



**Original Article**

**The Mechanism of miR-155/miR-15b Axis Contributed to Apoptosis of CD34+ Cells by Upregulation of PD-L1 in Myelodysplastic Syndromes**

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**Abstract.** Myelodysplastic syndromes (MDS) are a group of heterogeneous myeloid clonal diseases that are characterized by ineffective bone marrow hematopoiesis. Since studies have confirmed the significance of miRNAs in ineffective hematopoiesis in MDS, the current report elucidated the mechanism mediated by miR-155-5p. The bone marrow of MDS patients was collected to detect miR-155-5p and to analyze the correlation between miR-155-5p and clinicopathological variables. Isolated bone marrow CD34<sup>+</sup> cells were transfected with lentiviral plasmids that interfere with miR-155-5p, followed by apoptosis analysis. Finally, miR-155-5p-targeted regulation of RAC1 expression was identified, as well as the interaction between RAC1 and CREB, the co-localization of RAC1 and CREB, and the binding of CREB to miR-15b. As measured, miR-155-5p was upregulated in the bone marrow of MDS patients. Further cell experiments validated that miR-155-5p promoted CD34<sup>+</sup> cell apoptosis. miR-155-5p could reduce the transcriptional activity of miR-15b by inhibiting RAC1, dissociating the interaction between RAC1 and CREB, and inhibiting the activation of CREB. Upregulating RAC1, CREB, or miR-15b could reduce miR-155-5p-mediated apoptosis promotion on CD34<sup>+</sup> cells. Additionally, miR-155-5p could force PD-L1 expression, and this effect was impaired by elevating RAC1, CREB, or miR-15b. In conclusion, miR-155-5p mediates PD-L1-mediated apoptosis of CD34<sup>+</sup> cells in MDS by RAC1/CREB/miR-15b axis, thereby inhibiting bone marrow hematopoiesis.

**Keywords:** miR-155-5p; RAC1; CREB; miR-15b; PD-L1; Myelodysplastic syndrome.

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**Introduction.** Myelodysplastic syndromes (MDS) are a myeloid neoplasm characterized by abnormal differentiation and development of myeloid cells, manifested as ineffective hematopoiesis, refractory cytopenia, hematopoietic failure, and a high risk of transformation to acute myeloid leukemia.<sup>1</sup> For low-risk MDS, anemia, thrombocytopenia, multiple and/or refractory cytopenias are preferred, while for high-risk diseases, allogeneic HCT and hypomethylating agents are clinically recommended.<sup>2</sup> Prediction of prognosis is complicated by the fact that MDS is primarily a disease of the elderly, with frequent comorbidities and/or a patient population with environmental or therapeutic exposure to mutagens.<sup>3</sup> Genomics in MDS is needed today to improve diagnosis, assign classification and define prognosis, allow patient-tailored treatment, and even provide strategies to detect minimal residual disease.<sup>4</sup>

Theoretically, there are complex interactions between genetic and epigenetic factors, including miRNAs, ranging from clonal hematopoiesis of uncertain potential to hematological malignancies. It is underlined that miRNAs with differential expression may represent pathogenic implications of MDS in part by regulating hematopoiesis, and miR-155-5p is an upregulated miRNA in MDS.<sup>5</sup> More importantly, miR-155 transcript maintains a high level in CD34<sup>+</sup> MDS cells<sup>6</sup> and miR-155 can decrease RAC1 protein to regulate MDS neutrophils migration.<sup>7</sup> RAC1, a member of the Rho GTPases family, functions in various hematopoietic cell lineages<sup>8</sup> and RAC1 activation promotes human hematopoietic stem and progenitor cell maintenance.<sup>9</sup> Here, the work makes a thorough inquiry on the interlink between miR-155-5p and RAC1 in CD34<sup>+</sup> MDS cell apoptosis.

RAC1 can regulate and activate CREB, a key transcription factor regulating cell cycle, apoptosis, metabolism, DNA repair, etc.<sup>10</sup> Activated CREB can bind to the corresponding sites of other genes and regulate transcriptional expression of target genes,<sup>11,12</sup> including miR-15b.<sup>13</sup> A report discusses the combined loss-of-function of miR-15/16 drives the pathogenesis of acute myeloid leukemia.<sup>14</sup> However, whether miR-15b modulates CD34<sup>+</sup> MDS cell apoptosis is still an unsolved question.

Taking into consideration all factors, the present study mainly discussed and explained the mechanism of miR-155-5p in CD34<sup>+</sup> MDS cells through targeting RAC1 to manipulate CREB/miR-15b interaction, determining to carry forward molecule-based therapy for MDS.

## Materials and Methods.

*Clinical sample collection.* Forty cases of bone marrow specimens were obtained from healthy donors undergoing orthopedic surgery and MDS patients in

Guangzhou Women and Children's Medical Center, Guangzhou Medical University, respectively. The current study complied with the ethical standards of the ethics committee of Guangzhou Women and Children's Medical Center, Guangzhou Medical University. In addition, all patients and healthy donors obtained written informed consent. Patients are classified according to the World Health Organization Classification 2022. Prognostic impact was assessed using the Revised International Prognostic Scoring System (IPSS-R) and Molecular International Prognostic Scoring System (IPSS-M).<sup>15,16</sup> **Table 1** shows patients' clinical characteristics. Cytogenetic data of MDS are presented in **Supplementary Table 1**.

