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Research paper

Long noncoding RNA MIAT promotes the progression of acute myeloid leukemia by negatively regulating miR-495

Gaoyan Wang, Xuerong Li, Liang Song, Hua Pan, Jian Jiang, Lirong Sun*

Department of Pediatrics, The Affiliated Hospital of Qingdao University, No. 16 Jiangsu Road, Qingdao, Shandong, 266001, China

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ABSTRACT

Acute myeloid leukemia (AML) is a malignant myeloid hematopoietic stem and progenitor cell disease. Studies have shown that the long noncoding RNA (lncRNA) myocardial infarction associated transcript (MIAT) is abundantly expressed in multiple human solid tumors. However, the expression and role of MIAT in AML has not been explored previously. In this study, we find that MIAT is overexpressed in AML patient specimens and AML cell lines. Importantly, upregulation of MIAT is closely related with poor clinical outcome. Further investigations reveal that knockdown of MIAT inhibits the colony formation and proliferation, meanwhile, accelerates the apoptosis of AML cells *in vitro*. Consistently, MIAT knockdown slows AML progression in immunodeficient mice. Mechanistically, we confirm that MIAT can function as a sponge to inhibit microRNA-495 (miR-495), a tumor suppressor, in AML cells. Collectively, our results demonstrate that MIAT is involved in promoting the progression of AML, at least partly, through negative regulation of miR-495, and therefore provide a promising target for treatment of AML.

1. Introduction

Acute myeloid leukemia (AML), as the most common hematologic cancer, originates from the malignant myeloid hematopoietic stem and progenitor cells [1,2]. The abnormal proliferation of these primitive cells suppresses normal hematopoiesis in the bone marrow and periphery, which leads to anemia, bleeding, infection and even death [3,4]. As we know, AML is a kind of complicated disease with high incidence and death rates [5,6]. In the past few decades, many efforts have been made to diagnose and treat AML, whereas the clinical outcome of this disease remains unsatisfactory. Hence, it is required to deeply understand the underlying molecular mechanisms in the occurrence and development of AML.

It has been confirmed that mutations in genes implicated in regulating cell proliferation and apoptosis, such as FLT3, RUNX1, NPM1, DNMT3A, are associated with AML oncogenesis, progression and poor prognosis [4,7]. In recent years, epigenetic regulations, such as DNA methylation, histone acetylation and non-coding RNAs including microRNAs (miRNAs) and long non-coding RNA (lncRNAs) also play a crucial role in the initiation and progression of AML [7–9]. LncRNAs that is a type of non-protein coding RNAs having at least 200 nucleotides in length are involved in the modulation of multiple biological processes *via* transcriptional and posttranscriptional mechanisms [10,11]. More importantly, accumulating researches have observed the dysregulation of lncRNAs in human diseases especially in tumors [12]. Previously, studies have reported that several lncRNAs, including NEAT1, H19, LINC00152 and ANRIL, affect AML biology [5,6,13,14]. However, the exact role and intrinsic molecular mechanism of lncRNAs in the pathogenesis of AML have not been completely explored.

Myocardial infarction associated transcript (MIAT), also known as RNCR2 (retinal non-coding RNA 2) or Gomafu, is located on chromosome 22 [15–17]. Studies have demonstrated that MIAT participates in multiple pathological and physiological processes, including microvascular dysfunction, myocardial infarction, paranoid schizophrenia and neurogenic commitment [15,16,18–20]. Notably, it was reported that MIAT is abundantly expressed in many kinds of human solid tumors, and plays a critical role in facilitating the proliferation, invasion and metastasis of cancer cells [21–24]. In addition, a recent study found that MIAT is upregulated in aggressive chronic lymphocytic leukemia [25]. However, the expression and function of MIAT in AML is still unclear.

Abbreviations: AML, acute myeloid leukemia; LncRNA, long noncoding RNA; MIAT, myocardial infarction associated transcript; RNCR2, retinal non-coding RNA 2; miRNA, microRNA; BM, bone marrow; FAB, French–American–British; FBS, fetal bovine serum; CCK-8, Cell Counting Kit-8; qRT-PCR, quantitative real-time PCR; TCGA, The Cancer Genome Atlas; HSC, hematopoietic stem cell; LSCs, leukemia stem cells; CMP, common myeloid progenitor; GMP, granulocyte monocyte progenitor

* Corresponding author.
E-mail address: sunlirong2018@163.com (L. Sun).

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In the present study, we show that MIAT is overexpressed in AML specimens from patients, as well as AML cell lines. Of note, the upregulation of MIAT is closely associated with adverse prognosis. Further researches display that MIAT promotes AML progression by targeting an anti-tumor miR-495. Collectively, our data indicate that MIAT plays an oncogenic role in AML, and may serve as a potential therapeutic target.

2. Materials and methods

2.1. Patients and bone marrow (BM) specimens

All AML patients recruited in this study were diagnosed by the French–American–British (FAB) criteria, and received standard AML therapy following the protocol provided from the Dutch-Belgian Hematology-Oncology Cooperative Group. BM specimens were enrolled from 121 AML patients that do not have any other kinds of malignancy and 32 healthy donors from The Affiliated Hospital of Qingdao University. All volunteers have written the informed consents, and this research was authorized by the Ethics Committee (Qingdao University).

2.2. Cell lines and cell culture

Human bone marrow stromal cell (HS-5), human AML lines (NB-4, HL-60, THP-1, MOLM-14 and KG1a) and human embryonic kidney cell line (293 T) were purchased from BeNa Culture Collection (Beijing, China). HS-5, NB-4, HL-60, THP-1 and MOLM-14 cells were cultured using 90% RPMI-1640 (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; HyClone). KG1a cells were cultured in IMDM (HyClone) supplemented with 10% FBS. 293 T cells were cultured in DMEM (high glucose; HyClone) supplemented with 10% FBS. All cells were grown in 37 ˚C incubator containing 5% CO_2.

2.3. Cell proliferation assay

Cell proliferation assay was carried out using the Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) as described [13]. Briefly, cells were plated into 96-well plates at a density of 3×10^3/well. At indicated time (0, 24, 48, 72 or 96 h) after culture, 10 μl of CCK-8 solution was added into each well. After cultured for another 2 h at 37 ˚C, the samples were detected by a microplate reader (Thermo Fisher Scientific, Waltham, MA) set at 450 nm.

2.4. Cell cycle analysis

Cells were collected and washed twice in phosphate buffer saline (PBS). Then, samples were fixed by 75% ethanol. After treated with RNase, samples were incubated with propidium iodide (PI) for 15 min., followed by flow cytometric analysis (FACSCantoII flow cytometer; BD Biosciences, San Jose, CA, USA).

