_0072386	HMGCS1	21.492129	7.622E-05	miR-411-5p	miR-625-3p	miR-448	miR-205-5p	miR-526b-5p
hsa_circRNA_100834	hsa_circ_0022392	FADS2	19.371187	6.838E-05	miR-873-5p	miR-23b-5p	miR-181a-2-3p	miR-93-3p	miR-299-3p
hsa_circRNA_070616	hsa_circ_0070616	PAPSS1	16.533145	4.447E-05	miR-4786-3p	miR-6841-3p	miR-642a-5p	miR-5704	miR-574-5p
hsa_circRNA_100833	hsa_circ_0022383	FADS2	14.416911	2.634E-05	miR-765	miR-495-3p	miR-665	miR-193b-5p	miR-124-5p
hsa_circRNA_004390	hsa_circ_0004390	LPAR3	14.046012	4.443E-06	miR-2682-5p	miR-6511a-5p	miR-198	miR-4692	miR-6780b-5p
hsa_circRNA_022382	hsa_circ_0022382	FADS2	13.382935	0.000553	miR-5586-5p	miR-4726-5p	miR-4640-5p	miR-3138	miR-8080
hsa_circRNA_103716	hsa_circ_0006935	PAPSS1	12.879616	6.585E-05	miR-769-5p	miR-574-5p	miR-499a-3p	miR-520a-5p	miR-338-3p
hsa_circRNA_005965	hsa_circ_0005965	PAPSS1	12.29766	0.0001026	miR-6841-3p	miR-3119	miR-769-5p	miR-5704	miR-574-5p
hsa_circRNA_087856	hsa_circ_0087856	RAD23B	12.224208	0.001805	miR-3692-5p	miR-6511b-5p	miR-4763-3p	miR-4518	miR-1266-5p

Table 2. Comparison of repeated implantation failure (RIF) versus the control for the top 10 down-regulated expression of circRNAs (fold change > 2 and p < 0.05) sorted by their fold change (FC)

circRNA	circRNA alias	Symbol	FC	p-value	MRE1	MRE2	MRE3	MRE4	MRE5
hsa_circRNA_405443	-	NDE1	17.4299507	0.004255058	miR-7109-5p	miR-6780b-5p	miR-548k	miR-6761-5p	miR-4653-5p
hsa_circRNA_023461	hsa_circ_0023461	ARAP1	16.9511166	0.001613405	miR-6720-5p	miR-4512	miR-6836-5p	miR-766-3p	miR-3918
hsa_circRNA_104121	hsa_circ_0076767	TRAM2	12.2055249	0.001172624	miR-508-5p	miR-8062	miR-580-5p	miR-4758-5p	miR-1238-5p
hsa_circRNA_403556	-	LINC00340	11.3602467	0.002793942	miR-508-5p	miR-8062	miR-580-5p	miR-4758-5p	miR-1238-5p
hsa_circRNA_000367	hsa_circ_0000367	SIAE	11.3138545	2.2919E-05	miR-331-3p	miR-4646-5p	miR-4797-5p	miR-3919	miR-3190-3p
hsa_circRNA_406237	-	OXNAD1	10.3736491	0.001778372	miR-6868-3p	miR-6739-3p	miR-4639-3p	miR-632	miR-6886-5p
hsa_circRNA_100395	hsa_circ_0015278	KLHL20	10.2643198	0.001008832	miR-141-3p	miR-588	miR-660-3p	miR-136-5p	miR-200a-3p
hsa_circRNA_102446	hsa_circ_0049356	CARM1	10.1892307	0.001657153	miR-377-5p	miR-658	miR-889-5p	miR-23b-5p	let-7i-5p
hsa_circRNA_102442	hsa_circ_0049271	KEAP1	9.0496654	0.000975836	miR-509-5p	miR-593-5p	miR-376b-5p	miR-214-3p	miR-23a-5p
hsa_circRNA_001264	hsa_circ_0000086	ST6GALNAC3	8.8903771	0.001667582	miR-18a-3p	miR-607	miR-632	miR-654-3p	miR-10b-3p

were selected to be further validated out using another 6 samples with 3 control samples and 3 test samples. Primer sequences were presented in Table 3 and clinical characteristics of the patients is shown as Table 4.