*CD34<sup>+</sup> cell isolation.* Bone marrow specimens were collected in 2 mM EDTA, from which monocytes were isolated using Ficoll-Paque and CD34<sup>+</sup> cells were positively selected using the CD34 Bead Kit (Miltenyi Biotec, USA). Before RNA preparation, CD34<sup>+</sup> cells were tested by flow cytometry (CD34<sup>+</sup> cells make up more than 90% of isolated cells). Purified CD34<sup>+</sup> cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum at 37°C and 5% CO<sub>2</sub>-saturated humidity.

*Cell transfection.* miR-155-5p overexpression or knockout stable cell system was constructed in CD34<sup>+</sup> cells: Lentiviral plasmids, including Lv-miR-155-5p mimic, Lv-miR-155-5p inhibitor, Lv-RAC1, Lv-CREB, Lv-miR-15b mimic, and the corresponding empty controls were produced. Then, 293T cells were conditioned to lentiviral plasmid transfection, and CD34<sup>+</sup> cells were infected with the virus fluid and added with polybrene. The stably expressed cell clones were screened with puromycin.

*MTT.* CD34<sup>+</sup> cells at 80% confluence were trypsinized, prepared into a single-cell suspension, and seeded into 96-well plates (3 × 10<sup>3</sup> cells/well). After 72 h, a medium containing 10% MTT solution (5 g/L, GD Y1317; Guduo Biotechnology Co, Ltd, Shanghai, China) was added for 4 h, and DMSO (D5879 100ML; Sigma Aldrich, USA) was supplemented at 100 μL/well. Optical density values were measured using a microplate reader (Nanjing Iron & Steel Co, Ltd, Nanjing, Jiangsu, China) at 490 nm.

*Flow cytometry.* CD34<sup>+</sup> cells conditioned to 48-h culture were detached with 0.25% trypsin, centrifuged (4°C, 1000 rpm, 5 min), and fixed with pre-chilled 70% ethanol overnight at 4°C. Next, cells were incubated with 10 μL of RNase enzyme at 37°C for 5 min, stained with 1% propidium iodide (40710ES03; Qcbio, Shanghai, China) for 30 min, and placed in a FACSCalibur (BD Bioscience, USA) to test cell cycle by measuring red fluorescence at 488 nm.

**Table 1** Clinical features of patients with MDS.

Items	MDS patients (n=40)
Age (years), Median (range)	70 (45-85)
Hemoglobin (g/dL), Median (range)	9.6 (7.1-14.0)
ANC (10 <sup>3</sup> /mL), Median (range)	2000 (150-28000)
Platelets (10 <sup>3</sup> /mL), Median (range)	94000 (13000-716000)
BM blasts (%), Median (range)	6 (2-60)
Overall survival (months), Median (range)	16 (1-45)
Gender, n (%)	
Female	28 (70.0)
Male	12 (30.0)
Chromosome abnormalities, n (%)	18 (45.0)
WHO 2022 classification, n (%)	
MDS-5q-	1 (2.5)
MDS-SF3B1	3 (7.5)
MDS-biTP53	3 (7.5)
MDS-LB	14 (35.0)
MDS-h	4 (10.0)
MDS-IB1	8 (20.0)
MDS-IB2	5 (12.5)
MDS-f	2 (4.0)
IPSS-R risk, n (%)	
Very low	5 (12.5)
Low	9 (22.5)
Intermediate	10 (25.0)
High	10 (25.0)
Very high	6 (15.0)
IPSS-M risk, n (%)	
Very low	3 (7.5)
Low	7 (17.5)
Moderate low	6 (15.0)
Moderate high	5 (12.5)
High	10 (25.0)
Very high	9 (22.5)
Mutated genes per patient, median (range)	2 (0-6)

Note: ANC, Neutrophil absolute value; BM, Bone Marrow; WHO, World Health Organization; MDS, myelodysplastic syndromes; 5q-, isolated 5q deletion; MDS-SF3B1, MDS with low blasts and SF3B1 mutation; biTP53, biallelic TP53 inactivation; LB, low blasts; MDS-h, MDS hypoplastic; IB1/2, increased blasts type1/2; MDS-f, MDS with fibrosis; IPSS-R, Revised International Prognostic Scoring System; IPSS-M, International Prognostic Scoring Systems-Molecular.

CD34<sup>+</sup> cells after 48-h culture were detached with EDTA-free trypsin and centrifuged (4°C, 1000 rpm, 5 min). Apoptosis was determined by Annexin V FITC/PI Apoptosis Detection Kit (CA1020; Solarbio Beijing, China). In detail, cells were washed with a binding buffer, combined with a 1:40 mixture (Annexin V FITC + binding buffer) for 30 min, and reacted with another mixture of PI and binding buffer for 15 min. Finally,

apoptosis rates were analyzed by a BD FACS Aria (BD Biosciences).

**Hoechst staining.** CD34<sup>+</sup> cells were seeded into 12-well plates for 48 h and combined with Hoechst 33342 (Sigma-Aldrich) for 1 h. Darkly stained condensed nuclei indicate apoptosis.<sup>17</sup>

**RT-qPCR.** Total RNA from CD34<sup>+</sup> cells was extracted using the Trizol kit (15596 026; Invitrogen), reverse transcribed into cDNA using the reverse transcription kit (K1621; Fermentas, USA), and analyzed by PCR on an ABI 7500 system. Primer sequences are listed in **Table 2** (GeneChem, Shanghai, China). Gene expression was standardized by U6 or GAPDH, and determined by the 2<sup>-ΔΔCt</sup> method.