2.5. Cell viability assay

Cells were collected and stained with trypan blue (Invitrogen, Carlsbad, USA). Five mins later, dead cells were counted using the Zeiss microscope (Jena, Germany).

2.6. Apoptosis analysis

Cells were collected and washed twice in cold PBS. After that, cell apoptosis was detected using the Annexin V-FITC/PI Kit (Biolegend, San Diego, CA, USA) following the manufacturer's guidelines.

2.7. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from BM specimens or cultured AML cells with TRIzol reagent (Invitrogen). For detecting lncRNA, RNA was reverse transcripted using the PrimeScript RT reagent kit purchased from Takara Bio Inc. (Tokyo, Japan), and then qRT-PCR was conducted using the SYBR Premix Ex Taq kit (Takara Bio Inc.). Primers of GAPDH and MIAT are as follows: MIAT (forward: 5′-TTTACTTTAACAGACCA GAA-3′, reverse: 5′-CTCCTTTGTTGAATCCAT-3′

[20]), GAPDH (forward: 5′-CTGGGCTACACTGAGCACC-3′, reverse: 5′- AAGTGGTCGTTGAGGGCAATG-3′). The expressions of miRNAs were detected using the mirVana qRT-PCR miRNA Detection kit (Ambion, Austin, TX, USA) following the manufacturer's guidelines. GAPDH and U6 were used as internal references for lncRNA and miRNA, respectively. The primers of miRNAs were obtained from RIBOBio Co. Ltd. (Guangzhou, China).

2.8. Western blot analysis

Western blot was conducted as reported [14,26]. The following anti-human antibodies were used in this study: Bcl-2 (Abcam, Cambridge, UK), Bax (Abcam), Clvaved caspase3 (Cell Signaling Technology, Danvers, MA, USA), Caspase3 (Cell Signaling Technology), Clvaved caspase9 (Cell Signaling Technology), Caspase9 (Cell Signaling Technology), PBX3 (Abcam), MEIS1 (Abcam) and GAPDH (Beyotime, China).

2.9. Luciferase reporter assay

The fragment of MIAT containing the predicted miR-495 binding site, as well as the corresponding mutated MIAT, were amplified and cloned into the pmirGLO vector obtained from Promega Corporation (Madison, WI, USA). Then, miR-495 mimic or miRNA-neagive control (miR-NC), along with wild type (wt)-MIAT or mutant (mut)-MIAT, were transfected into 293 T cells using lipofectamine 2000 (Invitrogen). Forty-eight hours later, the relative luciferase activity was detected.

2.10. Lentivirus transduction and cell transfection

The lentivirus expressing scrambled small hairpin RNA (sh-NC) or sh-MIAT (Hanbio, Shanghai, China) were transduced into AML cell lines using polybrene (Hanbio). miR-inhibitor negative control (miR-inhibitor-NC) or miR-495 inhibitor (GenePharma CO., LTD, Shanghai, China) were transfected into cells using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The target sequence of shRNA anainst MIAT is as follows: 5′-GAGCTTGTA TATGAAGCCATTGTAA-3′ [22].

2.11. In vivo studies

These assays were conducted as previously reported [5]. In brief, 5×10^6 HL-60 or THP-1 cells transfected with sh-NC or sh-MIAT were transplantated into immunodeficient (NOD-SCID) mice (Shanghai Model Organisms Center, China) by caudal vein injection. Twenty days after transplantation, the percentage of GFP + HL-60 or THP-1 cells in recipient' BM and spleen analyzed by flow cytometry, or the spleen weight was measured. For survival analysis, mice were transplantated with HL-60 or THP-1 cells transfected with sh-NC or sh-MIAT. After transplantation, the survival rates were monitored. The animal experiments were performed following the institutional ethical guidelines.

2.12. Bioinformatic analysis

The targeting relationships between lncRNA–miRNA were predicted using Starbase v3.0 (http://starbase.sysu.edu.cn/) and LncBase Predicted v.2 (http://carolina.imis.athena-innovation.gr/diana_tools/web/).

2.13. Statistical analysis

All experiments were performed independently at least 3 times and the data was analyzed using SPSS 19.0 and GraphPad Prism 6.0. Student's *t*-test was used to compare the differences between groups, and one-way ANOVA was used to compare the differences among multiple groups. Kaplan-Meier method and Cox regression models (univariate and multivariate) were conducted to evaluate the effect of MIAT expression on overall survival. All data were showed as the mean ± SD. P value < 0.05 was considered as statistically significant.

3. Results

3.1. MIAT is overexpressed in AML patient specimens and AML cell lines

To assess the role of MIAT in AML, we detected the BM specimens obtained from AML patients and healthy controls by qRT-PCR. Compared with healthy controls, MIAT was distinctly upregulated in AML specimens (Fig. 1A). In line with this, MIAT expression was higher in AML cell lines (NB-4, HL-60, THP-1, and MOLM-14) than bone marrow stroma cells (HS-5) (Fig. 1B). We then analyzed the correlation between MIAT expression and clinical characteristics in AML patients. It was found that high MIAT expression was significantly related to higher WBC count and unfavorable cytogenetics (Supplementary Table 1). However, there was no significant association of MIAT expression with other clinical features including age, gender, BM blasts and FAB classification, as well as NPM1, FLT3-ITD and CEBPA gene mutation (Supplementary Table 1). Particularly, the overall survival of AML patients with high MIAT expression was lower than those with low MIAT expression (Fig. 1C). Importantly, multivariate analysis showed that high MIAT expression was an independent predictive factor for poor overall survival in AML patients (Supplementary Table 2). All of these findings were confirmed in The Cancer Genome Atlas (TCGA) database (Supplementary Tables 3 and 4; Supplementary Fig. 1A and B). These findings suggest that MIAT may play a potential role in AML progression.

3.2. Knockdown of MIAT inhibits the colony formation and proliferation of AML cells

To investigate whether MIAT is involved in the pathogenesis of AML, we then knocked down the MIAT in AML cells by lentivirus-mediated shRNA (Fig. 2A). It was found that knockdown of MIAT significantly decreased colony formation and proliferation of AML cells *in vitro* (Fig. 2B and C). Consistently, cell cycle analysis by flow cytometry revealed that MIAT knockdown evidently increased the frequency of cells in G0/G1 phases, while reduced the frequency of cells in S phase (Fig. 2D), hinting that cell cycle progression is arrested in G1/S transition. These results indicate that MIAT may control the proliferation of AML cells.

