Identification of circular RNAs as novel biomarkers and potentially functional competing endogenous RNA network for myelodysplastic syndrome patients

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Abstract
Circular RNAs (circRNAs) have been identified to exert vital biological functions and can be used as new biomarkers in a number of tumors. However, little is known about the functions of circRNAs in myelodysplastic syndrome (MDS). Here, we aimed to investigate circRNA expression profiles and to investigate the functional and clinical value of circRNAs in MDS. Differential expression of circRNAs between MDS and control subjects was analyzed using circRNA arrays, in which we identified 145 upregulated circRNAs and 224 downregulated circRNAs. Validated by real-time quantitative PCR between 100 MDS patients and 20 controls, three upregulated (hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_104634) and three downregulated (hsa_circRNA_103846, hsa_circRNA_102817, and hsa_circRNA_102526) circRNAs matched the arrays. The receiver operating characteristic curve analysis of these circRNAs showed that the area under the curve was 0.7266, 0.8676, 0.7349, 0.7091, 0.8806, and 0.7472, respectively. Kaplan-Meier survival analysis showed that only hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817 were significantly associated with overall survival. Furthermore, we generated a competing endogenous RNA network focused on hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817. Analyses using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes showed that the three circRNAs were linked with some important cancer-related functions and pathways.

KEYWORDS
circRNA, diagnosis, myelodysplastic syndrome, prognosis biomarker

1 | INTRODUCTION

Myelodysplastic syndrome is characterized by clonal expansion of BM myeloid cells. This results in increased apoptosis in one or more BM lineages and peripheral cytopenia, showing a high susceptibility for AML. The molecular mechanisms that lead to the genesis of MDS and its development are not yet fully understood.

Abbreviations: AUC, area under the ROC curve; BM, bone marrow; circRNA, circular RNA; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MDS, myelodysplastic syndrome; miRNA, microRNA; PB, peripheral blood; qPCR, quantitative PCR; qRT-PCR, quantitative real-time PCR; RAEB, refractory anemia with excess blasts; RCMRD, refractory cytopenia with multilineage dysplasia; ROC, receiver operating characteristic; RQ, relative quantity.

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Circular RNAs are a new type of endogenous RNA, whose 5' and 3' ends are joined together by splicing to form a covalent closed continuous loop. This structure is typically nonpolyadenylate and resistant to exonucleases, hence making them more stable than their linear counterparts. Circular RNAs are abundant, conserved, and stable in the cytoplasm. They have tissue-specific and developmentally stage-specific expression patterns and act as miRNA sponges and RNA-binding proteins to regulate gene expression at the transcriptional and posttranscriptional level. Circular RNAs have also been reported to be abundant and stable in human serum, especially in exosomes. Their expression patterns are significantly different between tumor samples and normal tissues, suggesting that circRNAs could be used as a biomarker for tumor diagnosis and prognosis. Researchers have identified the clinical value of circRNAs in a number of cancers, including, lung cancer, hepatocellular carcinoma, and colorectal cancer. However, little is known about the roles of circRNAs in MDS.

In this study, we aim to discover novel functional circRNAs and potential diagnostic and prognostic values of MDS by identification of differentially expressed circRNAs in BM of MDS patients. The circRNA expression profile was analyzed by circRNA array and then validated by qPCR. Receiver operating characteristic curve analysis and the Kaplan-Meier method with the log-rank test were used to assess the diagnostic and prognostic value of circRNAs. Bioinformatic analysis was also used to set a circRNA-miRNA-mRNA interaction network and predict possible functions of circRNAs.