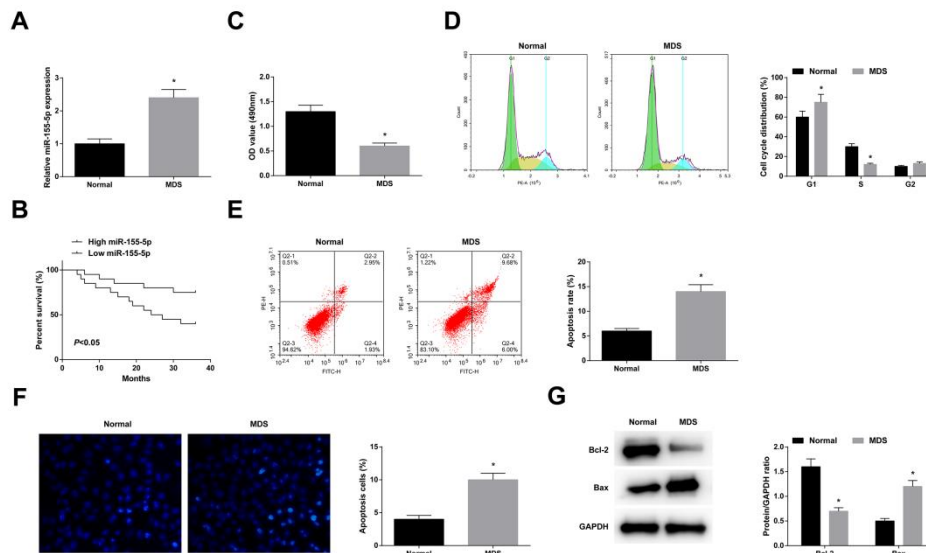
**Immunoblotting.** Protein lysis (R0010; Solarbio) was added to CD34<sup>+</sup> cells to isolate total protein, which was then separated using 12% SDS-polyacrylamide gel electrophoresis (P1200; Solarbio) and loaded onto a polyethylene difluoride membrane (HVLPO4700; Millipore). Next, nonfat milk-blocked membranes were added with primary antibodies RAC1 (1:1000; 05-389; MilliporeSigma), CREB (1:1000; 9198; Cell Signaling Technology), p-CREB (1:1000; 9197; Cell Signaling Technology), PD-L1 (1:1000; ab205921) Abcam), Bcl-2 (1:1000; ab32124; Abcam), Bax (1:1000; ab32503; Abcam) and GAPDH (1:1000; ab8245; Abcam) overnight at 4°C, and supplemented with horseradish peroxidase-conjugated secondary antibody (1:2000, ab6721; Abcam) for 2 h. Following diaminobenzidine development, imaging was done under Gel Doc Xr (Bio-Rad).

**Luciferase reporter gene assay.** Constructs with wild-type (WT) or mutant (MUT) miR-155-5p binding sites were established and designated RAC1-WT or RAC1-MUT. CD34<sup>+</sup> cells were seeded into 96-well plates for 24 h and co-transfected with the constructs, along with

**Table 2** Primer sequences for RT-qPCR.

Genes	Primer sequences (5'-3')
miR-155-5p	F: TAATCGTGATAGGGGTTT R: GGAGTCAGTTGGAGGC
miR-15b	F: AACTCCAGCTGGGTAGCAGCACATCATG R: GGAGTCAGTTGGAGGC
RAC1	F: GGCTAAGGAGATTGGTGCTGTA R: ACGAGGGGCTGAGACATTTAC
U6	F: CTCGCTTCGGCAGCACA R: AACGCTTCACGAATTTGCGT
GAPDH	F: CACCCACTCCTCCACCTTTG R: CCACCACCCTGTTGCTGTAG

Note: miR-155-5p, microRNA-155-5p; miR-15b, microRNA-15b; RAC1, Ras-related C3 botulinum toxin substrate 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase



**Figure 1** miR-155-5p levels in bone marrow CD34<sup>+</sup> cells of MDS patients. miR-155-5p expression in the bone marrow CD34<sup>+</sup> cells of MDS patients (A). Kaplan-Meier test of patient's survival (B). CD34<sup>+</sup> cell proliferation (C), cell cycle arrest (D), apoptosis cells (E), Bcl-2 and Bax protein expression (F-G) in MDS patients.

miR-155-5p mimic or mimic NC using Lipofectamine 3000. After 48 h, CD34<sup>+</sup> cells were obtained and analyzed using a luciferase reporter gene assay system.

**Co-immunoprecipitation (co-IP).** CD34<sup>+</sup> cells were lysed in HEPES lysis buffer containing 20 mM HEPES (pH 7.2), 50 mM NaCl, 0.5% Triton X-100, 1 mM NaF, 1 mM dithiothreitol and a cocktail of protease and phosphatase inhibitors. Cell lysates were incubated with anti-RAC1 or normal rabbit IgG (1:800, N101, Sigma-Aldrich) for 3 h and added with protein A/G-agarose beads (SC-2003; Santa Cruz) overnight at 4°C. After washing with HEPES buffer 3 times, immunoprecipitates were collected by 12% SDS-polyacrylamide gel electrophoresis and tested by immunoblotting.<sup>18</sup>

**Immunofluorescence.** Cells were fixed in 4% paraformaldehyde for 20 min and incubated in 50 mM glycine for 20 min. Cells permeabilized in 0.1% Triton X-100 for 30 min were blocked with 10% goat serum (Wako Pure Chemical Industries, Ltd.) for 60 min, and sequentially incubated with anti-RAC1 and anti-CREB, followed by co-incubation with Alexa 488-labeled anti-rabbit IgG antibody (1:1,000, A11031, Thermo Fisher Scientific). After DAPI staining, images were viewed using a Digital Eclipse C1 TE2000-E confocal microscope (Nikon).