3.3. Knockdown of MIAT reduces the viability and accelerates the apoptosis of AML cells

We next evaluated whether knockdown of MIAT affects AML cells viability and apoptosis. Indeed, placenta blue staining showed that MIAT knockdown reduced the viability of AML cells (Fig. 3A). In addition, flow cytometric analysis displayed knockdown of MIAT significantly accelerated the apoptosis of AML cells (Fig. 3B and C). Meanwhile, the anti-apoptotic protein (Bcl-2) was downregulated, while several pro-apoptotic proteins (including Bax, Cleaved caspase3, Cleaved caspase9) were upregulated in AML cells after MIAT knockdown by western blot analysis (Fig. 3D). These data confirm that MIAT is required to promote the survival of AML cells.

3.4. MIAT knockdown slows AML progression in NOD/SCID mice

Further, we utilized an immunodeficient mouse model to evaluate the role of MIAT *in vivo*. AML cells with or without knockdown of MIAT were transplantated into NOD/SCID mice *via* caudal vein, followed by flow cytometric analysis, and spleen weight measurement, survival monitoring (Fig. 4A and Supplementary Fig. 2A). It was found that knockdown of MIAT remarkably reduced the percentage of GFP^+ cells in recipients' bone marrow (BM) and spleen (Fig. 4B and Supplementary Fig. 2B). Meanwhile, mice transplantated with MIAT-knochdown AML cells displayed alleviated splenomegaly (Fig. 4C and Supplementary Fig. 2C). Specifically, knockdown of MIAT prolonged the survival time of mice transplantated with AML cells (Fig. 4D and Supplementary Fig. 2D). Thus, these findings illustrate that MIAT contributes to AML progression *in vivo*.

3.5. MIAT acts as a sponge to negatively regulate miR-495 in AML cells

Considerating that lncRNAs can act as a sponge to combine miRNAs, we therefore speculated that MIAT may inhibit some anti-tumor miRNAs in AML cells. Bioinformatics analysis using Starbase v3.0 and LncBase Predicted v.2 revealed that several previously recognized anti-tumor miRNAs, including miR-29a/b/c [27–29], miR-181a/b/c/d [30], miR-222 [8] and miR-495 [31], have potential binding sites in MIAT. Interestingly, we observed that the expression of miR-495, but not other miRNAs, was markedly increased in AML cells after MIAT knockdown (Fig. 5A and Supplementary Fig. 3A and B). In accordance with this

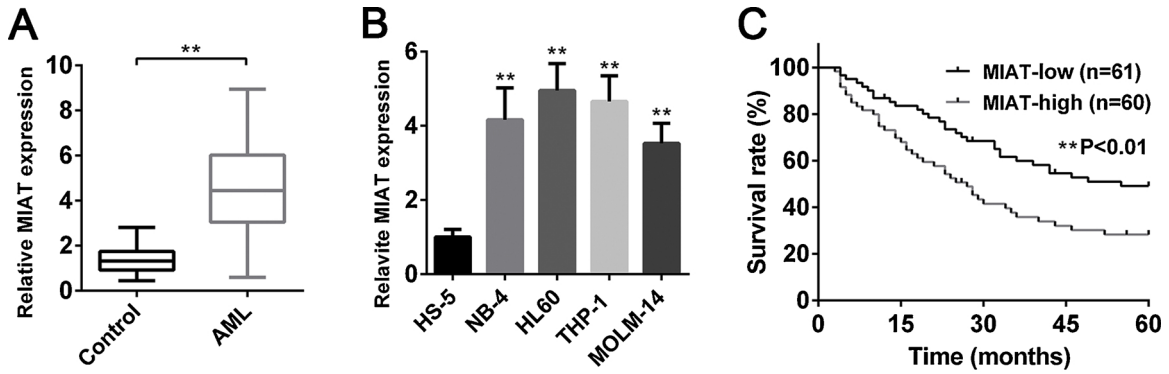

Figure 1

Fig. 1. MIAT is overexpressed in AML patient specimens and AML cell lines. (A) The relative MIAT expression in BM specimens obtained from 32 healthy controls and 121 AML patients, determined by qRT-PCR. (B) The relative expression of MIAT in HS-5, NB-4, HL-60, THP-1 and MOLM-14 cells, determined by qRT-PCR. (C) The overall survival of AML patients with high or low MIAT expression (based on the median expression levels). **p < 0.01.