2 MATERIAL AND METHODS

2.1 Patient recruitment and sample description

One hundred MDS patients (refractory anemia, n = 2; refractory anemia with ringed sideroblasts, n = 2; RCMD, n = 83; RAEB-1, n = 4; RAEB-2, n = 6; and MDS, unclassifiable, n = 3) and 20 control subjects were recruited from Shanghai Huashan hospital between 2007 and 2016. Patients with RCMD were newly diagnosed based on the 2008 WHO criteria and had not yet undergone treatment. Controls refer to samples from patients with benign blood diseases. Bone marrow aspiration is a traumatic procedure, and it is not widely accepted by patients. Together with ethical considerations, it is difficult to collect BM samples from healthy people. Therefore, samples of patients with benign blood diseases were enrolled as controls. Due to hematological count anomalies or hypersplenism, these patients had undergone BM tests and no abnormalities were found. After 2 years of follow-up, the BM of these patients remained normal. Bone marrow samples (5 mL) were collected from all 100 MDS patients and 20 control subjects. Blood samples (3 mL) were collected using an EDTA anticoagulated vacutainer from the 100 MDS patients. Bone marrow and PBMCs were isolated from all subjects using a Ficoll solution within 12 hours after sample collection. The study was approved by the institutional review board at Huashan Hospital of Fudan University and informed consent was obtained from each study participant.

2.2 RNA isolation and reverse transcription

Total RNA was extracted from each sample of BM and PBMCs using the QIamp RNA Blood Mini Kit (Qiagen) following the manufacturer’s instructions. RNA quality was assessed using the Bio-Rad Experion electrophoretogram instrument (Bio-Rad). The purity of RNA was assessed by measuring the optical density of 260/280 ratio using a NanoDrop spectrophotometer. Samples were used in reverse transcription reactions when the A260/A280 was between 1.8 and 2.0. RNA samples were reverse transcribed into cDNA using Takara PrimeScript RT Master Mix (Takara) according to the manufacturer’s protocol and stored at −80°C until further use.

2.3 Quantitative real-time PCR assay

Real-time qPCRs were carried out using the CBX 1000 Sequence Detection System (Applied Biosystems). SYBR Premix Ex Taq PCR reagents (Takara) were used for amplification and detection following the manufacturer’s instructions. Specifically, 10 μL reaction mixture containing 5 μL SYBR Green premix, 0.2 μL of each 10 mol/L primer, 1 μL cDNA, and 3.6 μL diethylpyrocarbonate-treated water was heated at 95°C for 30 s and subsequently subjected to 40 cycles of 95°C for 5 s and 60°C for 30 s. The Ct value was the fractional cycle number at which the fluorescence exceeded the given threshold. We used GAPDH to normalize the qPCR. The relative expression levels of circRNAs were determined using the 2−ΔΔCT method. Divergent primers were selected for circRNAs and primers for GAPDH were synthesized by Biosune. All qRT-PCR primer sequences are illustrated in Table 1.

| TABLE 1 | Quantitative real-time PCR primer sequences of circular RNAs (circRNAs) in myelodysplastic syndrome |
|-----------------------------------------------|--------------------------------------------------------------------------------------------------------------------------|
| Target                                     | Primer sequences (5’-3’)                                                                                               |
| hsa_circRNA_100352                          | GGTTGAAGAGAAAGGTGGCT ACAGAGTCATCCCGCACAGAC                                                                         |
| hsa_circRNA_104056                          | GCACTCAGGAAAGCTCAGTG TTGACACTGTGATGTCC                                                                             |
| hsa_circRNA_104634                          | CCAAAACGAAAGGACTCCCTGTTAT CGCCAGCAATGCAACT                                                                      |
| hsa_circRNA_102526                          | CAATGGGCGAGTGGAGGACC CAGTACATTTCCCTGACC                                                                            |
| hsa_circRNA_103846                          | GTTCTGATCCGAAAGGCAGCTTAT TGAAGAGGAGAACCTAGG                                                                      |
| hsa_circRNA_102817                          | CAGTGCGTGTCCCTATGGCCTTCCGCTTCCAACG TCCAGTTTCCTGTTGCTGTTGTGA                                                          |
| GAPDH                                      | ACCACACCTGCATTGCGATC TCCACACCTGGTGGTGA                                                                   |
2.4 | Sanger sequencing

Products of qRT-PCR were sent for Sanger sequencing to determine the full-length sequence and confirm the splice junctions of the selected circRNAs. Sanger sequencing was carried out by Biosune.