As a result, all of them showed the same trends of up- or down-regulation as the microarray data. Particularly, hsa_circRNA_070616, hsa_circRNA_103716, hsa_circRNA_104854 and hsa_circRNA_104001 are significantly up-regulated in patients with repeated implantation failure (Fig. 3A), while hsa_circRNA_004183, hsa_circRNA_044353 and hsa_circRNA_404686 are significantly down-regulated (Fig. 3B). This indicated that the results of qRT-PCR was well consistent with microarray results, demonstrating the high reliability of the microarray expression results.

Table 3. Primer sequences were presented

gene	Primer sequences	PCR product (bp)
β-actin	F:5'GTGGCCGAGGACTTTGATTG3' R :5'CCTGTAACAACGCATCTCATATT3'	73
hsa_circRNA_103716	F:5'GTGGAACTTCTACAGGAACGGG3' R:5'TGGGCTTGGTAGGTGACATTG3'	59
hsa_circRNA_070616	F:5'TGAACAGAGGGATGTCAAAGG3' R:5'GGTAGGTGACATTGGTTGCTCT3'	87
hsa_circRNA_104854	F:5'ATGTCAATGGGCTATGAACGA3' R:5'AGTTGTAGCTGGTGCTGGTGT3'	126
hsa_circRNA_104001	F:5'CTGGGAGAGAATAAAAAGGAAGA3' R:5'AAATTTTCTTCTTGTAACTGCTCC3'	119
hsa_circRNA_004183	F:5'ATGCCGTCCACGCCAGAC3' R:5'GCCGACTGCGAGCTATCTGAT3'	87
hsa_circRNA_044353	F:5'AAGGAGACCAGCCTAAGAGATGT3' R:5'CATCCTACACCAAAAGCCCA3'	53
hsa_circRNA_404686	F:5'TCTCAGAACAAGAGCGTCCAT3' R:5'GTAGAGGGGCAACCGGTATT3'	120

Table 4. Clinical characteristics of the patients

Case number	Age	Cause of infertility	Year of infertility	Total number of cycles	Endometrial thickness(cm)
1	34	Tubal factor	3	3	1.0
2	36	Tubal factor	14	4	1.0
3	28	Tubal factor	7	3	1.0
4	33	Tubal factor	7	3	1.0
5	33	Tubal factor	5	3	1.0
6	32	Tubal factor	3	3	1.0

MRE analysis of differentially expressed circRNAs in patients with repeated implantation failure

Because the circRNA may affect the development of repeated implantation failure through the miRNAs, interactions between circRNAs and their target miRNAs were predicted with arraystar's homemade miRNA target prediction software. Table 1 and Table 2 displayed the potential complementary binding miRNAs of top 10 up-regulated and down-regulated circRNA, respectively. Furthermore, endometrial receptive relevant miRNAs previously described as differentially expressed in pa-

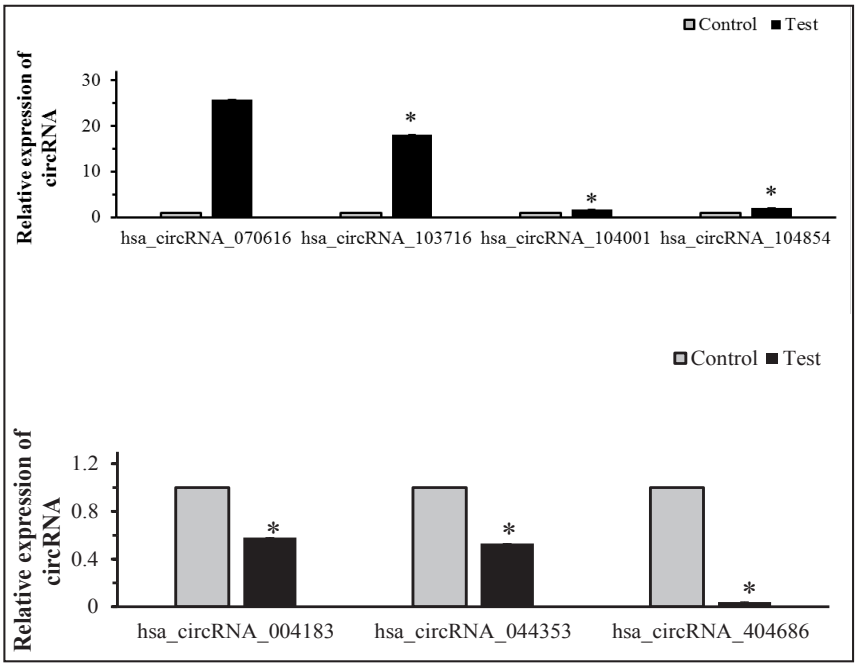

Fig. 3. Quantitative RT-PCR validation for five circRNAs from the microarray data. Fold changes were calculated by the 2 ^ (- ΔΔ Ct) method and β-actin expression is used as a control. (A) The expression level of up-regulated circRNAs. (B) The expression level of down-regulated circRNAs.