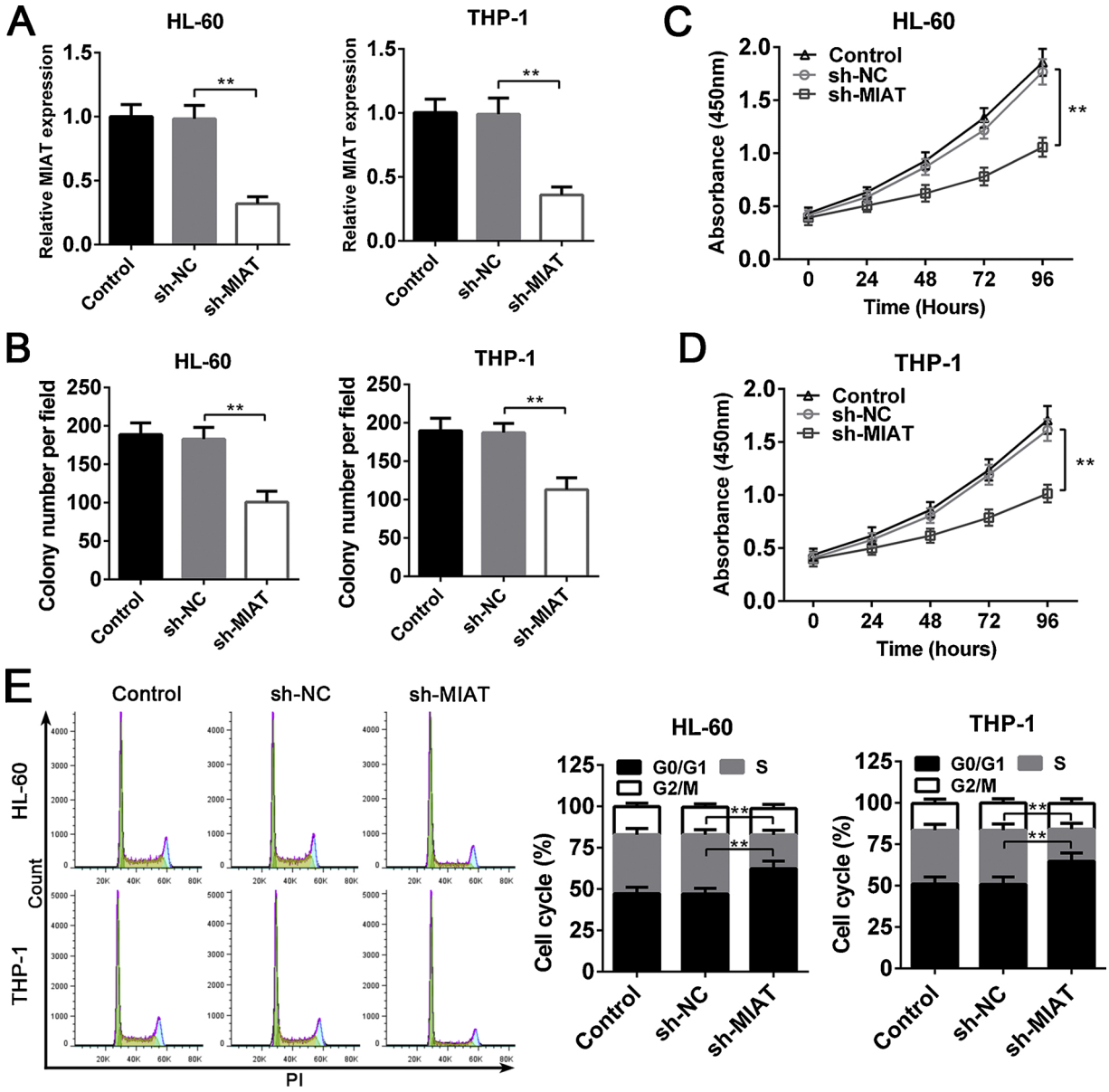

Fig. 2. Knockdown of MIAT inhibits the colony formation and proliferation of AML cells. (A) The knockdown efficiency of MIAT in HL-60 and THP-1 cells after transfected with lentivirus carrying sh-NC or sh-MIAT, determined by qRT-PCR. (B) The colony formation of HL-60 and THP-1 cells with or without knockdown of MIAT, determined by Wright-Giemsa staining. (C, D) The proliferation of (C) HL-60 and (D) THP-1 cells with or without knockdown of MIAT, determined by CCK-8 assay. (E) Cell cycle analysis of HL-60 and THP-1 cells with or without knockdown of MIAT, detected by flow cytometry. **p < 0.01.

finding, miR-495 expression was significantly decreased in AML patient specimens (Fig. 5B). Simultaneously, miR-495 expression was negatively correlated with MIAT levels (Fig. 5C). These results indicate that MIAT may function as a sponge to inhibit miR-495 in AML cells. To further validated this notion, we performed a luciferase reporter assay and found that MIAT was able to bind miR-495 (Fig. 5C and D). Besides, PBX3 and MEIS1, two miR-495 target genes [31], that have been shown to facilitate AML progression, were distinctly downregulated in AML cells when MIAT was knocked down (Fig. 5F). Taken together, these data suggest that MIAT promotes AML progression probably by sponging miR-495.

3.6. MIAT/miR-495 axis modulates AML progression

Finally, we set out to determine whether MIAT promotes AML progression by inhibiting the tumor suppressor, miR-495. In fact, we noticed that inhibition of miR-495 using a miR-495 inhibitor significantly recovered the proliferation and cell cycle, and reduced the apoptosis of AML cells with MIAT knockdown (Fig. 6A–D and Supplementary Fig. 4A–D). Furthermore, miR-495 suppression also markedly increased the expressions of PBX3 and MEIS1 in MIAT knockdown-AML cells (Fig. 6E and Supplementary Fig. 4E). Collectively, our findings demonstrate that MIAT/miR-495 axis plays a critical role in modulating the pathogenesis of AML.

4. Discussion

AML is a rapidly progressing disease that has high heterogeneity in regard to pathogenesis [2,3]. Although many advances in diagnosis and treatment of AML have been made, the prognosis of this disease is still poor [3]. Previous studies have confirmed that lncRNAs are functional RNA molecules which are involved in diverse biological processes including tumor biology by regulation of gene expression [10,11]. Here, we show that the lncRNA MIAT promotes the progression of AML by sponging miR-495.

