Original Article

Access this article online
Quick Response Code:
Website:
https://journals.lww.com/ijhm
DOI:
10.4103/ijh.ijh_34_25

Comparative analysis of circular RNA expression in bone marrow and peripheral blood samples of patients with acute myeloid leukemia

Ammar Basim Al-Asadi[1,2], Jinan M. J. Al-Saffar[1]

Abstract:

BACKGROUND: The noncoding RNAs with covalently closed loops, known as circular RNAs (circRNAs), represent essential regulators of cancer biology. CircRNAs demonstrate stability combined with regulatory properties that position them as biomarker candidates for the diagnosis of hematologic malignancies such as acute myeloid leukemia (AML).

OBJECTIVE: The research investigates and compares the expression levels of *hsa_circ_0001947* and *hsa_circ_0009581* circRNAs in bone marrow (BM) and peripheral blood (PB) extracted from AML patients to evaluate their biological function and clinical value in AML development.

MATERIALS AND METHODS: The study enrolled 73 AML patients who received their treatment at Baghdad Teaching Hospital and the Hematology Center in the Medical City of Baghdad. The study obtained PB from 50 patients while extracting BM aspirates from 23 patients. RNA extraction was followed by cDNA synthesis. The assessment of circRNA expression through quantitative real-time polymerase chain reaction PCR (qRT-PCR) utilized the U6 housekeeping gene as a normalization factor.

RESULTS: Analysis of *hsa_circ_0001947* expression showed lower expression in BM than PB. The expression level of *hsa_circ_0009581* in BM was significantly higher than that in PB samples, which indicates how this circulatory RNA contributes to AML development in BM microenvironments.

CONCLUSION: The differential expression of circRNAs between BM and PB highlights their potential involvement in AML biology. Specifically, *hsa_circ_0009581* may serve as a promising molecular target or biomarker for AML diagnosis and monitoring. These findings contribute to the growing understanding of circRNA-mediated gene regulation in leukemia.

Keywords:

Acute myeloid leukemia, circular RNA, gene expression, *hsa_circ_0001947*, *hsa_circ_0009581*

[1]Department of Biotechnology, College of Science, University of Baghdad, [2]Department of Quality Assurance and Performance Evaluation, Ibn Sina University of Medical and Pharmaceutical Sciences, Baghdad, Iraq

Address for correspondence:
Mr. Ammar Basim Al-Asadi, PHD Student at University of Baghdad and Lecturer at Ibn Sina University, Baghdad, Iraq.
E-mail: ammar.alasadi@ibnsina.edu.iq

Submission: 21-04-2025
Revised: 17-05-2025
Accepted: 18-05-2025
Published: 20-08-2025

Introduction

Hematopoiesis is a normal formation of different blood cellular components that occurs during the early weeks of fetal development and during adulthood to make and replenish the blood system.[1,2] Red bone marrow (BM) contains hematopoietic stem cells that produce all blood cells. Red BM is the only marrow source in newborns until yellow BM takes over due to decreased erythropoietin, which activates red blood cells in BM. The amount of red marrow reduces significantly by puberty and reaches <30% in elderly individuals.[3] Rapid blood and BM cancer AML shows the signs of clonal myeloid progenitor cells, which stop differentiating into blood cells while causing multilineage cytopenias.[4,5] The clinical course of AML contains varied outcomes while revealing strong disease diversity.

How to cite this article: Al-Asadi AB, Al-Saffar JM. Comparative analysis of circular RNA expression in bone marrow and peripheral blood samples of patients with acute myeloid leukemia. Iraqi J Hematol 2025;14:186-91.

Genetic abnormalities, together with molecular-level changes and chromosome translocations, can lead to the formation of this disease. The research indicates that genetic mutations occur in 97% of observed cases.[6] Multiple genetic alterations within normal hematopoietic stem cells or their progenitors result in autonomous clones that dominate and produce marrow failure.[7] Sequential mutation acquisition causes clonal myeloid progenitor proliferation to increase and differentiation to fail, ultimately resulting in resulting in fulminant disease.[8]