**Chromatin Immunoprecipitation.** Following the instructions of ChromaFlash High Sensitivity ChIP Kit (Epigentek), CD34<sup>+</sup> cells were transfected with HA-CREB for 24 h, cross-linked with 1% formaldehyde for 5 min, and added with glycine to 125 mM concentration. CD34<sup>+</sup> cells were sonicated to 100-700 bp, and interacted with HA rabbit antibody (Abcam), CREB rabbit

antibody (Abcam), or rabbit IgG at 4°C. The extracted and purified ChIP DNA was PCR-amplified with primer pairs. miR-15b promoter, F: AACCTTTTCTCACCAGGTCCA; miR-15b promoter, R: TTCAGAAGAGGGGCGGGCTC. PCR products were separated by electrophoresis and visualized by UV.

**Statistical analysis.** All data were processed by SPSS 21.0, and measurement data were expressed in the form of mean ± standard deviation. Measurement data following normal distribution were analyzed by t test, one-way ANOVA and Tukey's post-hoc test. Survival analysis was implemented with the Kaplan-Meier test. *P* < 0.05 represents statistical significance.

## Results.

**miR-155-5p levels in bone marrow CD34<sup>+</sup> cells of MDS patients.** RT-qPCR found that miR-155-5p was upregulated in the bone marrow CD34<sup>+</sup> cells of MDS patients (Figure 1A). MDS patients were divided into miR-155-5p high expression group and low expression group according to the median expression of miR-155-5p. Kaplan-Meier test reported poor survival of MDS patients in the miR-155-5p high expression group (Figure 1B). Analysis of clinicopathological variables in Table 3 validated that miR-155-5p expression was associated with BM blast percentage, IPSS-R, IPSS-M, and mutated genes per patient. In the bone marrow of MDS patients, it tested reduced CD34<sup>+</sup> cell proliferation, cell arrest in the G1 phase, increased apoptosis rates, decreased Bcl-2, and elevated Bax protein expression (Figure 1C-G).

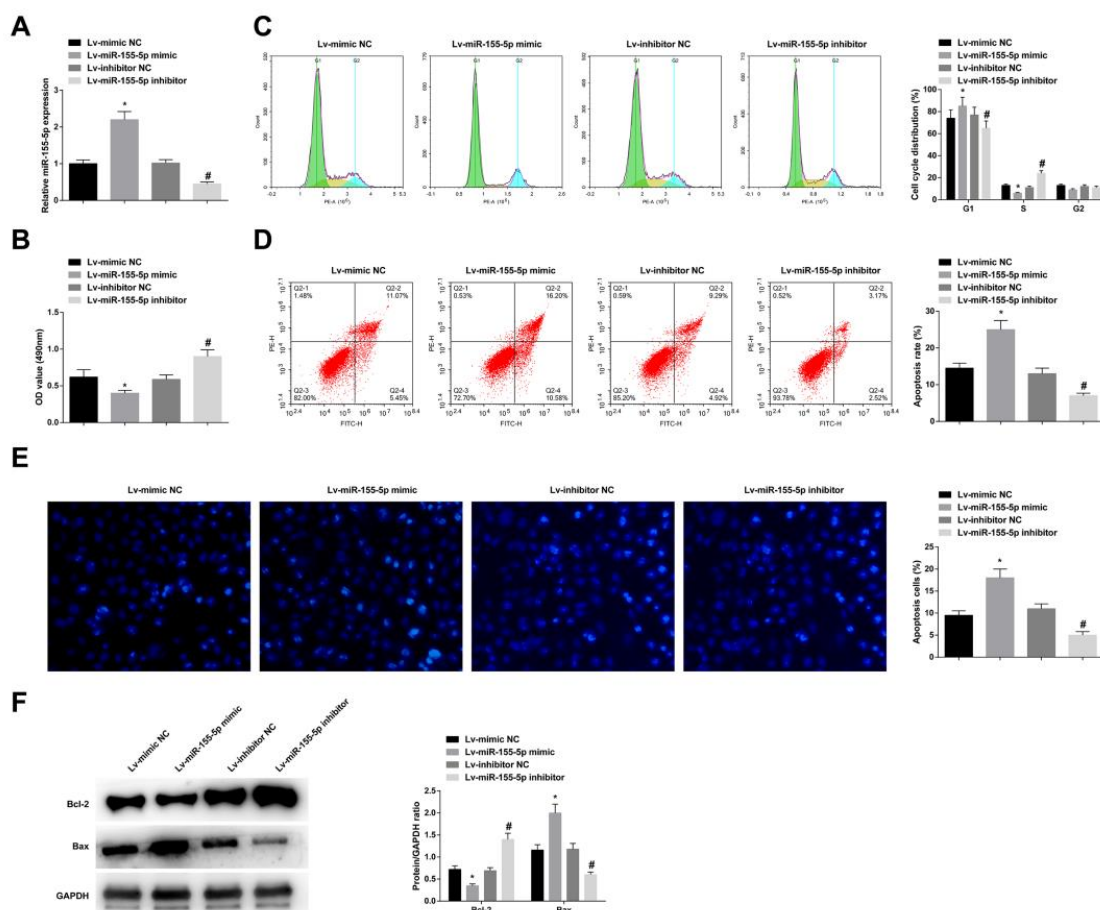
**miR-155-5p promotes apoptosis of bone marrow CD34<sup>+</sup> cells.** Lv-miR-155-5p mimic, Lv-miR-155-5p inhibitor,



