The knockdown of MAGI2-AS3 inhibits drug resistance in acute myeloid leukemia by up-regulating miR-155

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Background: Long-chain non-coding RNA (lncRNA) has been confirmed to be involved in the process of many diseases, including acute myeloid leukemia (AML). However, the role of lncRNA MAGI2-AS3 in AML is not clear. This study sought to evaluate the effect and mechanism of MAGI2-AS3 in AML.

Methods: The levels of MAGI2-AS3, miR-155, and zinc finger protein 238 (ZNF238) mRNA were assessed by quantitative reverse transcription PCR (QRT-PCR), and the proliferation and drug sensitivity of AML cells were examined by Cell Counting Kit-8 (CCK-8) assays. Apoptosis was validated by flow cytometry. Further, western blot was used to assess the level of ZNF238, caspase-3, Bax, MRP1, and P-GP. Additionally, a bioinformatics analysis, double luciferase reporter assays, RNA immunoprecipitation assays, and RNA pull-down assays were used to predict and prove the combination of MAGI2-AS3 and miR-155.

Results: Consistent with the results of the Gene Expression Profiling Interactive Analysis (GEPIA) database, the levels of MAGI2-AS3 in AML bone marrow samples and AML cells were decreased. Further, the low levels of MAGI2-AS3 were found to promote the proliferation of AML bone marrow cells, and inhibit apoptosis and sensitivity to Adriamycin (ADM). Additionally, in AML, miR-155 was proven to be the target of MAGI2-AS3, and ZNF238 was proven to be the target of miR-155. Further experiments demonstrated that the low expression of MAGI2-AS3 targeted miR-155, and regulated the proliferation, apoptosis, and sensitivity to ADM of AML bone marrow cells.

Conclusions: MAGI2-AS3 downregulates the expression of ZNF238 by upregulating miR-155, and thus increases drug resistance, and promotes the cell viability and apoptosis of AML cells. These findings may provide a theoretical and experimental basis for the clinical diagnosis and treatment of AML in the future.

Keywords: MAGI2-AS3; acute myeloid leukemia (AML); proliferation; apoptosis; drug resistance

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Introduction

Acute myeloid leukemia (AML) is a common malignant tumor of the hematopoietic system, and accounts for about 70% of acute leukemias. A study has shown that the global incidence of AML is about 1.62 per 100,000 (1), and its incidence in China is trending upwards. As most patients diagnosed with AML have a serious illness, their prognosis is poor and their overall survival rate is low; thus, AML has
become one of the major diseases seriously endangering the health of people (2). During the onset of AML, a great deal of abnormal primordial cells and immature cells in the bone marrow proliferate and inhibit normal hematopoiesis. These cells can infiltrate various organs, such as the liver, spleen and lymph nodes, and lead to anemia, hemorrhage, infection, and infiltration (3,4). At present, chemotherapy is still the main treatment for AML. However, multidrug resistance to major cytotoxic drugs is the main challenge and obstacle facing AML (5). Thus, finding early sensitive biomarkers and exploring effective treatment methods will be of great significance to the effective control of AML.

In recent years, long-chain non-coding RNA (lncRNA) has received more and more attention from scholars. LncRNA has been proven to play a remarkable role in regulating subcellular structure distribution, growth and development, cell directional differentiation, human evolution, and disease occurrence (6,7). Additionally, lncRNA is involved in leukemia; for example, in AML cell lines, lncRNA RP4-576H24.2 is upregulated, and is related to AML susceptibility, risk stratification, and survival (8). Conversely, lncRNA LINP1 accelerates the process of AML by activating the HNF4α/AMPK/WNT5A signaling pathway (9). LncRNA MAGI2-AS3 is located on human chromosome 7q21.11, and has been to be extraordinarily expressed in numerous cancers (10-12). Similarly, Chen et al. confirmed that MAGI2-AS3 can inhibit self-renewal of leukemic stem cells (13). On the other hand, MAGI2-AS3 is involved in cancer drug resistance (14). Whereas, the role and mechanism of MAGI2-AS3 in AML cell drug resistance need to be further explored. However, the effect of MAGI2-AS3 in leukemia is unclear.