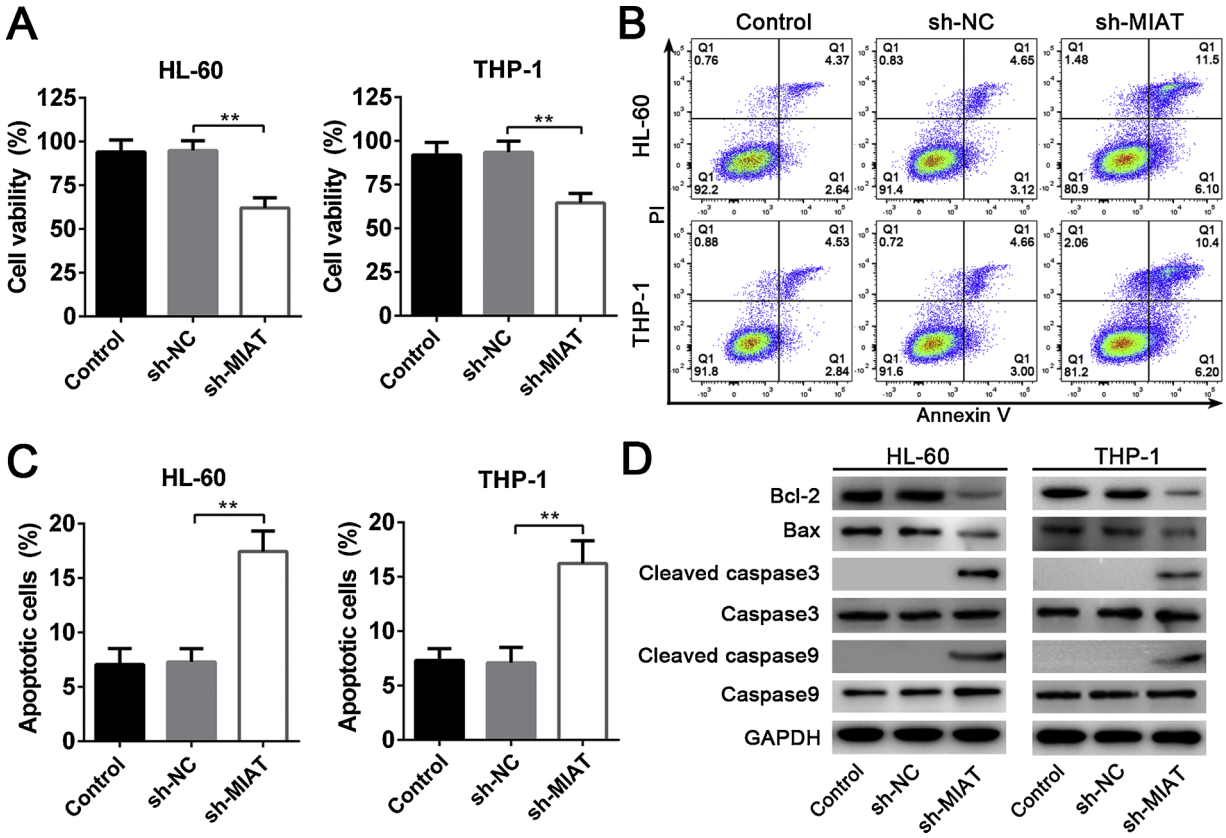

Fig. 3. Knockdown of MIAT reduces the viability and accelerates the apoptosis of AML cells. (A) The viability of HL-60 and THP-1 cells with or without knockdown of MIAT, analyzed by trypan blue staining. (B, C) Flow cytometry analysis of the apoptosis of HL-60 and THP-1 cells with or without knockdown of MIAT. (D) The expressions of Bcl-2, Bax, Cleaved caspase3, Caspase3, Cleaved caspase9 and Caspase9 in HL-60 and THP-1 cells with or without knockdown of MIAT, determined by western blot analysis. **p < 0.01 (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Recently, studies using high-throughput sequencing found that many human cancers show abnormal expression of lncRNAs [12]. Specifically, several lncRNAs have been shown to promote the development and progression of AML [5,14,26,32]. Indeed, MIAT is abundantly expressed in multiple human solid tumors, whereas the expression of MIAT in AML cells has not been explored. In the present study, we observed that MIAT was overexpressed in BM specimens obtained from AML patients, suggesting that MIAT may regulate AML biology. Furthermore, we found that AML patients with high MIAT expression had lower survival rate, and MIAT expression was an independent predictive factor for overall survival in AML. Thus, our findings indicate that MIAT may also serve as a potential prognostic marker for AML.

Cancer cells are characterized by uncontrolled proliferation and decreased apoptosis [33,34]. Studies have reported that MIAT is able to promote cell proliferation and inhibit cell apoptosis [15,17], while the exact function of MIAT in AML cells is still uncharacterized. In this research, we found that MIAT knockdown significantly suppressed the proliferation, cell cycle and survival of AML cells *in vitro*. Consistent with these findings, knockdown of MIAT slowed the progression of AML in immunodeficient mice. Therefore, our data indicate that MIAT is a proto-oncogene in AML.

Previous studies have reported that MIAT is involved in various types of cancers through targeting miRNAs. For instance, MIAT can facilitate the development of hepatocellular carcinoma through regulation of miR-214 or miR-520d-3p [23,35]. Besides, MIAT can promote the growth and metastasis of gastric cancer cells by modulating miR-141/DDX5 axis [22]. Moreover, MIAT is able to accelerate the proliferation and metastasis of pancreatic carcinoma *via* interaction with miR-133 [24]. In the study, we observed that knockdown of MIAT markedly upregulated miR-495 expression in AML cells. It was reported that miR-495 is an important tumor suppressor in AML or other tumor cells [31,33,36,37]. As we know, miRNAs are widely involved in regulating cell behaviors mainly through degrading target mRNA or inhibiting the translation of target gene [34,38]. Importantly, the luciferase reporter assay revealed that MIAT can function as a competing endogenous RNA to sponge miR-495. In addition, we found that the expressions of two miR-495 target genes (PBX3 and MEIS1) that have been reported to promote AML progression were evidently decreased in AML cells after MIAT knockdown. Further, suppression of miR-495 in MIAT-knockdown AML cells significantly recovered the proliferation and cell cycle, and reduced the apoptosis. These results confirm that MIAT promotes the progression of AML, at least in part, by inhibiting the tumor suppressor, miR-495. Hence, targeting MIAT may be a promising strategy for treating AML. Interestingly, we did not find significant difference in the expressions of other predicted miRNAs, which may be due to the fact that lncRNAs are heterogeneous in different tissues and cells [10]. On the other hand, given that lncRNAs can regulate cell biological function by other ways, such as localizing to target gene loci, scaffolding regulatory proteins or shaping three-dimensional nuclear structure [10]. Therefore, we can not deny that there are may be other mechanisms that mediate the function of MIAT in AML.

Furthermore, data from previous studies showed that, among healthy stem/progenitor cells, MIAT expression was significantly higher in hematopoietic stem cells (HSC) than common myeloid progenitor (CMP) and granulocyte monocyte progenitor (GMP) [39]. It was well accepted that AML is a malignant disease caused by the leukemia stem cells (LSCs) that have aberrant self-renewal capacity [2,40]. Like normal HSCs, the majority of LSCs resides in G0 phase, which leads to chemotherapy resistance [40,41]. Consequently, new therapeutic measures are required to target not only the rapidly proliferative AML