**Table 3** Correlation between miR-155-5p and clinicopathological variables in MDS patients.

Variable	Total	miR-155-5p		P
		High (n = 20)	Low (n = 20)	
BM blasts				0.001
≤ 10%	28	9	19	
> 10%	12	11	1	
IPSS-R risk				< 0.001
Very low or low	14	1	13	
Intermediate	10	6	4	
High or very high	16	13	3	
IPSS-M risk				0.001
Very low or low	10	1	9	
Moderate low or Moderate high	11	4	7	
High or very high	19	15	4	
Mutated genes per patient				0.002
≤ 3	26	8	18	
> 3	14	12	2	

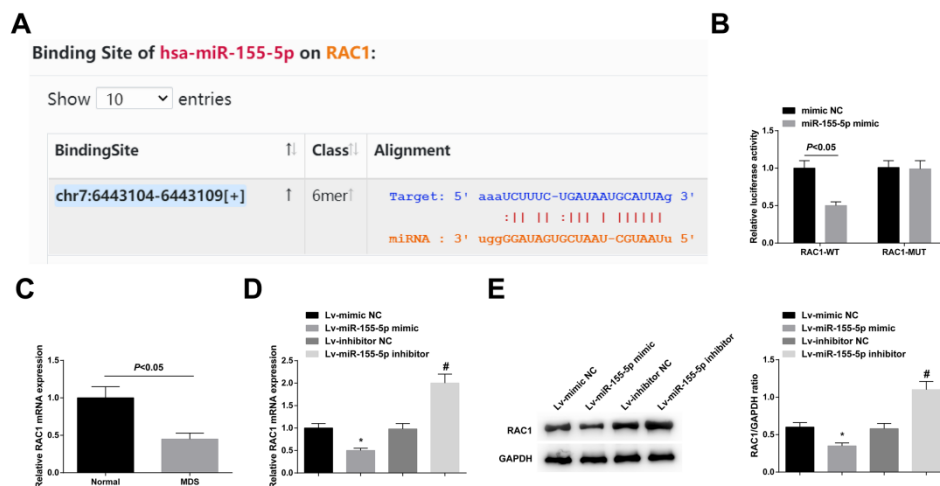
Note: BM, Bone Marrow; WHO, World Health Organization; IPSS-R, Revised International Prognostic Scoring System; IPSS-M, International Prognostic Scoring Systems-Molecular.



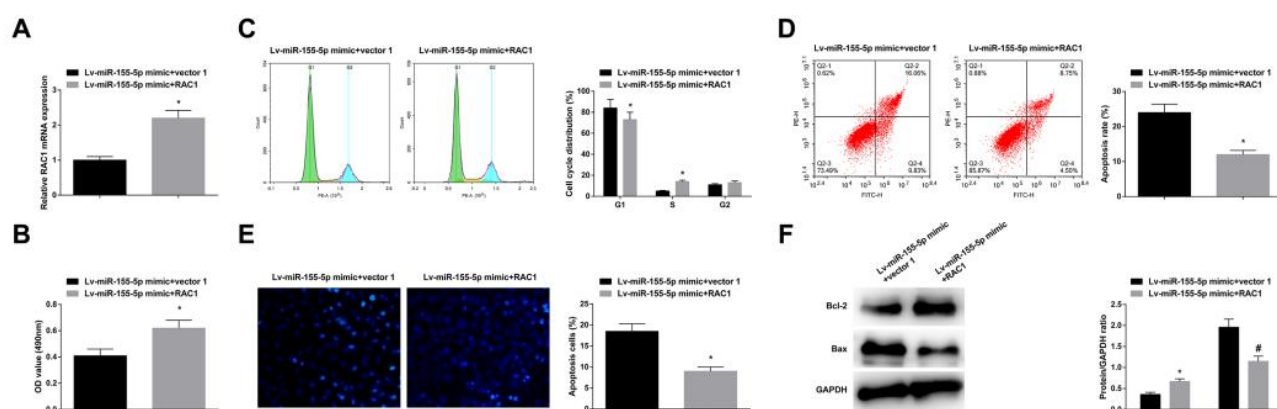
**Figure 2** miR-155-5p promotes apoptosis of bone marrow CD34<sup>+</sup> cells. Transfection efficacy of Lv-miR-155-5p mimic and Lv-miR-155-5p inhibitor in CD34<sup>+</sup> cells (A). Transfection-mediated alternations in CD34<sup>+</sup> cell proliferation (B), cell cycle arrest (C), apoptosis cells (D-E), Bcl-2 and Bax protein expression (F).

or negative control was transfected into CD34<sup>+</sup> cells. RT-qPCR verified that the lentiviral vector was successfully transfected (Figure 2A). MTT proliferation analysis

found that after upregulating and downregulating miR-155-5p, CD34<sup>+</sup> cell proliferation was obstructed and forced, respectively (Figure 2B). Flow cytometry



**Figure 3 miR-155-5p targets RAC1.** Binding site between miR-155-5p and RAC1 (A). Luciferase reporter assay results (B). RAC1 expression in bone marrow CD34<sup>+</sup> cells of MDS patients (C). miR-155-5p transfection-mediated changes in RAC1 expression in CD34<sup>+</sup> cells (D-E).



**Figure 4 miR-155-5p modulates CD34<sup>+</sup> cell apoptosis by interacting with RAC1.** Transfection efficacy of Lv-miR-155-5p mimic + RAC1 in CD34<sup>+</sup> cells (A). Transfection-mediated alternations in CD34<sup>+</sup> cell proliferation (B), cell cycle arrest (C), apoptosis cells (D-E), Bcl-2 and Bax protein expression (F).