2.5 | Circular RNA expression profile in MDS patients

Circular RNA transcripts were detected using a circRNA array for nine human samples, four control subjects, and five newly diagnosed MDS patients with no prior treatment. Due to financial constraints, we could not send all subtypes of MDS for circRNA array analysis as the experimental group needs at least five sample repetitions to obtain reliable results. Therefore, samples from patients with the intermediate state of MDS, MDS-RAEB-1, were chosen for examination, thus hoping to obtain maximum homogeneity results within limited conditions. The control subjects were four patients with benign blood diseases, whose BM tests showed no abnormalities. The characteristics of the nine patients were shown in Table 2. Data were quality controlled and normalized. P value and fold change were calculated using the Student’s t test. Differentially expressed circRNAs were selected based on threshold values of fold change greater than or equal to 2 and P value less than or equal to .05.

2.6 | Bioinformatics analysis

Circular RNA-miRNA interaction was predicted by TargetScan and miRanda. Target mRNAs of miRNAs were analyzed by TargetScan, miRDB, and miRWalk. Cytoscape 3.8.0 software was used to visualize the circRNA-miRNA-mRNA network. Gene Ontology annotation and KEGG analysis were undertaken using an R package named clusterProfiler.

2.7 | Statistical analysis

All data were verified as nonnormal distributed using Shapiro-Wilk tests and presented as median (25%, 75%). The Mann-Whitney U test was used to analyze the data, with a significant value cut-off of P < .05. The clinical diagnostic value of any given circRNA was verified by ROC curve analysis, in which AUC > 0.5 and P < .05 indicated diagnostic value. The cut-off value and corresponding sensitivity and specificity were identified through ROC curve analysis. Kaplan-Meier survival analysis was carried out to analyze the prognostic value and the statistical significance was obtained using the log-rank test. The χ²-test or Fisher’s exact test was used to compare the difference of categorical variables between patients with lower and higher circRNA expression. The correlation between circRNA expression levels in BM and PB samples was evaluated using Spearman’s correlation test. Statistical analyses were undertaken using GraphPad Prism 6.

3 | RESULTS

3.1 | Bone marrow circRNA expression profile in MDS patients

To identify differentially expressed circRNAs between MDS patients and control subjects, we extracted BM total RNA from five MDS patients and four control subjects for circRNA array assay analysis. Three hundred and sixty-nine circRNAs were differently expressed out of the 3459 circRNAs in the array, of which 145 circRNAs were upregulated and 224 were downregulated in MDS patient samples.

3.2 | Validating the results of circRNA array

Circular RNAs were chosen for further qPCR detection according to the following three criteria: (a) sequence analysis showed that

TABLE 2 Clinicopathological characteristics of nine patients included in circular RNA array analysis

| Group | No. | Sex  | Age (y) | Diagnosis    | Karyotype                  | IPSS risk subgroup |
|-------|-----|------|---------|--------------|----------------------------|-------------------|
| MDS   | 1   | Male | 70      | MDS-RAEB-1   | 45,X−Y[20]                 | Int-1             |
| MDS   | 2   | Male | 67      | MDS-RAEB-1   | 47,XY,+8[20]               | High              |
| MDS   | 3   | Male | 71      | MDS-RAEB-1   | 47,XY,del(20)(q11.2q12),+21[16]/48,1dem,+21[4] | Int-2             |
| MDS   | 4   | Male | 74      | MDS-RAEB-1   | 46,XY,del(3)[q13.2q25]/{15}/47,XY,+8[4]/46,XY[1] | Int-2             |
| MDS   | 5   | Male | 72      | MDS-RAEB-1   | 46,XY[20]                  | Int-1             |
| Control | 6 | Male | 60      | Hypersplenism | 46,XY[20]                  | –                 |
| Control | 7 | Female | 51    | Thrombocytopenia | 46,XX[20]                  | –                 |
| Control | 8 | Female | 59    | Thrombocytopenia | 46,XX[20]                  | –                 |
| Control | 9 | Male | 65      | Hypersplenism  | 46,XY[20]                  | –                 |