Table 5. Endometrial receptive relevant miRNAs previously described as differentially expressed in patients with repeated implantation failure and corresponding circRNA matches

microRNA	circRNA match	Author
Up-regulated microRNA		
hsa-miR-145-5p	hsa_circRNA_102324, hsa_circRNA_101206, hsa_circRNA_091419, hsa_circRNA_101139, hsa_circRNA_003484, hsa_circRNA_102293, hsa_circRNA_100422, hsa_circRNA_400759, hsa_circRNA_031896, hsa_circRNA_102910, hsa_circRNA_104821	Ariel et al.[17]
hsa-miR-27b-3p	hsa_circRNA_104503, hsa_circRNA_101876, hsa_circRNA_003653	Ariel et al. [17]
hsa-miR-652	hsa_circRNA_104293, hsa_circ_0086419, hsa_circRNA_048474	Ariel et al. [17]
hsa-miR-195	hsa_circRNA_101853, hsa_circRNA_101852, hsa_circRNA_103457, hsa_circRNA_102747	Ariel et al. [17]
hsa-miR-342-3p	hsa_circRNA_001459, hsa_circRNA_102485, hsa_circRNA_000966	Ariel et al. [17]
hsa-miR-150-5p	hsa_circRNA_001405	Ariel et al. [17]
hsa-miR-23b-3p	hsa_circRNA_007059	Ariel et al. [17]
hsa-miR-139-5p	hsa_circRNA_104168	Ariel et al. [17]
hsa-miR-99a-5p	hsa_circRNA_102646	Ariel et al. [17]
hsa-miR-1972	hsa_circRNA_405698, hsa_circRNA_006290	Youngsok et al. [18]
hsa-miR-29b-1-5p	hsa_circRNA_101139, hsa_circRNA_101787, hsa_circRNA_104748, hsa_circRNA_104503, hsa_circRNA_101184, hsa_circRNA_104392, hsa_circRNA_101356, hsa_circRNA_102378, hsa_circRNA_102039, hsa_circRNA_100776, hsa_circRNA_406051, hsa_circRNA_102923, hsa_circRNA_406866, hsa_circRNA_104101, hsa_circRNA_104664, hsa_circRNA_008761, hsa_circRNA_400890	Youngsok et al. [18]
hsa-miR-1246	hsa_circRNA_400850, hsa_circRNA_105055	Youngsok et al. [18]
hsa-miR-1273f	hsa_circRNA_406326	Youngsok et al. [18]
hsa-miR-363-3p	hsa_circRNA_001729	Youngsok et al. [18]
hsa-miR-654-3p	hsa_circRNA_001264, hsa_circRNA_101000, hsa_circRNA_103754, hsa_circRNA_103139	Youngsok et al. [18]
hsa-miR-34b-3p	hsa_circRNA_405698	Youngsok et al. [18]
hsa-miR-138-1-3p	hsa_circRNA_027612	Youngsok et al. [18]
hsa-miR-654-3p	hsa_circRNA_101000, hsa_circRNA_001264	
hsa-miR-346	hsa_circRNA_102324, hsa_circRNA_104293, hsa_circRNA_100834	Youngsok et al. [18]
Down-regulated microRNA		
hsa-miR-32-5p	hsa_circRNA_400322	Ariel et al. [17]
hsa-miR-628-5p	hsa_circRNA_103717, hsa_circRNA_104000, hsa_circRNA_103670, hsa_circRNA_103715, hsa_circRNA_104267	Ariel et al. [17]
hsa-miR-874-5p	hsa_circRNA_101805, hsa_circRNA_104143, hsa_circRNA_103221, hsa_circRNA_102451	Ariel et al. [17]
hsa-miR-3187-3p	hsa_circRNA_403782	Youngsok et al. [18]
hsa-miR-21-3p	hsa_circRNA_100186, hsa_circRNA_102452, hsa_circRNA_076989, hsa_circRNA_102747, hsa_circRNA_102434, hsa_circRNA_100604	Youngsok et al. [18]
hsa-miR-181c-5p	hsa_circRNA_102949, hsa_circRNA_102950, hsa_circRNA_101762	Youngsok et al. [18]
hsa-miR-299-3p	hsa_circRNA_008952, hsa_circRNA_100834, hsa_circRNA_102324	Youngsok et al. [18]
hsa-miR-495-3p	hsa_circRNA_100833, hsa_circRNA_104175, hsa_circRNA_100735, hsa_circRNA_104174	Youngsok et al. [18]
hsa-miR-205-5p	hsa_circRNA_103827, hsa_circRNA_103754, hsa_circRNA_103828	Youngsok et al. [18]
hsa-miR-188-5p	hsa_circRNA_100246, hsa_circRNA_400994	Youngsok et al. [18]
hsa-miR-551b-5p	hsa_circRNA_101609, hsa_circRNA_103706, hsa_circRNA_104536	Youngsok et al. [18]
hsa-miR-485-5p	hsa_circRNA_048728, hsa_circRNA_009181, hsa_circRNA_102001, hsa_circRNA_020596, hsa_circRNA_102056, hsa_circRNA_103339, hsa_circRNA_103219, hsa_circRNA_104913, hsa_circRNA_001225, hsa_circRNA_100790, hsa_circRNA_104165, hsa_circRNA_102597, hsa_circRNA_102598, hsa_circRNA_101979, hsa_circRNA_074491, hsa_circRNA_100935, hsa_circRNA_100934	Youngsok et al. [18]