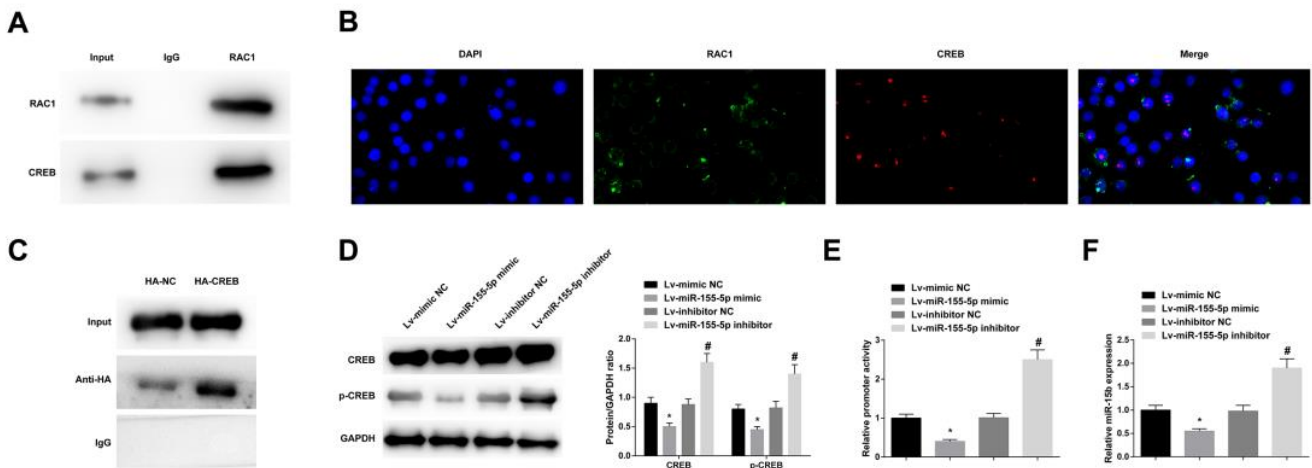
detection discovered an increased number of CD34<sup>+</sup> cells arrested in the G1 phase and promoted apoptosis rates when miR-155-5p was overexpressed; upon miR-155-5p knockdown, the number of CD34<sup>+</sup> cells in the G1 phase arrest was decreased, along with low apoptosis rates (Figure 2C, D). Hoechst staining manifested that elevating miR-155-5p levels was supportive for CD34<sup>+</sup> cell apoptosis, while reducing miR-155-5p levels was suppressive for cellular apoptosis (Figure 2E). Moreover, immunoblotting for apoptosis-related proteins further measured that Bcl-2 decreased and Bax increased in CD34<sup>+</sup> cells overexpressing miR-155-5p; the expression trend of the two proteins in CD34<sup>+</sup> cells lowly-expressing miR-155-5p were contrary to that in CD34<sup>+</sup> cells overexpressing miR-155-5p (Figure 2F). After treatment of CD34<sup>+</sup> cells with the Bcl-2 inhibitor venetoclax, cell proliferation decreased and apoptosis increased (supplementary Figure 1A-E), similar to the results after upregulation of miR-155-5p.

*miR-155-5p targets RAC1.* Searched by the online software starBase 3.0, it was found a binding site between miR-155-5p and RAC1 (Figure 3A). Luciferase

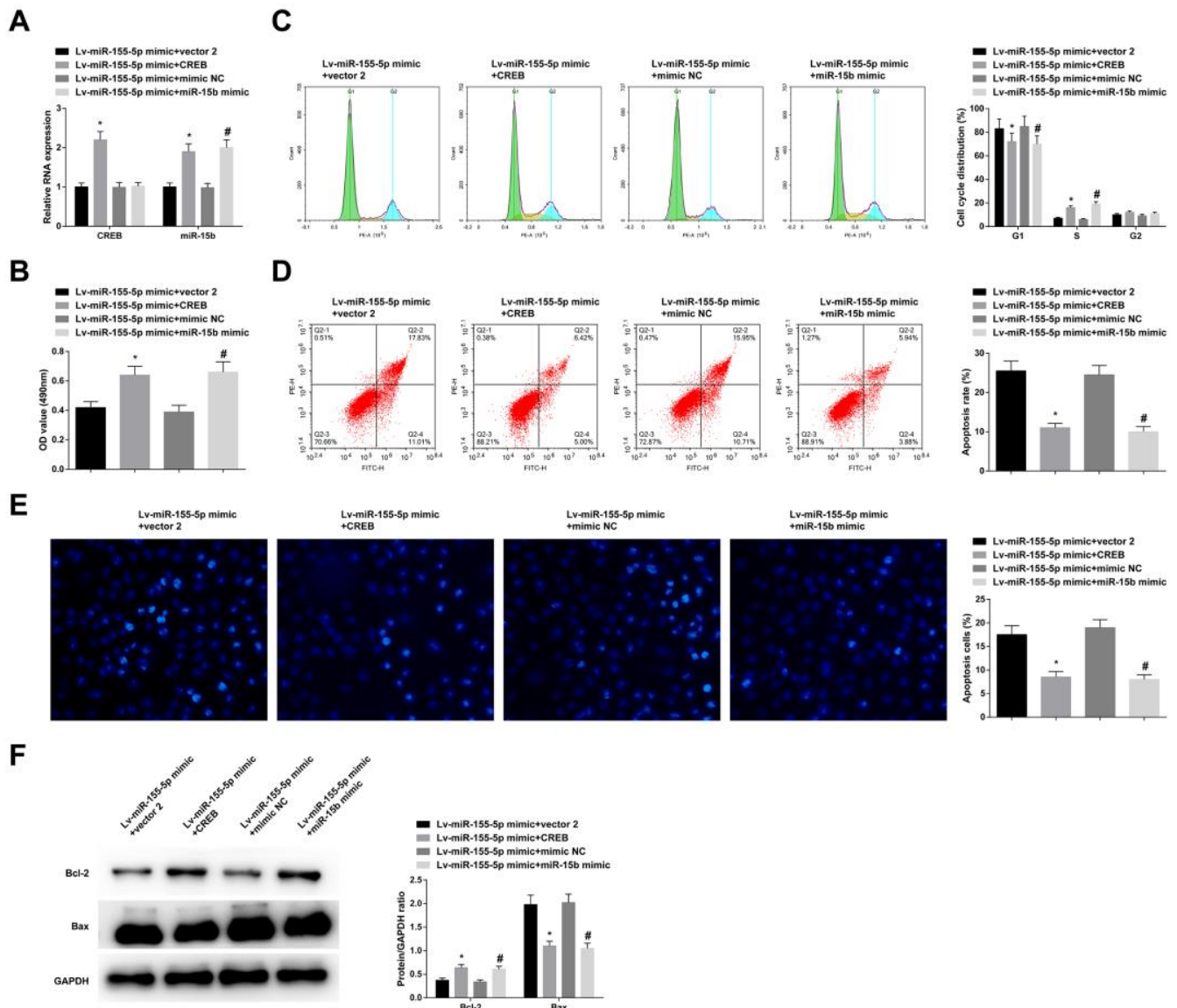
reporter assay supported that miR-155-5p mimic reduced the luciferase activity of the RAC1-WT (Figure 3B). RAC1 was downregulated in bone marrow CD34<sup>+</sup> cells of MDS patients (Figure 3C). Moreover, RAC1 expression was enhanced in CD34<sup>+</sup> cells transfected with Lv-miR-155-5p mimic, or decreased in those transfected with Lv-miR-155-5p inhibitor (Figure 3D, E).