MicroRNAs (miRNAs) are a class of endogenous non-coding single-stranded small RNA molecules (about 18–26 nucleotides in length), and are involved in post-transcriptional gene regulation. MiRNAs play a prominent role in the differentiation of normal blood cells, and are also involved in biological processes, such as cell proliferation, apoptosis, and hematopoiesis (15,16). The anomalous expression of miRNAs might be closely related to the occurrence and process of hematological diseases. For example, miR-485-5p is decreased in AML cells, and it prevents the viability of AML cells by decreasing SALL4 (17). MiR-155 has been confirmed to be highly expressed in thyroid cancer, breast cancer, oral squamous cell carcinoma, and other malignant tumors (18-20). Additionally, miR-155 is involved in the drug resistance of AML cells. Yu et al. confirmed that LncRNA MEG3 downregulates ALG9 through sponging miR-155 in drug-resistant AML cells (21). Further, the targeting of miR-155 by MAGI2-AS3 promotes the malignant phenotype of non-small cell lung cancer (11). Similarly, Chen et al. confirmed that MAGI2-AS3 inhibits the self-renewal of leukemic stem cells (13). However, the function and mechanism of MAGI2-AS3 in AML need to be further explored. In addition, in leukemia, the expression of miR-155 is up-regulated in leukemia. The up-regulation of miR-155 is related to the poor prognosis of leukemia, and promotes the proliferation of acute lymphocytes by targeting ZNF238 (22).

In view of the above research, this study sought to reveal the effect and mechanism of MAGI2-AS3 in AML. In this study, the low expression of MAGI2-AS3 enhanced AML HL-60 cell proliferation, inhibited apoptosis and sensitivity to doxorubicin, and the low expression of MAGI2-AS3 was related to a poor prognosis. MiR-155 was the targeted miRNA of MAGI2-AS3. Further, functional gain and loss experiments confirmed that the low expression of MAGI2-AS3 inhibited AML by regulating the miR-155/zinc finger protein 238 (ZNF238) axis. These findings lay the foundation for future clinical research on AML. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6853/rc).

Methods

Patient sample

The bone marrow specimens of 48 AML patients newly diagnosed at The First Affiliated Hospital of China Medical University from February 2019 to March 2020 were selected. All the patients were confirmed to have AML by morphology, immunology, cytogenetics, molecular biology (MICM) typing; the diagnostic criteria used have been detailed previously in the literature (23). Forty-eight bone marrow samples were also collected from healthy control patients. The samples were immediately preserved and stored in liquid nitrogen.

All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). All the bone marrow patients signed informed consent forms before the collection of the samples. The research plan was approved by the ethics board of The First Affiliated Hospital of China Medical University (No. 20170325).
Cellular cultivation and transfection

Bone marrow stromal HS-5 cells (the control cells) and AML cells (KG-1, HL-60, and CCRF-CEM) were purchased from CCTCC (PRC). The cells were cultivated in Roswell Park Memorial Institute Medium 1640 intermediary with 10% fetal bovine serum and 1% penicillin/streptomycin in an incubating device at 37 °C with a 5% volume fraction of carbon dioxide. 0.25% trypsin was used for digestion and passage in the log-growth phase of the cell. HL-60 cells in the log-growth phase were seeded in 6-well dishes, and when the cellular density reached 40–50%, MAGI2-AS3 low expression plasmids, miR-155 inhibitors, and mimics and their respective controls were subjected to transfection with HL-60 cells by Lipofectamine 2000 transfection reagent (ThermoFisherScience, Waltham, MA, USA). For small-inhibitory RNA (siRNA) transfection, the siRNAs were developed and prepared by Genepharma (PRC) and afterwards employed to realize the knockdown of MAGI2-AS3 in HL-60 (si-MAGI2-AS3#1: 5’-CCAGCUAGCGCUACUGAG-3’, si-MAGI2-AS3#2: 5’-CCTTCACACTTTGCTGTA-3’, si-MAGI2-AS3#3: 5’-CCACAGACACUUAACACAA-3’, si-NC: 5’-UUCUCGCCAGUGUCACGU-3’). After 6 hours, the cells were cultured in complete medium, and transfection efficiency was detected 24 hours after transfection.