Abbreviations: –, not applicable; Int, intermediate; IPSS, International Prognostic Scoring System; MDS, myelodysplastic syndrome; RAEB-1, refractory anemia with excess blasts-type 1.
the amplified product of circRNA was consistent with the CircBase sequence; (b) results of the Sanger sequencing confirmed the back-spline junctions of the circRNAs selected; and (c) the specificity of the amplified circRNA product was confirmed by electrophoresis. These inclusion criteria for selection proved that the selected circRNAs naturally existed as a loop in BM and could be amplified by qRT-PCR. To identify the most clinically applicable biomarker, we chose six circRNAs that showed the highest fold differences ($P < .05$) between MDS and control subjects. Information of circRNAs is listed in Table 3. Then we verified the six circRNAs by qRT-PCR in a cohort consisting of 20 control subjects and 100 MDS patients. The results were consistent with the circRNA array (Figure 1).

3.3 Receiver operating characteristic analysis of six circRNAs in MDS patients

To compare the diagnostic value of the previously selected six circRNAs as candidate biomarkers of MDS, we undertook ROC curve analysis for each circRNA with the same cohort of 20 control subjects and 100 MDS patients. As shown in Figure 2, the AUC was larger than 0.500 ($P < .05$) for six circRNAs, suggesting their potential diagnostic value. Notably, the AUC of hsa_circRNA_104056 was 0.89 ($P < .001$), which was the largest among the selected circRNAs. The sensitivity and specificity of each circRNA were determined based on the cut-off value (shown in Table 4). Hsa_circRNA_104056 had the highest sensitivity and specificity (0.8 and 0.8421, respectively).

3.4 Kaplan-Meier survival analysis of six circRNAs in MDS patients

We then detected whether the alteration of each circRNA expression could predict the prognosis of MDS patients. The survival curves for patients with MDS according to different circRNA expression levels are shown in Figure 3. We found that the elevated expression levels of hsa_circRNA_100352/hsa_circRNA_104056/hsa_circRNA_102817 showed significant relationships with poorer

| TABLE 3 | Information of six selected circular RNAs (circRNAs) from the array that showed the highest fold differences ($P < .05$) between myelodysplastic syndrome patients and control subjects |
|---|---|---|---|---|---|---|---|
| CircRNA | Fold change | P value | FDR | Regulation | CircRNA type | Gene symbol |
| hsa_circRNA_104634 | 7.26 | 0.0045 | 0.0502 | Up | Exonic | ASPH |
| hsa_circRNA_104056 | 5.26 | 0.0126 | 0.0860 | Up | Exonic | RREB1 |
| hsa_circRNA_100352 | 2.35 | 0.0409 | 0.1470 | Up | Exonic | ASH1L |
| hsa_circRNA_103846 | 3.20 | 0.0035 | 0.0422 | Down | Exonic | DEPDC1B |
| hsa_circRNA_102817 | 2.37 | 0.0000 | 0.0045 | Down | Exonic | SAP130 |
| hsa_circRNA_102526 | 2.22 | 0.0459 | 0.1540 | Down | Exonic | UBA2 |

FIGURE 1 Expression levels of circular RNAs (circRNAs) compared between patients with myelodysplastic syndrome (MDS) and control subjects
patient overall survival \((P < .05)\). Hence, these three circRNAs are preferable and potential biomarkers for MDS, not only for diagnosis but also for prognosis.