*miR-155-5p modulates CD34<sup>+</sup> cell apoptosis by interacting with RAC1.* Lv-miR-155-5p mimic + RAC1 were co-transfected into CD34<sup>+</sup> cells, and the transfection success was verified (Figure 4A). In response to RAC1 overexpression, the impacts of miR-155-5p overexpression came to a reversal, resulting in enhanced proliferation (Figure 4B), cell cycle arrest, reduced apoptosis rates (Figure 4C-E), elevated Bcl-2, and reduced Bax levels (Figure 4F).

*miR-155-5p reduces the transcriptional activity of miR-15b by RAC1/CREB axis.* Immunoprecipitation data validated the interaction of RAC1 with CREB, demonstrating RAC1 binding to CREB (Figure 5A). Then, immunofluorescence staining revealed that RAC1



**Figure 5** miR-155-5p reduces the transcriptional activity of miR-15b by RAC1/CREB axis. Immunoprecipitation validation of the interaction of the RAC1 with CREB (A). RAC1 and CREB co-localization (B). Binding relationship of CREB to miR-15b promoter (C). miR-155-5p transfection-mediated changes in CREB and p-CREB levels (D). miR-155-5p transfection-mediated changes in the transcriptional activity of miR-15b promoter and miR-15b expression (E-F).



**Figure 6** Overexpression of CREB or miR-15b suppresses miR-155-5p-induced apoptosis of CD34<sup>+</sup> cells. Transfection efficacy of Lv-miR-155-5p mimic + CREB or Lv-miR-155-5p mimic + miR-15b mimic in CD34<sup>+</sup> cells (A). Transfection-mediated alternations in CD34<sup>+</sup> cell proliferation (B), cell cycle arrest (C), apoptosis cells (D-E), Bcl-2 and Bax protein expression (F).

and CREB co-localized in the nucleus (**Figure 5B**). Furthermore, chromatin immunoprecipitation found that CREB could bind to the miR-15b promoter (**Figure 5C**). It was further measured that elevating miR-155-5p reduced CREB and p-CREB levels, while downregulating miR-155-5p exerted oppositely (**Figure 5D**). In addition, overexpression of miR-155-5p decreased the transcriptional activity of the miR-15b promoter and miR-15b expression, while underexpression of miR-155-5p had the contrary effect (**Figure 5E, F**).

*Overexpression of CREB or miR-15b suppresses miR-155-5p-induced apoptosis of CD34<sup>+</sup> cells.* In CD34<sup>+</sup> cells transfected with Lv-miR-155-5p mimic + CREB or Lv-miR-155-5p mimic + miR-15b mimic, the transfection efficacy was checked to be successful (**Figure 6A**). Due to CREB or miR-15b overexpression, miR-155-5p upregulation-mediated effects on CD34<sup>+</sup> cell proliferation (**Figure 6B**), cell cycle arrest, apoptosis rates (**Figure 6C-E**), and levels of Bcl-2 and Bax (**Figure 6F**) were all blocked.

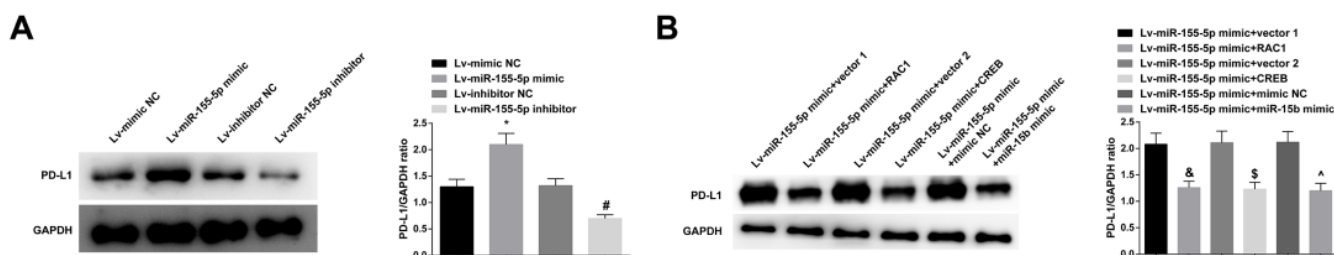
*miR-155-5p promotes PD-L1 expression by RAC1/CREB/miR-15b axis.* Immunoblotting for PD-L1 in CD34<sup>+</sup> cells found that overexpression of miR-155-5p could promote PD-L1 expression, low expression of miR-155-5p could inhibit PD-L1 expression, while overexpression of RAC1, CREB or miR-15b could prevent the promoting effect of miR-155-5p on PD-L1 expression (**Figure 7A, B**).