tients with repeated implantation failure [17, 18]and corresponding circRNA match which are differently expressed in our circRNA microarray results are shown in Table 5. The 2D structure were compiled to show the MRE sequence of validated circRNA and the target miRNA seed type (Fig. 4).

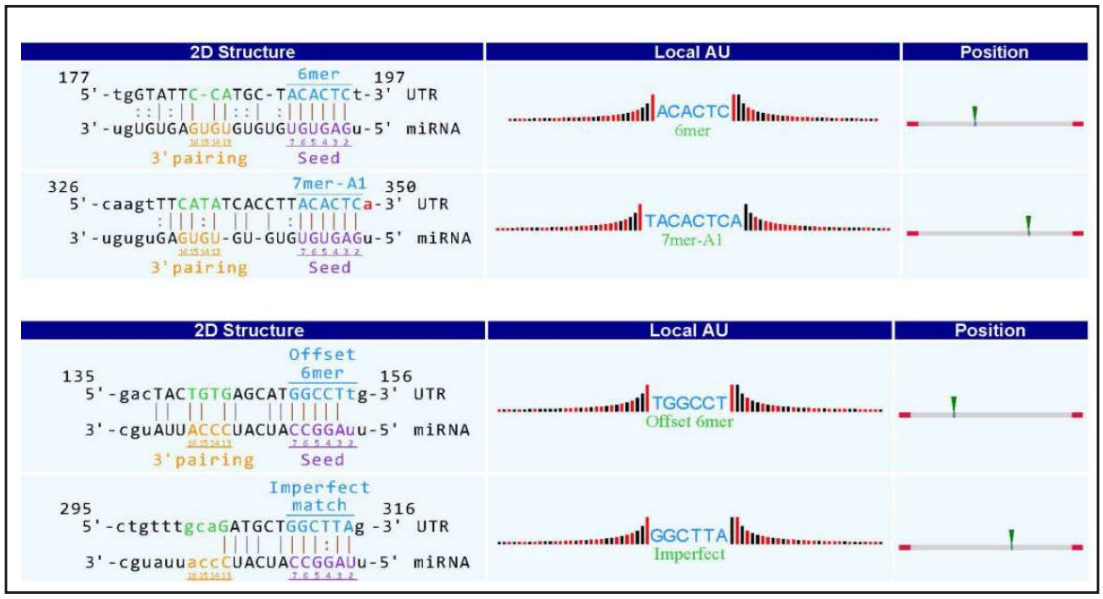

Fig. 4. Binding sites of miR-574-5p in 3′UTR of circRNA_103716 and circRNA_070616.

Discussion

In the present study, the differentially expressed profiles of circRNAs in repeated implantation failure patients were analyzed and validated. To our knowledge, this is the first study to evaluate the differential expression of circRNAs in the endometrium from patients with repeated implantation failures, indicating that the repeated implantation failure-associated circRNAs could be exploited as new candidates for diagnosis and treatment of embryo implantation failures.