### 3.5 Association between circRNA expression level with clinicopathological characteristics of MDS patients

To determine the clinical significance of expression level of circRNAs, we divided the MDS patients into two groups based on the median RQ value of circRNAs. As shown in Table 5, we found that higher hsa_circRNA_100352 expression was more frequently seen in patients younger than 60 years of age \((P = .020)\), or patients with lower Revised International Prognostic Scoring System (IPSS-R) scores \((P = .019)\). Uregulation of hsa_circRNA_104056 was significantly associated with cytogenetics \((P = .033)\). The cytogenetic of patients with higher hsa_circRNA_100352 expression were more likely to be good. However, the expression level of hsa_circRNA_102817 has no significant correlation with any of these clinicopathologic characteristics.
3.6 | Spearman’s correlation test showed that there was no correlation between expression level of circRNAs in BM and PB samples

As BM aspiration is invasive and not easily accepted by patients, we sought to analyze the correlation between circRNA expression levels in PB and BM in these MDS patients to determine whether circRNA expression levels in PB can be used as a viable approach for MDS diagnosis, instead of BM. We analyzed the expression levels of six circRNAs in 100 MDS PB samples and undertook a Spearman’s correlation test with circRNA levels in BM and PB. Our results shown that none of the six circRNAs levels in BM correlated with levels in PB samples (Figure 4). The results suggested that circRNA levels in PB could not be a substitute for circRNA measurements in BM.

3.7 | Circular RNA-miRNA-mRNA network

Arraystar’s homemade miRNA target prediction software based on database TargetScan and miRanda were used to predicted miRNAs that could bind to hsa_circRNA_100352, hsa_circRNA_104056, or hsa_circRNA_102817. The results are listed in Table 6 and Figure 5. Target mRNAs of miRNAs were analyzed using three databases, TargetScan, miRDB, and miRWalk. Targets that have been annotated by all databases were filtered out. The circRNA-miRNA-mRNA network was developed to predict potential target mRNAs of circRNAs based on the mechanism that circRNAs can bind to miRNAs, which can further silence certain gene expression by degrading the mRNA transcripts. The circRNA-miRNA-mRNA network diagram was drawn using Cytoscape 3.8.0 (Figure 6).

3.8 | Gene Ontology and KEGG analyses

The lists of all the predicted genes were analyzed by the GO and KEGG approaches in R. Specifically, these genes were most enriched in the biological process of response to transforming growth factor-β and cell cycle arrest (P < .01), cellular component in the histone deacetylase complex and transcription regulator complex (P < .01), molecular function in SMAD binding, and cadherin binding (P < .05) (Figure 7A). The KEGG analysis revealed that these genes were enriched in viral carcinogenesis, transcriptional misregulation in cancer, and p53 signaling pathway (Figure 7B).

4 | DISCUSSION

Circular RNAs, endogenous small RNAs that regulate gene expression in mammals, can work as miRNA sponges and regulators of splicing and transcription, and participate in ribosomal RNA processing.13,14 Circular RNAs have been confirmed as important biological regulators in various cancers. Due to their relative tolerance to exonucleases, circRNAs can also serve as diagnostic and prognostic biomarkers.5-9 However, few studies have focused on the roles that circRNAs could play in MDS.
In our study, we profiled circRNA expression in the BM of MDS patients and controls by circRNA array. Here, 145 and 224 circRNAs were detected as upregulated and downregulated with statistical significance, respectively. Validated by qPCR, three upregulated (hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817) and three downregulated (hsa_circRNA_103846, hsa_circRNA_103846, and hsa_circRNA_103846) were identified.