**Discussion.** MDS consists of a constellation of bone marrow failure diseases and ineffective hematopoiesis is the main obstacle to disease management. CD34 family is a marker for type I transmembrane sialomucin and HSCs, and bioinformatics analysis in CD34<sup>+</sup> cells from MDS patients is applicable for evaluating miRNA-mRNA interaction.<sup>19</sup> To improve MDS, targeting treatment strategies to increase the effectiveness of hematopoiesis is of great therapeutic potential.<sup>20</sup> Similar to the treatment theory, the study from the aspect of miRNA evaluated the action of miR-155-5p in CD34<sup>+</sup> MDS cell activities and eventually summarized that miR-155-5p induces CD34<sup>+</sup> MDS cell apoptosis by

modulating RAC1-mediated CREB/miR-15b axis and activating PD-L1 checkpoint.

miR-155-5p is one of the best-characterized miRNAs and exerts a key role in various physiological and pathological processes including hematopoietic lineage differentiation.<sup>21</sup> In the course of MDS, miR-155-5p has been validated to present an up-trend expression level and is associated with high-risk MDS.<sup>5</sup> Furthermore, the increase of miR-155-5p has been also checked in the bone marrow of acquired aplastic anemia patients.<sup>22</sup> miR-155-5p concentration is elevated in the serum of AML patients and is feasible to distinguish AML patients.<sup>23</sup> Moreover, M-K Chuang *et al.* have measured that hsa-miR-155-5p is high in de novo acute myeloid leukemia patients and is an independent indicator of poor prognosis.<sup>24</sup> The current paper also supported the upward expression pattern of miR-155-5p in MDS, as manifested in CD34<sup>+</sup> cells, further validated the relationship between high expression of miR-155-5p and low survival rates of MDS patients, and confirmed the correlation between miR-155-5p and clinicopathological variables including BM blasts percentage, IPSS-R, IPSS-M, and mutated genes per patient. According to a study report, it is known that miR-155 knockdown can prevent the development of myeloproliferative-like disease and cytokine induction in RBPJ<sup>-/-</sup> mice.<sup>25</sup> Notably, it is previously elaborated that AML inhibits hematopoiesis by producing exosomes carrying miR-150 and miR-155 to target c-MYB.<sup>26</sup> With regard to hematopoiesis, this research obtained the findings that miR-155-5p overexpression reduced proliferation and induced apoptosis of CD34<sup>+</sup> cells, as demonstrated in cell cycle, apoptosis rates, expression of apoptosis-related proteins, and PD-L1 expression. Oppositely, miR-155-5p silencing attenuated MDS CD34<sup>+</sup> cell apoptosis.

Confirmation was obtained regarding the targeting relationship between miR-155-5p and RAC1 in the study. Activation of RAC1 promotes AML development by enhancing homing and retention of leukemia cells in the niche,<sup>27</sup> and RAC1 inhibitors have suggested an anti-proliferative effect on AML cells.<sup>28</sup> Additionally, it is reported that blocking RAC1 results in impaired migration by upregulating a dominant negative form of RAC1 in CD34<sup>+</sup> cells from leukemia patients.<sup>29</sup> Intriguingly, activating RAC1 contributes to the



**Figure 7** miR-155-5p promotes PD-L1 expression by RAC1/CREB/miR-15b axis. PD-L1 expression changes in CD34<sup>+</sup> cells after transfection.



promotion of HSC regeneration, hematologic recovery, and HSC survival under the treatment of PTP $\alpha$  inhibitors.<sup>30</sup> In particular, this study discovered that RAC1 was lowly expressed in MDS CD34<sup>+</sup> cells and RAC1 induction was protective to prevent miR-155-5p-induced cellular apoptosis, thereby enhancing bone marrow hematopoiesis. As mechanistically determined, miR-155-5p reduced the transcriptional activity of miR-15b by inhibiting RAC1, dissociating the interaction between RAC1 and CREB, and inhibiting CREB activation. Also, either overexpressing CREB or miR-15b reduced miR-155-5p-mediated apoptosis of CD34<sup>+</sup> cells. CREB has been commonly accepted as a pro-oncogene in AML,<sup>31,32</sup> but its regulatory action in MDS has been rarely reported. It is established that loss of miR-15/16 can lead to MDS-AML transformation.<sup>14</sup>

**Conclusions.** The paper illustrates that miR-155-5p mediates PD-L1 and promotes CD34<sup>+</sup> cell apoptosis in MDS by regulating the RAC1/CREB/miR-15b axis, thereby inhibiting bone marrow hematopoiesis. To some extent, the findings may open up a new avenue for the development of molecule-based pharmaceuticals for MDS. However, the relevant mechanism was only studied in

cells, but not verified in animal experiments. Also, more rigorous experimental protocols should be designed to validate the role and mechanism of miR-155-5p in MDS.

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