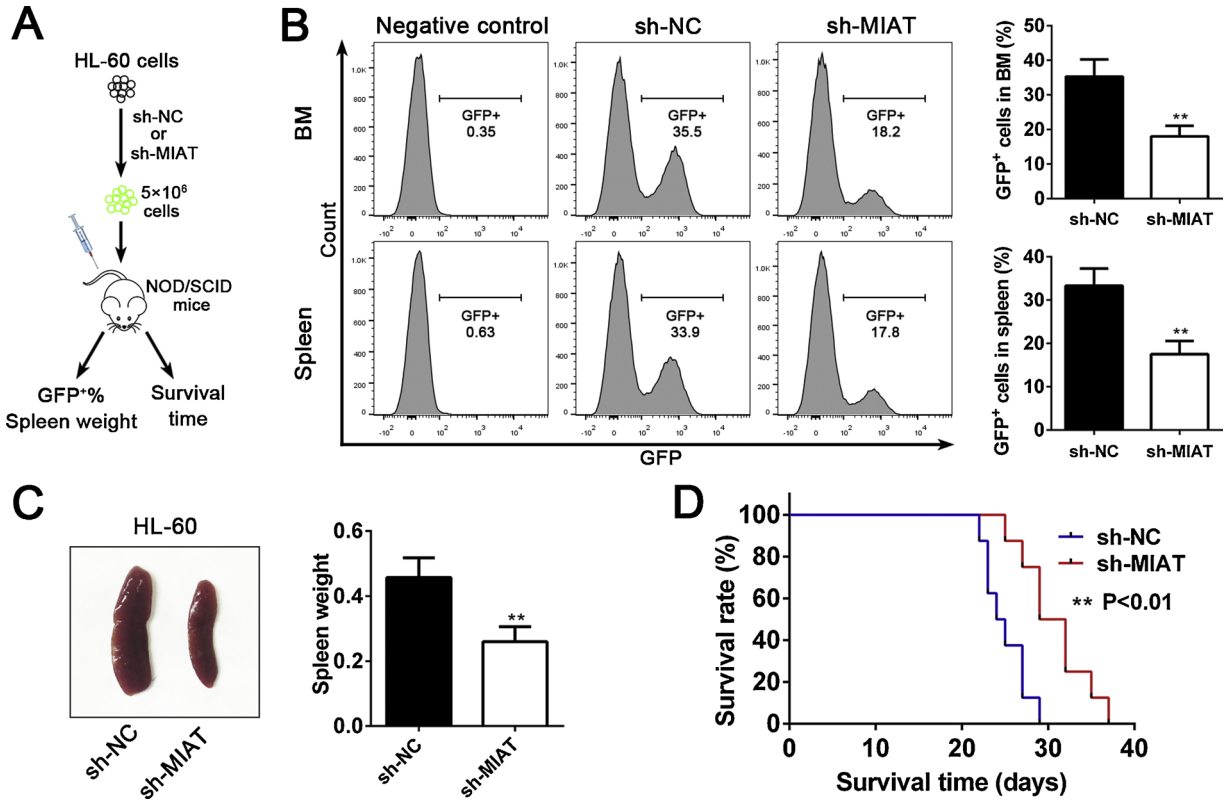

Fig. 4. MIAT knockdown slows AML progression in NOD/SCID mice. (A–C) The immunodeficient (NOD/SCID) mice were transplantated with HL-60 cells transfected with sh-NC or sh-MIAT by caudal vein injection. (A) The experimental procedure. (B, C) Twenty days after transplantation, (B) the percentage of GFP$^+$ HL-60 cells in the BM and spleen of recipient mice were detected by flow cytometry, and (C) spleen weight was measured (n = 5 mice per group). (D) Kaplan-Meier curve showing the survival rates of mice transplantated with HL-60 cells with or without knockdown of MIAT (n = 8 mice per group). **p < 0.01.

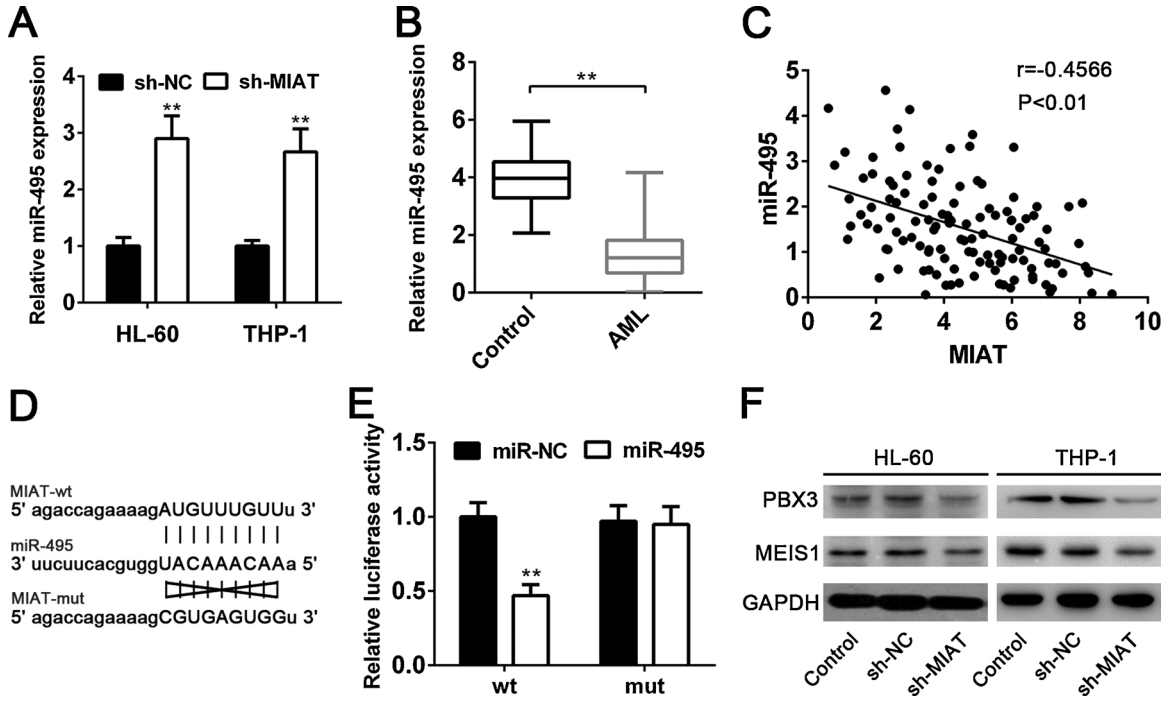

Fig. 5. MIAT acts as a sponge to negatively regulate miR-495 in AML cells. (A) The relative expression of miR-495 in HL-60 and THP-1 cells with or without knockdown of MIAT, determined by qRT-PCR. (B) The relative MIAT expression in BM specimens obtained from 32 healthy controls and 121 AML patients, determined by qRT-PCR. (C) Pearson correlation analysis revealing the significantly negative relationship between miR-495 and MIAT expression in AML patient specimens (r = -0.4566, p < 0.01). (D) StarBase 3.0 analysis revealing the specific binding site of miR-495 with wild-type (wt) and mutant (mut) MIAT. (E) 293 T cells were co-transfected with wt-MIAT or mut-MIAT and miR-495 or miR-NC. Then, the relative luciferase activity in each group was measured. (F) The expressions of PBX3 and MEIS1 in HL-60 and THP-1 cells with or without knockdown of MIAT, determined by western blot. **p < 0.01.