### TABLE 5

|                      | hsa_circRNA_100352 | hsa_circRNA_104056 | hsa_circRNA_102817 |
|----------------------|--------------------|--------------------|--------------------|
|                      | Low (%)            | High (%)           | P value            |
| Age at diagnosis (y) |                    |                    |                    |
| ≥60                  | 26 (49.1)          | 14 (26.9)          | .020               |
| <60                  | 27 (50.9)          | 38 (73.1)          |                    |
| Gender               |                    |                    |                    |
| Male                 | 32 (60.4)          | 22 (42.3)          | .064               |
| Female               | 21 (39.6)          | 30 (57.7)          |                    |
| WBC                  |                    |                    |                    |
| <4 × 10^9/L          | 45 (84.9)          | 49 (94.2)          | .119               |
| ≥4 × 10^9/L          | 8 (15.1)           | 3 (5.8)            |                    |
| ANC                  |                    |                    |                    |
| <1.5 × 10^9/L        | 36 (67.9)          | 42 (80.8)          | .132               |
| ≥1.5 × 10^9/L        | 17 (32.1)          | 10 (19.2)          |                    |
| Hgb                  |                    |                    |                    |
| <10g/dL              | 46 (86.8)          | 40 (76.9)          | .189               |
| ≥10g/dL              | 7 (13.2)           | 12 (23.1)          |                    |
| Platelet             |                    |                    |                    |
| <100 × 10^9/L        | 37 (69.8)          | 37 (71.2)          | .880               |
| ≥100 × 10^9/L        | 16 (30.2)          | 15 (28.8)          |                    |
| Blast %              |                    |                    |                    |
| <5                   | 46 (88.5)          | 51 (98.1)          | .072               |
| 5-10                 | 1 (1.9)            | 1 (1.9)            |                    |
| >10                  | 5 (9.6)            | 0 (0.0)            |                    |
| IPSS risk subgroup   |                    |                    |                    |
| Low                  | 7 (13.1)           | 5 (9.6)            | .076               |
| Int-1                | 40 (75.5)          | 47 (90.4)          | .376               |
| Int-2                | 3 (5.7)            | 0 (0.0)            |                    |
| High                 | 3 (5.7)            | 3 (5.8)            |                    |
| IPSS-R               |                    |                    |                    |
| ≤1.5                 | 2 (3.8)            | 8 (15.4)           | .019               |
| 1.5-3                | 26 (49.0)          | 19 (36.5)          |                    |
| 3-4.5                | 14 (26.4)          | 22 (42.3)          |                    |
| 4.5-6                | 8 (15.1)           | 3 (5.8)            |                    |
| >6                   | 3 (5.7)            | 0 (0.0)            |                    |
| Cytogenetic scoring system |            |                    |                    |
| Very good/ good      | 40 (75.5)          | 46 (88.5)          | .082               |
| Intermediate         | 9 (17.0)           | 6 (11.5)           |                    |
| Poor/very poor       | 4 (7.5)            | 0 (0.0)            |                    |

Abbreviations: ANC, absolute neutrophil count; Int, intermediate; Hgb, hemoglobin; IPSS, International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; WBC, white blood cell count.
hsa_circRNA_102817, and hsa_circRNA_102526) circRNAs were chosen for further analysis.

The diagnosis and follow-up of MDS are based on the morphologic quantification of BM blasts and conventional cytogenetics. However, these methods have limitations: interobserver variability exists in blast counts and cytogenetics requires long cell culturing (more than 2 days). In contrast, the detection of circRNA using qPCR is fast and simple. The 5′ and 3′ ends of circRNA are joined together by splicing to covalently link and form a closed continuous loop. The stability of circRNA is more than 10 times that of homologous linear RNA. This property suggests that circRNAs could serve as potential biomarkers. Researchers have identified the diagnostic and prognostic value of circRNAs in a number of cancers. We explored the diagnostic and prognostic value of the six selected circRNAs as MDS biomarkers in 100 MDS patients and 20 control subjects. The circRNAs hsa_circRNA_100352, hsa_circRNA_104056, hsa_circRNA_104634, hsa_circRNA_103846, hsa_circRNA_102817, and hsa_circRNA_102526 showed potential value for the diagnosis of MDS, with AUC values of 0.7266, 0.8676, 0.7349, 0.7091, 0.8806, and 0.7472 respectively. Only three circRNAs, hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817, were significantly associated with overall survival. These results confirmed the clinical value of circRNAs in MDS, especially hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817.

The acquisition of BM samples requires BM aspirate smears and biopsy, which is invasive and not easily accepted by patients. Thus, we detected the expression level of the six selected circRNAs both in BM and PB and used Spearman’s correlation test to investigate whether PB could be substituted for BM samples to detect the selected circRNA biomarkers of MDS. However, the Spearman coefficients of each circRNA were all less than 0.5, which were too small and had no significance. The results suggested that circRNA expression levels in PB cannot replace BM as a viable approach for MDS diagnosis or prognosis.