Circular RNA (circRNA) represents a rapidly expanding field within RNA research that corresponds to microRNA (miRNA) research despite being an endogenous noncoding RNA. The circular structure of circRNA enables protection against RNA exonuclease degradation because it lacks conventional linear RNA terminal ends. The circular shape of circRNA confers resistance to degradation which protects against degradation by exonucleases.[9,10] Multiple miRNA-binding sites found in circRNAs produce miRNA sponge activity. Abundant circRNA types can link to miRNAs through their complementary nature, which removes these miRNAs from target mRNA proteins.[11] CircRNAs have gained considerable attention as molecular markers for accurate cancer diagnosis and prevention because of their stability properties and ribonuclease resistance.[12] Various blood cell populations contain circRNAs, which show distinct expression patterns that depend on both the cellular origin and developmental maturity of those cells. Changes in circRNAs suggest their potential to regulate the hematopoietic system, while malignant hematopoiesis seems connected to their expression modifications.[13]

This study aims to investigate and compare the expression levels of *hsa_circ_0001947* and *hsa_circ_0009581* in BM and peripheral blood (PB) samples from patients with AML, with the goal of evaluating their potential roles as diagnostic or prognostic biomarkers.

Materials and Methods

A total of 73 patients with AML were treated at the Baghdad Teaching Hospital and the Hematology Center at the Medical City in Baghdad between December 2023 and April 2024 and 30 healthy controls were included in this study. Fifty AML patients had PB samples taken, and 23 patients had BM aspirates taken for comparative molecular analysis. Patients in the investigated group ranged in age of 15–77 years, with an average age of 45.46 ± 2.38 at presentation. A sterile Eppendorf tube containing 300 µl of each sample was filled with 600 µl of TRIzol reagent to extract the RNA.

Inclusion criteria

- Patients (≥14 years of age) with a confirmed diagnosis of AML
- Individuals in apparent good health with no clinical or laboratory evidence of AML, malignancies, or other hematologic disorders.

Exclusion criteria

- Patients with incomplete clinical records
- Insufficient RNA sample quality.

Ethical approval

The Ethics Committee of the University of Baghdad's College of Science gave its approval to this work (Ref.: CSEC/0724/0048). All procedures were performed under the ethical guidelines of the institutional research committees and the 1964 Declaration of Helsinki and its later amendments.

Extraction of RNA and synthesis of cDNA

The extraction of total RNA containing circRNA proceeded from samples using TransZol Up Plus RNA Kit from Transgene/China based on manufacturer recommendations. The Quantus™ Fluorometer was used to determine the RNA concentration and purity of extracted RNA. EasyScript® one-step gDNA removal and cDNA synthesis SuperMix Kit was used to transform total RNA into cDNA.

Primer design

The primer sequences for *hsa_circ_0001947* and *hsa_circ_0009581* were designed based on the circRNA information retrieved from circBase (https://www.circbase.org/). This database provided validated circRNA back-splice junction sequences necessary for specific primer targeting.[14] All primers were synthesized in lyophilized form by Macrogen (Korea).

Primer sequences for circRNA and housekeeping gene (U6) used in this study are shown in Table 1.

Performing real-time polymerase chain reaction and gene expression calculation

Detection of cDNA using Quantitative RT-PCR (qPCR) with PerfectStart® Green qPCR SuperMix. The reaction mixtures contain 20 µl total volume using 0.5 µl of primers along with 10 µl PerfectStart® Green qPCR SuperMix (2X), 0.4 µl passive reference dye, 4 µl cDNA from each sample, and 4.6 µl nuclease-free water. Real-time PCR was applied for the duration of 30 s at a temperature of 94°C to initiate the activity of the polymerase enzyme. Subsequently, the double-stranded cDNA was denatured for 5 s at 94°C and then annealed for 35 s at 60°C using channel scanning, repeating this process 45 times. Finally, the dissociation step for 15 s at 60°C–90°C repeated 100 times. The Ct value of circRNA

Table 1: Sequences of the primers used in this study

Name of primers	Sequences (5′→3′)	Primer size (bp)	Temperature
hsa_circ_0001947			
Forward	ACAAGTGAAACAAACAAAGGTGA	23	57.54
Reverse	TCCAAGCGTGTTCTGGACTC	20	59.97
hsa_circ_0009581			
Forward	CTACAGAGGACGGCTGTGTC	20	59.8
Reverse	TCCACTCGGGCTTTAAACTCT	21	59.4
U6			
Forward	GACCTGCTCTGGTGGTCTTG	20	60.32
Reverse	GAACCACACTCTGGGACAGG	20	59.96

was normalized using the U6 housekeeping gene. The expression of circRNA was assessed using a relative quantitative technique, which involved the comparative Ct formula and the $2^{-\Delta\Delta CT}$ analysis.[15]

Statistical analysis

Data were analyzed using the Statistical Analysis System software (Version 9.6; SAS Institute Inc., Cary, NC, USA) to evaluate differences among various patient samples. A one-way analysis of variance was conducted to examine the differences in means among groups. In addition, the Chi-square test was used to evaluate relationships between categorical variables. Statistical significance was considered at probability levels of 0.05 and 0.01.