CircRNAs are recently discovered as unique class of endogenous non-coding RNAs that can affect the expression of the target genes of miRNA by binding and preventing miRNAs expression with multiple miRNA binding sites [8-10]. It has been reported that circRNAs participate in many essential biological processes and play important role as miRNA sponge [19-23]. However, there is no studies to date have reported the effects of circRNAs in patients with repeated implantation failure.

In this study, there were 423 circRNAs significantly up-regulated and 433 circRNAs significantly down-regulated in endometrial tissue from patients with repeated implantation failure compared to normal controls. In which, hsa_circRNA_103717, hsa_circRNA_103828, hsa_circRNA_100834, hsa_circRNA_070616, hsa_circRNA_100833 were up-regulated and hsa_circRNA_405443,

hsa_circRNA_023461, hsa_circRNA_104121, hsa_circRNA_403556, hsa_circRNA_000367 were down-regulated as top 5, respectively. Furthermore, the differential expression of circRNA whose matched miRNA is related with endometrial receptive was analyzed. The up-regulated hsa_circRNA_070616, hsa_circRNA_103716, hsa_circRNA_104854, hsa_circRNA_104001 and down-regulated hsa_circRNA_004183, hsa_circRNA_044353 and hsa_circRNA_404686 were validated by qRT-PCR analysis. Consistent results were observed, indicating that the altered expression levels of circRNAs may be related to the involvement of repeated implantation failure. Most of these differential circRNAs (hsa_circRNA_070616, hsa_circRNA_103716, hsa_circRNA_104854, hsa_circRNA_004183, hsa_circRNA_044353) are reported expressed in the mammalian brain [24]. Li et al. [25] have reported that hsa_circRNA_004183 play an important regulatory role in hepatic steatosis, but there is no report about the detailed mechanism. Hsa_circRNA_070616 and hsa_circRNA_103716 are selected for further analysis

because they are both predicted as sponge of hsa-miR-574-5p. The previous study about the function of hsa-miR-574-5p are mainly focus on the invasion and metastasis of small/non-small cell lung cancer [26, 27]. On the contrary, Cui et al. [28] have reported that hsa-miR-574-5p suppress the liver metastasis of colorectal cancer by reducing the expression of MACC-1. The study of Ma et al. [29] have reported that overexpression of miR-574-5p inhibit the growth and metastasis in cervical cancer. Notably, Zhang et al. [30] have reported that hsa-miR-574-5p play an important role in hyperplastic endometria and could be a biomarker as estrogen over-exposure. A few studies have reported that supplement of estrogen may improve the thickness of endometrial of patients with repeated implantation failure [31, 32]. However, the relationship between hsa-miR-574-5p and endometrial receptive, the network of hsa_circRNA_070616/hsa_circRNA_103716- hsa-miR-574-5p-mRNA need to be further studied. CircRNAs were believed to function as miRNA sponge. Our results implied that it is worthwhile to further investigate these novel dysregulated circRNAs as miRNA sponges and their potential biological functions in the development of endometrial receptive with patients of repeated implantation failure.

Conclusion

In conclusion, for the first time, our study revealed a unique set of circRNA expression signatures in endometrial tissue from patients with repeated implantation failure. Furthermore, the potential roles of dysregulated circRNAs (hsa_circRNA_070616/hsa_circRNA_103716) were investigated by bioinformatic analysis and they may function as sponge of hsa-miR-574-5p. In summary, the present study could improve our knowledge of the molecular mechanism of repeated implantation failure and would be helpful for further studies on diagnostic, therapeutic and functional of circRNAs in patients with repeated implantation failure. However, differential expression of circRNA related with repeated implantation failure should be validated further in larger studies and the detailed interaction mechanisms between hsa_circRNA_070616/hsa_circRNA_103716 and its target miRNAs are preliminary and need to be validated in future.

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Lin Liu and Xuehong Zhang conceived and designed the experiments; Lin Liu, Yiqing Wang and Feng Yue performed the experiments; Liyan Wang and Panpan Jin analyzed the data; Lifei Li and Xiaoling Ma contributed reagents/materials/analysis tools; Lin Liu wrote the paper.

Disclosure Statement

The authors declare no conflict of interest.

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