Quantitative reverse transcription PCR (QRT-PCR)

The overall RNA in the tissues or cells was abstracted using Trizol reagent as per the supplier’s protocol (Invitrogen, USA). The RNA level and purity were detected using a nanodrop spectral photometer. As per the supplier’s instructions, our team used the PrimeScript-RT Kit (Madison, USA) to prepare the complementary DNA from 1 μg of overall RNA. Our team then used the SYBR® Premix-Ex-Taq chips (Takara, TX, USA) and ABI7300 systems for the QRT-PCR. The overall volume of the PCR system was 30 μL, and every specimen involved 300 ng of cDNA. The magnification program was first denatured under 95 °C for 10 min, followed by another 45 cycles (i.e., 95 °C for 10 s, 60 °C for 0.5 min, and 85 °C for 20 s). Our team transformed the entire fluorescent data into comparative quantitation data. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was the inner reference of MAGI2-AS3 and ZNF238, and U6 was the inner reference of miR-155. All the QRT-PCR responses were repeated 3 times. The specific primer sequence data are presented in Table 1.

Flow cytometry

The two groups of cells in the log-growth stage were seeded into a 96-well plate at 1×10⁴ cells per well. Prior to the 24 h of cultivation, they were cleaned 2 times in phosphate-buffered saline (PBS), subjected to fixation in 70% ethanol, and stored overnight at 4 °C. The PBS was washed once, and the cellular density was modified to 1×10⁶ cells/mL. Propidium iodide dyeing liquor was supplemented for staining for 30 min at 4 °C with an eventual thickness of 0.05 mg. The cell cycle was analyzed by flow cytometry.

Table 1 Primer sequence

| Genes   | Primer sequence                  |
|---------|----------------------------------|
| MAGI2-AS3 | F: 5’-CTACAGGCAAGCTGATGAAAG-3’ |
|         | R: 5’-AGTGTGAAAGGTAGGAGTCA-3’   |
| MiR-155  | F: 5’-CGCGTTAATGCTAATCCTGTA-3’  |
|         | R: 5’-AGTTCAGGTCGCCAGTTAT-3’    |
| ZNF238   | F: 5’-GCAGGACTAGAAAGAAGGA-3’    |
|         | R: 5’-CTCTGCTCGCTAGACACTTG-3’   |
| U6       | F: 5’-CTCGTTCCGAGCAGAC-3’       |
|         | R: 5’-AACGCTCCAGAATTTGCGT-3’    |
| GAPDH    | F: 5’-CTGGGCTACACTGAGCACC-3’    |
|         | R: 5’-AAGTGGTCGTTGAGGCAATG-3’   |

Cell Counting Kit-8 (CCK-8) assays

AML cells in the logarithmic growth phase with a concentration of 1×10³/mL were seeded to 96-well dishes with 100 L/holes with 3 multiple holes in each group. After 24 hours, the medium was replaced by a new medium containing 80 g/mL of doxorubicin, and the blank control group was supplemented with an identical volume of dimethyl sulfoxide culture medium. After continued cultivation for 24, 48, and 72 h, 10 μL of CCK-8 reagent was added to each well. After 2 hours of culture, the optical density (OD) of every hole was identified at 450 nm by an automatic enzyme labeling instrument. The average value of the 3 holes was used to calculate the cell growth inhibition rate. The following equation was used to compute the inhibition rate: growth suppression rate = (1 − OD group/OD control group) × 100%. The IC₅₀ value was obtained by curve fitting using the least squares method.
Southern Biotechnology, Birmingham, AL, USA). Three compound holes were set in every group, and the assay was conducted repeatedly 3 times, and the average value was taken.