Results

According to the sex distribution of AML patients, the prevalence of illness was higher in males than in females in both study groups, as illustrated in Table 2.

Among the patients, 30.1% reported a positive family history, whereas 69.9% reported no family history, with the difference being statistically significant (*P* value = 0.0006) [Table 3].

The fold expression of *hsa_circ_0001947* was lower in AML BM (0.397 ± 0.101) samples compared to PB (0.584 ± 0.080), as shown in Table 4 and Figure 1.

The fold expression of *hsa_circ_0009581* was significantly higher in AML BM (19.147 ± 6.508) samples compared to PB (4.851 ± 1.018), as shown in Table 5 and Figure 2.

Discussion

Several studies have indicated that males are at a higher risk of developing AML, while females demonstrate a reduced mortality risk upon completing therapy, possibly due to the impact of mutational factors in males.[16,17] AML incidence was increased by family history; there are expected to be resemblances between genetic and environmental causes of myeloid malignancies, as well as the ways these factors might affect different phenotypes varying. Familial risks tended to be higher for myeloid malignancies diagnosed at a younger age, indicating a possible stronger genetic predisposition in early-onset cases.[18]

Studies showed that *hsa_circ_0001947* expression levels decreased in AML patients because of its interaction with miR-329-5p, which leads to cell growth inhibition and apoptosis.[19-21] By acting as an hsa-miR-329-5p sponge, *hsa_circ_0001947* restored the expression of the CREBRF target gene. Scientific evidence through the circRNA-miRNA-mRNA network revealed that *hsa_circ_0001947* acted as a key element in AML progression.[22]

Hu *et al.* found that *hsa_circ_0009581* was significantly upregulated in AML patients. They identified interactions between circRNAs and miR-150-5p that influence AML progression. *Hsa_circ_0009581* has been shown to promote leukemia development through its interaction with miR-150-5p. This interaction highlights the complex regulatory networks involving miRNAs and circRNAs in AML pathogenesis. The *hsa_circ_0009581*/hsa-miR-150-5p regulatory network includes multiple axes involving RAD51, CDK4, PKMYT1, MYB, HSP90B1, ZNF346, and PIK3R1.[23] The majority of AML cases demonstrate reduced expression of miR-150, while this downregulation occurs independently from DNA variations or methylation or mutation patterns. MiR-150 gene expression was lower in patients who had AML at diagnosis than in healthy participants but levels returned to normal in patients who reached complete remission.[24]

Different patterns of gene expression emerge in BM versus PB samples collected from AML patients because of differences in both cell types present alongside environmental factors influencing their expression. Researchers compared 1271 genes from differential expression in paired blood marrow and PB AML patient samples. The analysis detected three genes that exhibited differential expression, including CD5L, CXCL12, and C7, while many others belonged to immune system pathways, thus highlighting differences in immune components between BM and PB samples.[25] Studies analyzing the surface phenotypes of CD34+ myeloblasts isolated from the blood and BM of patients with AML detected equivalent surface characteristics. The homing capability was higher in CD34+ cells derived from PB. Gene expression analysis revealed that CD34+ myeloblasts from peripheral blood showed decreased activity of proteins associated with G-protein signaling, prostaglandin signaling, chemotaxis, stress response, cell proliferation, and apoptosis, likely due to the absence of regulatory influences from the bone marrow microenvironment.[26] Another study using real-time polymerase chain reaction on paired BM and PB samples from AML patients found significant differences in the expression of certain genes. HOXA9 and LTC4S were expressed at higher levels in BM, while cystatin C and LYN were more prominent in PB. These differences may reflect the distinct microenvironments and cellular compositions of BM and PB.[27]

These studies highlight that gene expression differences between BM and PB in AML patients are influenced by specific cell populations and their functional states, as well as the microenvironmental context.