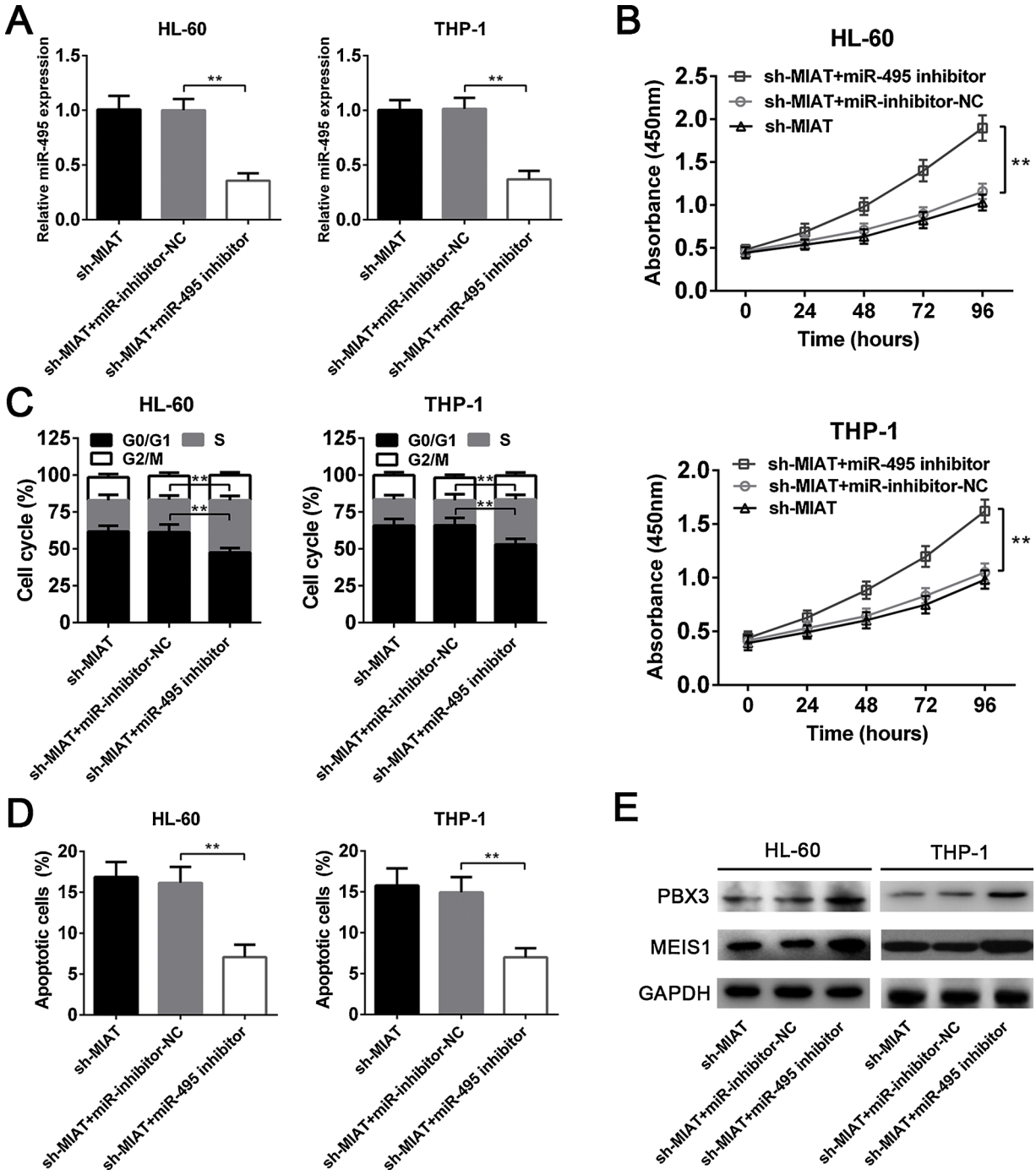

Fig. 6. MIAT/miR-495 axis modulates AML progression. (A–E) HL-60 and THP-1 cells with knockdown of MIAT were treated with miR-inhibitor-NC or miR-495 inhibitor. After that, (A) the expression of miR-495 was measured by qRT-PCR. Then, the (B) proliferation, (C) cell cycle and (D) apoptosis were detected by the CCK-8 assay or flow cytometry. Finally, the expressions of PBX3 and MEIS1 were determined by western blot. **$p < 0.01$.

blasts but also the LSCs. However, the role of MIAT in normal stem/progenitor cells and LSCs still needs further observation.

In summary, our findings highlight the importance of MIAT in the progression of AML through sponging miR-495, meanwhile, provides a new sight for evaluation of AML prognosis and a potential therapeutic target for AML. Indeed, many studies have identified other specific lncRNAs that are correlated with AML patient outcome [39,42,43]. However, due to the high heterogeneity of AML, data obtained from different patients may not show exactly the same results. Thus, it still needs a large-sample research, as well as external experiments, such as single-cell sequencing and establishment of gene knockout mouse model, to further validate these findings.

Authorship

W. G. designed the study, performed experiments, analyzed data and wrote the paper. L. X. and S. L. performed some *in vitro* experiments and analyzed data. P. H. and J.J. participated in the animal experiments and data analysis. S. L. conceived and supervised the study, and revised the manuscript.

Declaration of Competing Interest

None.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.106265.