**Double luciferase reporter gene assays**

Luciferase reporter gene detection was completed using the dual-luciferase reporter assay system (Promega, USA). The targeted fragments of WTMAGI2-AS3 and MUT MAGI2-AS3 were constructed and integrated into the pGL3 vector (Promega, Madison, WI, USA) to construct the pGL3-MAGI2-AS3-wild type (MAGI2-AS3-wt) and pGL3-MAGI2-AS3-mutant (MAGI2-AS3-mut) reporter vectors. MAGI2-AS3-wt or MAGI2-AS3-mut and miR-155 mimetic substance or normal control (NC) were co-transfected into the HEK293T cells. Forty-eight hours before transfection, luciferase activity was determined according to the supplier's instructions.

**RNA immunoprecipitation assays**

A total of 2×10<sup>7</sup> AML cells were harvested and lysed with an identical volume of H1P lysate. The cells were centrifuged for 12,000 r/min. The diameter of centrifugation was 420 mm, and the centrifugal diameter was 10 min. The supernatant was obtained. As per the instructions of the RIP RNA binding protein immune precipitation kit, 900×1 RIP immune precipitation buffering solution (including RNase suppressor, protease suppressor, and DNase) and 100 μL of cellular lysate were supplemented to the EP tube containing magnetic beads, immunoglobulin G (IgG) or argonaute 2 (Ago2) anti-substance was supplemented, cultivated overnight at 4℃, subjected to centrifugation (12,000 r/min, with a centrifugal diameter of 420 mm) for 10 min, after which the supernatant was discarded. The RNA was purified by washing the cells 6 times with 500 L RIP Washing Buffering solution, and the RNA was dissolved and subjected to purification by 15 L of DEPC water. The cells were stored at −80℃. Those without antibodies were used as the positive control (Input) group, those with IgG antibodies were used as the negative control (anti-lgG) group, and those with Ago2 antibodies were used as the experimental (anti-Ago2) group.

**RNA pull-down assays**

Overall, 1×10<sup>7</sup> cells were collected and cracked by ultrasound. The MAGI2-AS3 probe was incubated with Cmurl-1 magnet beads (Life Technologies) at 25℃ for 2 hours to form the probe-coated beads. The cellular lysate containing the MAGI2-AS3 probe or oligonucleotide probe was cultivated at 4℃ overnight. Before cleaning in a washing buffering solution, the RNA mixture binding to the beads were subjected to elution using the RNasy Mini Kit (QIAGEN) for RT-PCR or real-time PCR.

**Western blot**

The cells were harvested, incubated on ice via precooled RIPA lysate for 20 min, and centrifuged at 13,000 r/min, at 4℃ for 20 min. Before centrifugation, the supernatant was taken, and the protein was quantified using the BCA protein quantitative kit, the protein concentration was adjusted, and boiled for denaturation for 5 min. The protein was electrophoretic by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis for 90 min, and moved onto polyvinylidene fluoride film. The film was sealed with Tris-buffered saline (TBST) solution of 3% Bovine serum albumin (BSA) at room temperature, and diluted primary antibodies were added to the anti-Caspase-3 (1:1,000, ab32351, Shanghai, China), anti-Bax (1:1,000, ab32503, PRC), anti-ZNF238 (1:1,000, ab118471, PRC), anti-MRP1 (1:1,000, ab233383, PRC) and anti-PGP (1:1,000, ab261736, PRC), and stored overnight at 4℃. TBST was used to clean the film 5 times, for 3 min each time. Diluted secondary antibodies (1:2,000) were added and shaken gently for 40 minutes under RT. The film was cleaned 6 times, for 3 min each time. The enhanced chemiluminescence (ECL) was developed, fixed, and scanned. The gray values of every band were studied via the software ImageJ, and the rate of the gray value of the targeted protein to the gray value of GAPDH was used as the protein relative expression value.

**Statistical analysis**

The entire assays were conducted in triplicate, and the data were studied via SPSS 20.0. The data are displayed as the average ± standard deviation. The statistical methods included an analysis of variance. The mean values of two independent samples were compared by t-tests. A P value <0.05 was considered statistically significant. All the experiments were repeated 3 times.