Table 2: Distribution of study groups according to sex

Sex	PB AML patients, *n* (%)	BM AML patients, *n* (%)	*P*
Male	28 (56)	12 (52)	0.760 (NS)
Female	22 (44)	11 (48)	
Total	50	23	-

NS=Nonsignificant, AML=Acute myeloid leukemia, BM=Bone marrow, PB=Peripheral blood

Table 3: Distribution of acute myeloid leukemia patients according to family history

Family history	*n* (%)	*P*
Yes	22 (30.1)	0.0006**
No	51 (69.9)	
Total	73 (100)	-

**$P \leq 0.01$

However, multiple studies indicate that circRNAs demonstrate different expression levels between BM and PB in medical conditions. Scientific data revealed that particular circRNAs exhibit different expression levels within BM stem cells of individuals who suffer from osteonecrosis of the femoral head.[28] In addition, another study found distinct circRNA expression profiles in PB mononuclear cells of rheumatoid arthritis patients.[29] Moussa Agha *et al*. highlight the role of circulating miRNAs in the BM of patients with AML, providing critical insights into their potential function within the tumor microenvironment (TME). The AML TME is characterized by a dynamic molecular landscape, where the expression of various biomolecules fluctuates in accordance with disease progression and response to treatment. MiR-150-5p expression levels in BM have been assessed as possible biomarkers for the diagnosis and prognosis of AML. Research has shown that miR-150 expression in BM is downregulated in patients with AML, indicating that it may be used as a noninvasive biomarker for disease identification. Furthermore, variations in miR-150 expression have been linked to

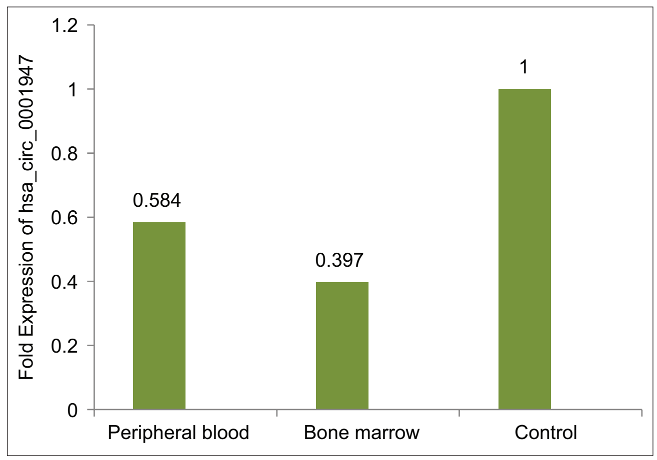

Figure 1: Fold of *hsa_circ_0001947* expression depending on the $2^{-\Delta\Delta CT}$ method

Table 4: Comparison between bone marrow and peripheral blood in fold expression of *hsa_circ_0001947* according to ΔCT calibrator method

Groups	Reference gene CT	Target gene CT	ΔCT	ΔΔCT	2^-ΔΔCT	Fold change
PB AML	19.388±0.240	28.004±0.332	8.616±0.337	1.016±0.337	1.041±0.142	0.584±0.080
BM AML	19.669±0.625	29.748±0.562	10.078±0.649	2.478±0.649	0.708±0.180	0.397±0.101
Control	18.926±0.235	26.536±0.287	7.610±0.363	0.010±0.363	1.785±0.337	1.00±0.00
P	0.3735NS	0.00001**	0.0021**	0.0021**	0.0073**	0.0073**

**$P \leq 0.01$. NS=Nonsignificant, AML=Acute myeloid leukemia, BM=Bone marrow, PB=Peripheral blood

Table 5: Comparison between bone marrow and peripheral blood in fold expression of *hsa_circ_0009581* according to ΔCT calibrator method

Groups	Reference gene CT	Target gene CT	ΔCT	ΔΔCT	$2^{-\Delta\Delta CT}$	Fold change
PB AML	19.388±0.240	25.874±0.332	6.486±0.263	−1.714±0.263	6.695±1.404	4.851±1.018
BM AML	19.669±0.625	24.857±0.695	5.187±0.495	−3.013±0.495	26.423±8.982	19.147±6.508
Control	18.926±0.235	27.117±0.231	8.190±0.263	−0.010±0.263	1.388±0.160	1.00±0.00
P	0.3735NS	0.0031**	0.00001**	0.00001**	0.00013**	0.00013**