References

[1] J.A. Pulikkan, M. Hegde, H.M. Ahmad, et al., CBFβ-SMMHC inhibition triggers apoptosis by disrupting MYC chromatin dynamics in acute myeloid leukemia, Cell 174 (2018) 172.e21-186.e21.
[2] D. Thomas, R. Majeti, Biology and relevance of human acute myeloid leukemia stem cells, Blood 129 (2017) 1577–1585.
[3] V. Stavropoulou, S. Kaspar, L. Brault, et al., MLL-AF9 expression in hematopoietic stem cells drives a highly invasive AML expressing EMT-Related genes linked to poor outcome, Cancer Cell 30 (2016) 43–58.
[4] G. Marcucci, T. Haferlach, H. Döhner, Molecular genetics of adult acute myeloid leukemia: prognostic and therapeutic implications, J. Clin. Oncol. 29 (2011) 475–486.
[5] L. Sun, X. Li, Y. Sun, et al., LncRNA ANRIL regulates AML development through modulating the glucose metabolism pathway of AdipoR1/AMPK/SIRT1, Mol. Cancer 17 (2018) 1–6.
[6] T. Zhang, J. Zhou, W. Zhang, et al., H19 overexpression promotes leukemogenesis and predicts unfavorable prognosis in acute myeloid leukemia, Clin. Epigenetics 10 (2018) 1–12.
[7] R. Garzon, S. Volinia, D. Papaioannou, et al., Expression and prognostic impact of lncRNAs in acute myeloid leukemia, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 18679–18684.
[8] A. Forrest, M. Kanamori-Katayama, Y. Tomaru, et al., Induction of microRNAs, mir-155, mir-222, mir-424 and mir-503, promotes monocytic differentiation through combinatorial regulation, Leukemia 24 (2010) 460–466.
[9] R. Garzon, S. Liu, M. Fabbri, et al., MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1, Blood 113 (2009) 6411–6418.
[10] J.M. Engreitz, N. Ollikainen, M. Guttman, Long non-coding RNAs: spatial amplifiers that control nuclear structure and gene expression, Nat. Rev. Mol. Cell Biol. 17 (2016) 756–770.
[11] J.J. Quinn, H.Y. Chang, Unique features of long non-coding RNA biogenesis and function, Nat. Rev. Genet. 17 (2016) 47–62.
[12] J. Beermann, M. Piccoli, J. Viereck, et al., Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches, Physiol. Rev. 96 (2016) 1297–1325.
[13] C. Zhao, S. Wang, Y. Zhao, et al., Long noncoding RNA NEAT1 modulates cell proliferation and apoptosis by regulating miR-23a-3p/SMC1A in acute myeloid leukemia, J. Cell. Physiol. 234 (2019) 6161–6172.
[14] X. Zhang, W. Tao, Long noncoding RNA LINC00152 facilitates the leukemogenesis of acute myeloid leukemia by promoting CDK9 through miR-193a, DNA Cell Biol. 38 (2019) 236–242.
[15] J. Liao, Q. He, M. Li, et al., LncRNA MIAT: myocardial infarction associated and more, Gene 578 (2016) 158–161.
[16] B. Yan, J. Yao, J. Liu, et al., lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA, Circ. Res. 116 (2015) 1143–1156.
[17] C. Sun, L. Huang, Z. Li, et al., Long non-coding RNA MIAT in development and disease: a new player in an old game, J. Biomed. Sci. 25 (2018) 1–7.
[18] E. Li, P. Zhao, J. Jian, et al., LncRNA MIAT overexpression reduced neuron apoptosis in a neonatal rat model of hypoxic-ischemic injury through miR-211/GDNF, Cell Cycle 18 (2019) 156–166.
[19] Y. Bai, W. Wang, Y. Zhang, et al., lncRNA MIAT suppression alleviates corneal angiogenesis through regulating miR-1246/ACE, Cell Cycle (2019) 1–9.
[20] X. Zhong, X. Ma, L. Zhang, et al., MIAT promotes proliferation and hinders apoptosis by modulating miR-181b/STAT3 axis in ox-LDL-induced atherosclerosis cell models, Biomed. Pharmacother. 97 (2018) 1078–1085.
[21] F. Crea, E. Venalainen, X. Ci, et al., The role of epigenetics and long noncoding RNA MIAT in neuroendocrine prostate cancer, Epigenomics 8 (2016) 721–731.
[22] M. Sha, M. Lin, J. Wang, et al., Long non-coding RNA MIAT promotes gastric cancer growth and metastasis through regulation of miR-141/DDX5 pathway, J. Exp. Clin. Cancer Res. 37 (2018) 1–12.
[23] Y. Xiang, Y. Huang, H. Sun, et al., Deregulation of miR-520d-3p promotes hepatocellular carcinoma development via lncRNA MIAT regulation and EPHA2 signaling activation, Biomed. Pharmacother. 109 (2019) 1630–1639.
[24] T. Li, J. Liu, S. Fu, The interaction of long non-coding RNA MIAT and miR-133 play a role in the proliferation and metastasis of pancreatic carcinoma, Biomed. Pharmacother. 104 (2018) 145–150.
[25] A. Sattari, H. Siddiqui, F. Moshiri, et al., Upregulation of long noncoding RNA MIAT in aggressive form of chronic lymphocytic leukemias, Oncotarget 7 (2016) 54174–54182.
[26] L. Ma, W.X. Kuai, X.Z. Sun, et al., Long noncoding RNA LINC00265 predicts the prognosis of acute myeloid leukemia patients and functions as a promoter by activating PI3K-AKT pathway, Eur. Rev. Med. Pharmacol. Sci. 22 (2018) 7867–7876.
[27] W. Blum, R. Garzon, R.B. Klisovic, et al., Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 7473–7478.
[28] A. Mims, A.R. Walker, X. Huang, et al., Increased anti-leukemic activity of decitabine via AR-42-induced upregulation of miR-29b: a novel epigenetic-targeting approach in acute myeloid leukemia, Leukemia 27 (2013) 871–878.
[29] A. Mims, A.R. Walker, X. Huang, et al., Increased anti-leukemic activity of decitabine via AR-42-induced upregulation of miR-29b: a novel epigenetic-targeting approach in acute myeloid leukemia, Leukemia 27 (2013) 871–878.
[30] H. Chen, T. Liu, J. Liu, et al., Circ-ANAPC7 is upregulated in acute myeloid leukemia and appears to target the MiR-181 family, Cell. Physiol. Biochem. 47 (2018) 1998–2007.
[31] X. Jiang, H. Huang, Z. Li, et al., miR-495 is a tumor-suppressor microRNA downregulated in MLL-rearranged leukemia, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 19397–19402.
[32] X. Dong, Z. Fang, M. Yu, et al., Knockdown of long noncoding RNA HOXA-AS2 suppresses chemoresistance of acute myeloid leukemia via the miR-520c-3p/S100A4 Axis, Cell. Physiol. Biochem. 51 (2018) 886–896.
[33] J. Guo, D. Jin, Y. Wu, et al., The miR 495-UBE2C-ABCG2/ERCC1 axis reverses cisplatin resistance by downregulating drug resistance genes in cisplatin-resistant non-small cell lung cancer cells, EBioMedicine 35 (2018) 204–221.
[34] Z.H. Fang, S.L. Wang, J.T. Zhao, et al., miR-150 exerts antileukemia activity in vitro and in vivo through regulating genes in multiple pathways, Cell Death Dis. 7 (2016) e2371-e2371.
[35] X. Huang, Y. Gao, J. Qin, et al., lncRNA MIAT promotes proliferation and invasion of HCC cells via sponging miR-214, Am. J. Physiol. Gastrointest. Liver Physiol. 314 (2018) G559–G565.
[36] C. Lv, Z. Bai, Z. Liu, et al., MicroRNA-495 suppresses human renal cell carcinoma malignancy by targeting SATB1, Am. J. Transl. Res. 7 (2015) 1992–1999.
[37] C. Liu, M. Jian, H. Qi, et al., MicroRNA 495 inhibits proliferation and metastasis and promotes apoptosis by targeting Twist1 in gastric Cancer cells, Oncol. Res. 27 (2019) 389–397.
[38] M. Gomez-Benito, A. Conchillo, M.A. Garcia, et al., EVI1 controls proliferation in acute myeloid leukaemia through modulation of miR-1-2, Br. J. Cancer 103 (2010) 1292–1296.
[39] D. Beck, J.A.I. Thoms, C. Palu, et al., A four-gene LincRNA expression signature predicts risk in multiple cohorts of acute myeloid leukemia patients, Leukemia (2018) 263–272.
[40] C. Pabst, A. Bergeron, V.P. Lavallee, et al., GPR56 identifies primary human acute myeloid leukemia cells with high repopulating potential in vivo, Blood 127 (2016) 2018–2027.
[41] K.B. Kaufmann, L. Garcia-Prat, Q. Liu, et al., A stemness screen reveals C3orf54/INKA1 as a promoter of human leukemia stem cell latency, Blood (2019) 2198–2211.
[42] R. Garzon, S. Volinia, D. Papaioannou, et al., Expression and prognostic impact of lncRNAs in acute myeloid leukemia, Proc. Natl. Acad. Sci. U. S. A. (2014) 18679–18684.
[43] A. Schwarzer, S. Emmrich, F. Schmidt, et al., The non-coding RNA landscape of human hematopoiesis and leukemia, Nat. Commun. (2017) 218.