**Results**

The expression of MAGI2-AS3 was declined in AML

To examine the effect of MAGI2-AS3 on AML, our team
studies the expression of MAGI2-AS3 in AML patients according to the Gene Expression Profiling Interactive Analysis (GEPIA) database (http://gepia.cancer-pku.cn/). As Figure 1A shows, MAGI2-AS3 was a lowly expressed lncRNA in AML cells. The expression of MAGI2-AS3 in AML bone marrow samples and AML myeloid cells was also downregulated. HL-60 cells with a significantly high expression of MAGI2-AS3 were selected for subsequent functional experiments (P<0.05; see Figure 1B,1C). A high proportion of AML non-M3 had an effect on the high expression of MAGI2-AS3. Further, a low level of MAGI2-AS3 was related to higher risk stratification (see Table 2).

**Silencing MAGI2-AS3 promoted the proliferation of AML bone marrow cells, and inhibited apoptosis and sensitivity to doxorubicin**

Subsequently, siRNAs for MAGI2-AS3 (i.e., si-MAGI2-AS3-1, si-MAGI2-AS3-2, and si-MAGI2-AS3-3) were synthesized, and the content of MAGI2-AS3 was assessed by QRT-PCR. The data showed that these siRNA significantly reduced the level of MAGI2-AS3. Among them, si-MAGI2-AS3-2 showed a higher transfection efficiency, and was thus selected for the follow-up experiment (P<0.05; see Figure 2A). Additionally, the viability of HL-60 cells was enhanced and the apoptosis was decreased after MAGI2-AS3 silencing (P<0.05; see Figure 2B,2C). Conversely, the expression of Bax and caspase-3 decreased after MAGI2-AS3 silencing (P<0.05; see Figure 2D). Further, the susceptibility of HL-60 cells to doxorubicin decreased (P<0.05; see Figure 2E).

**MiR-155 was a targeted miRNA of MAGI2-AS3**

The binding site between MAGI2-AS3 and miR-155 was proven to be a wild type (WT) binding spot (wt-MAGI2-AS3) or a MUT binding spot (mut-MAGI2-AS3) (see Figure 3A). As Figure 3B,3C show, the expression of miR-155 in AML myeloid specimens and AML bone marrow cells was higher than that in healthy myeloid specimens and healthy myeloid lineage cells. Additionally, the cotransfection of miR-155 mimic substance and luciferase vector involving wt-MAGI2-AS3 induced a remarkable reduction in comparative luciferase activity in HEK293T cells (P<0.05; see Figure 3D). Subsequently, to determine whether MAGI2-AS3 can be used as a RNA sponge of miR-155, we undertook a RIP analysis, and found that MAGI2-AS3 and miR-155 were more highly expressed in Ago2 particles than IgG particles (P<0.05; see Figure 3E). Moreover, the RNA pull-down experiment showed that the
MAGI2-AS3 level of the miR-155-bio probe was higher than that of the miR-155 probe in the control group (NC bio) (P<0.05; see Figure 3F). Additionally, low MAGI2-AS3 expression was found to regulate the level of miR-155 in HL-60 (P<0.05; see Figure 3G). The results of the Pearson correlation analyses showed that a negative association existed between the expression of MAGI2-AS3 and miR-155 in AML bone marrow samples (P<0.001; R$^2=0.562$; see Figure 3H). Thus, miR-155 can be used as a straight miRNA target of MAGI2-AS3, and is negatively regulated by MAGI2-AS3.