**$P \leq 0.01$. NS=Nonsignificant, AML=Acute myeloid leukemia, BM=Bone marrow, PB=Peripheral blood

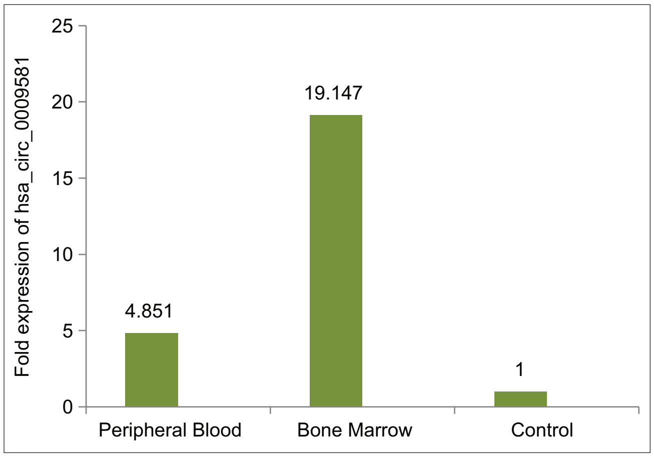

Figure 2: Fold of *hsa_circ_0009581* expression depending on the $2^{-\Delta\Delta CT}$ method

responses to treatment, suggesting that it has predictive significance.[30] Vascular endothelial cells need signaling pathways located within BM microenvironments, including bone morphogenetic protein signals, to keep genomes intact through RAD51-mediated maintenance mechanisms. External cellular signals function together with DNA repair mechanisms to preserve the operational capacity of the BM niche.[31] Multiple myeloma (MM) shows elevated RAD51 expression because it originates from plasma cells present in BM tissue. The elevated levels of RAD51 expression relate to better DNA restoration capacity, which makes cells less sensitive to DNA-destroying drugs like melphalan. Research has established that using peptide nucleic acids against RAD51 makes MM cells more susceptible to chemotherapy drugs which support its potential therapeutic application.[32]

Conclusion

This study demonstrates that *hsa_circ_0009581* is significantly upregulated while *hsa_circ_0001947* was downregulated in BM compared to PB samples of AML patients, suggesting its involvement in leukemogenesis and potential use as a biomarker. These findings underscore the importance of the BM microenvironment in regulating circRNA expression and support further research into circRNAs as molecular tools in AML diagnosis, prognosis, and therapy.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Calvanese V, Mikkola HK. The genesis of human hematopoietic stem cells. Blood 2023;142:519-32.
2. Alwan AF. Clinical features and therapeutic outcome of 30 patients diagnosed with primary myelofibrosis at the national center of hematology. J Fac Med Baghdad 2014;56:362-6.
3. Lee JY, Hong SH. Hematopoietic stem cells and their roles in tissue regeneration. Int J Stem Cells 2020;13:1-12.
4. Thomas D, Majeti R. Biology and relevance of human acute myeloid leukemia stem cells. Blood 2017;129:1577-85.
5. Hamad HM, Shabeeb ZA, Awad MM. Expressions of CD274 (PD-L1) and CD47 rReceptors on the sSurface of bBlast cCells in AML pPatients. Iraqi J Sci 2022;63:2373-87.
6. Kurzer JH, Weinberg OK. Updates in molecular genetics of acute myeloid leukemia. Semin Diagn Pathol 2023;40:140-51.
7. Almohsen F, Al-Rubaie HA, Habib MA, Nasr SA, Perni R, Al-Quraishi L. Circulating miR-126-3p and miR-423-5p expression in *de novo* adult acute myeloid leukemia: Correlations with response to induction therapy and the 2-year overall survival. J Blood Med 2022;13:83-92.
8. Wachter F, Pikman Y. Pathophysiology of acute myeloid leukemia. Acta Haematol 2024;147:229-46.
9. Zhao X, Zhong Y, Wang X, Shen J, An W. Advances in circular RNA and its applications. Int J Med Sci 2022;19:975-85.
10. Tan KE, Lim YY. Viruses join the circular RNA world. FEBS J 2021;288:4488-502.
11. Ikeda Y, Morikawa S, Nakashima M, Yoshikawa S, Taniguchi K, Sawamura H, *et al.* CircRNAs and RNA-binding proteins involved in the pathogenesis of cancers or central nervous system disorders. NcRNA 2023;9:23.
12. Pisignano G, Michael DC, Visal TH, Pirlog R, Ladomery M, Calin GA. Going circular: History, present, and future of circRNAs in cancer. Oncogene 2023;42:2783-800.
13. Rahmati A, Mafi A, Soleymani F, Babaei Aghdam Z, Masihipour N, Ghezelbash B, *et al.* Circular RNAs: Pivotal role in the leukemogenesis and novel indicators for the diagnosis and prognosis of acute myeloid leukemia. Front Oncol 2023;13:1149187.
14. Glazar P, Papavasileiou P, Rajewsky N. circBase: A database for circular RNAs. RNA 2014;20:1666-70. Available from: https://www.circbase.org. [Last accessed on 2024 Apr 15].
15. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25:402-8.
16. Stabellini N, Tomlinson B, Cullen J, Shanahan J, Waite K, Montero AJ, *et al.* Sex differences in adults with acute myeloid leukemia and the impact of sex on overall survival. Cancer Med 2023;12:6711-21.
17. Naji AS, Gata AK, Hamodi LE, Abass M, Yousif FS. Outcome of allogeneic bone marrow transplant for Iraqi patients with acute myeloid leukemia. Wiad Lek 2021;74:2247-54.
18. Sud A, Chattopadhyay S, Thomsen H, Sundquist K, Sundquist J, Houlston RS, *et al.* Familial risks of acute myeloid leukemia, myelodysplastic syndromes, and myeloproliferative neoplasms. Blood 2018;132:973-6.
19. Babin L, Andraos E, Fuchs S, Pyronnet S, Brunet E, Meggetto F. From circRNAs to fusion circRNAs in hematological malignancies. JCI Insight 2021;6:e151513.
20. Singh V, Uddin MH, Zonder JA, Azmi AS, Balasubramanian SK. Circular RNAs in acute myeloid leukemia. Mol Cancer 2021;20:149.
21. Mirazimi Y, Aghayan AH, Keshtkar A, Mottaghizadeh Jazi M, Davoudian A, Rafiee M. CircRNAs in diagnosis, prognosis, and clinicopathological features of multiple myeloma; a systematic review and meta-analysis. Cancer Cell Int 2023;23:178.
22. Han F, Zhong C, Li W, Wang R, Zhang C, Yang X, *et al.* hsa_circ_0001947 suppresses acute myeloid leukemia progression via targeting hsa-miR-329-5p/CREBRF axis. Epigenomics 2020;12:935-53.
23. Hu L, Zheng B, Yang Y, Chen C, Hu M. Construction of circRNA-miRNA-mRNA network reveal functional circRNAs and key genes in acute myeloid leukemia. Int J Gen Med 2023;16:1491-504.