According to present studies, most circRNAs harbor abundant miRNA binding sites and mainly act as miRNA sponges to regulate the expression of miRNA-mediated mRNA. To obtain a more comprehensive understanding, we generated a circRNA-miRNA-mRNA network focused on hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817 as they can serve as both diagnostic and prognostic biomarkers of MDS. The network consists of three circRNAs, 15 miRNAs, and 334 mRNAs. Eleven of the 15 predicted miRNAs have been researched in studies and they all participate in many vital cancer-related pathways, such as the miR-298/Wnt/β-catenin signaling, miR-616-3p/PTEN/Akt/mTOR, miR-377-3p/nuclear

![Figure 4](https://example.com/figure4.png)

**Figure 4** Spearman’s correlation test for circular RNA (circRNA) levels in bone marrow (BM) and peripheral blood (PB) samples from patients with myelodysplastic syndrome

**Table 6** Predicted microRNAs (miR) of chosen circular RNAs (circRNAs)

| CircRNAs       | MRE1          | MRE2          | MRE3          | MRE4          | MRE5          |
|----------------|---------------|---------------|---------------|---------------|---------------|
| hsa_circRNA_100352 | hsa-miR-627-3p | hsa-miR-329-5p | hsa-miR-25-3p | hsa-miR-766-5p | hsa-miR-143-5p |
| hsa_circRNA_104056 | hsa-miR-637   | hsa-miR-623   | hsa-miR-377-3p | hsa-miR-193b-5p | hsa-miR-616-3p |
| hsa_circRNA_102817 | hsa-miR-331-5p | hsa-miR-298   | hsa-miR-296-3p | hsa-miR-30c-1-3p | hsa-miR-588    |
factor-κB,\textsuperscript{18} and miR-143-5p/hypoxia-inducible factor-1α pathway.\textsuperscript{19} These miRNAs can influence cell proliferation, differentiation, invasion, and apoptosis, and play important roles in several tumors.

At the same time, GO analysis showed that most enriched genes were involved in the biological processes of response to transforming growth factor-β and cell cycle arrest (\(P < .01\)), cellular component in the histone deacetylase complex and transcription regulator complex (\(P < .01\)), molecular function in SMAD binding and cadherin binding (\(P < .05\)). Through KEGG analysis, we found viral carcinogenesis, transcriptional misregulation in cancer, and the p53 signaling pathway were most related. The results suggested that these three selected circRNAs might be of importance in the incidence and development of MDS.

In follow-up studies, we will focus on the biological functions and molecular mechanisms of hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817. The predicted circRNA-miRNA-mRNA network and GO/KEGG analysis helped us to clarify and explore the molecular mechanisms of circRNAs in MDS.

In summary, we determined the differences in expression levels of circRNAs in BM between MDS patients and controls. The ROC and Kaplan-Meier survival analyses based on qPCR suggest that hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817 in BM are valuable for diagnosis and prognosis of MDS. The circRNA-miRNA-mRNA network prediction and bioinformatics analysis provided a comprehensive understanding of these three circRNAs, which might play important roles in incidence and development of MDS. Nevertheless, further research is still needed to explore the molecular mechanisms of circRNAs in the development of MDS.

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DISCLOSURE

The authors have no conflict of interest.

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FIGURE 6 Cytoscape was used to generate a circular RNA (circRNA)-microRNA (miRNA)-mRNA network consisting of three circRNAs (red V), 15 miRNAs (blue diamond), and 334 mRNAs (green ellipse).

FIGURE 7 A, Gene Ontology analysis for mRNAs participating as competing endogenous RNAs (ceRNAs) of circular RNAs hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817. BP: biological process; CC: cellular component; MF: molecular function. B, Kyoto Encyclopedia of Genes and Genomes analysis for mRNAs participating as ceRNAs of hsa_circRNA_100352, hsa_circRNA_104056, and hsa_circRNA_102817.
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