24. Wang F, Ren X, Zhang X. Role of microRNA-150 in solid tumors. Oncol Lett 2015;10:11-6.
25. Caliskan G, Pawitan Y, Vu TN. Similarities and differences of bone marrow and peripheral blood samples from acute myeloid leukemia patients in terms of cellular heterogeneity and *ex-vivo* drug sensitivity. EJHaem 2024;5:721-7.
26. Cheung AM, Chow HC, Liang R, Leung AY. A comparative study of bone marrow and peripheral blood CD34+ myeloblasts in acute myeloid leukaemia. Br J Haematol 2009;144:484-91.
27. Sakhinia E, Farahangpour M, Tholouli E, Liu Yin JA, Hoyland JA, Byers RJ. Comparison of gene-expression profiles in parallel bone marrow and peripheral blood samples in acute myeloid leukaemia by real-time polymerase chain reaction. J Clin Pathol 2006;59:1059-65.
28. Xiang S, Li Z, Weng X. Changed cellular functions and aberrantly expressed miRNAs and circRNAs in bone marrow stem cells in osteonecrosis of the femoral head. Int J Mol Med 2020;45:805-15.
29. Zheng F, Yu X, Huang J, Dai Y. Circular RNA expression profiles of peripheral blood mononuclear cells in rheumatoid arthritis patients, based on microarray chip technology. Mol Med Rep 2017;16:8029-36.
30. Moussa Agha D, Rouas R, Najar M, Bouhtit F, Naamane N, Fayyad-Kazan H, *et al.* Identification of acute myeloid leukemia bone marrow circulating MicroRNAs. Int J Mol Sci 2020;21:7065.
31. Vattulainen-Collanus S, Southwood M, Yang XD, Moore S, Ghatpande P, Morrell NW, *et al.* Bone morphogenetic protein signaling is required for RAD51-mediated maintenance of genome integrity in vascular endothelial cells. Commun Biol 2018;1:149.
32. Alagpulinsa DA, Yaccoby S, Ayyadevara S, Shmookler Reis RJ. A peptide nucleic acid targeting nuclear RAD51 sensitizes multiple myeloma cells to melphalan treatment. Cancer Biol Ther 2015;16:976-86.