**ZNF238 was a direct target of miR-155**

To demonstrate the potential mechanism of miR-155 in AML, we performed a bioinformatics analysis to investigate the underlying downstream targets of miR-155. We found that ZNF238 may be the target of miR-155. The binding spot between miR-155 and ZNF238 is shown in Figure 4A. Next, a luciferase report analysis was conducted to evaluate the association between miR-155 and ZNF238. As Figure 4B shows, luciferase activity was significantly decreased in the HEK293T cells co-transfected with the miR-155 mimetic substance and ZNF238-wt. The expression of ZNF238 was downregulated and increased in HL-60 cells treated with the miR-155 mimetic substance and suppressors, respectively (P<0.05; see Figure 4C). Further, the outcomes of Pearson correlation analysis revealed that there was a negative correlation between the expression of ZNF238 and miR-155 in bone marrow specimens of AML (P<0.001; R$^2=0.532$), but positively correlated with MAGI2-AS3 (P<0.001; R$^2=0.537$; see Figure 4D,4E).

Additionally, as Figure 4F,4G show, the expression of ZNF238 in AML myeloid specimens and AML bone marrow cells was lower than that in normal myeloid specimens and healthy myeloid cell lines (P<0.05). The role of miR-155/ZNF238 in AML was further confirmed. The miR-155 mimics and overexpression plasmids of ZNF238 were co-transfected into the HL-60 cells. The CCK-8 analysis revealed that the survival rate of HL-60 cells increased after miR-155 mimics transfection, but decreased after the overexpression of ZNF238 (P<0.05; see Figure 4H). Further, the flow cytometry results revealed that the apoptosis of HL-60 cells was decreased before miR-155 mimics transfection, but increased after the overexpression of ZNF238 (P<0.05; see Figure 4I). Additionally, CCK-8 detection revealed that the susceptibility of HL-60 cells to doxorubicin was reduced after miR-155 mimics transfection, while the susceptibility of HL-60 cells to Adriamycin (ADM) was increased after the overexpression of ZNF238 (P<0.05; see Figure 4J). Thus, miR-155 could facilitate the growth of HL-60 cells, inhibit apoptosis and weaken the sensitivity of HL-60 cells to ADM by negatively modulating ZNF238.

### Table 2: The relationship between the expression of MAGI2-AS3 and clinical and genetic characteristics

| Characteristics                        | MAGI2-AS3 |       | P    |
|----------------------------------------|----------|------|------|
|                                          | High expression (n=24) | Low expression (n=24) |      |
| Age, mean ± SD                         | 35.24±8.25 | 34.36±8.42 | 0.716 |
| White blood cells, mean ± SD           | 42.52±5.82 | 39.45±4.61 | 0.071 |
| Platelets, mean ± SD                   | 67.43±7.43 | 70.47±7.36 | 0.161 |
| Hemoglobin, mean ± SD                  | 9.38±1.25  | 9.15±1.26  | 0.529 |
| FAB typing, n                          | 0.004     |      |      |
| M₃                                     | 8         | 18   |      |
| Non-M₃                                 | 16        | 6    |      |
| Risk stratification of cytogenetics, n | 0.000     |      |      |
| Good                                   | 15        | 4    |      |
| Intermediate                           | 6         | 10   |      |
| Poor                                   | 3         | 10   |      |

P<0.05 means the difference is statistically significant. FAB, French-American-British.
Figure 2 Silencing MAGI2-AS3 promoted the proliferation of AML bone marrow cells, and inhibited apoptosis and sensitivity to doxorubicin. (A) The knockdown efficiency of MAGI2-AS3 siRNA was tested by QRT-PCR. (B) CCK-8 assays were used to detect the proliferation of HL-60 cells after MAGI2-AS3 silencing. (C) Flow cytometry was used to detect the apoptosis of HL-60 cells after MAGI2-AS3 silencing. (D) Western blot was used to determine the expression of apoptosis-related proteins Bax and caspase-3 after MAGI2-AS3 silencing. (E) CCK-8 assays were used to detect the sensitivity of HL-60 cells to doxorubicin. *P<0.05; **P<0.01; ***P<0.001. AML, acute myeloid leukemia; QRT-PCR, quantitative reverse transcription PCR; CCK-8, Cell Counting Kit-8; NC, normal control; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Figure 3 MiR-155 was a targeted miRNA of MAGI2-AS3. (A) The bioinformatics analysis showed that there were conservative binding sites between MAGI2-AS3 and miR-155. (B) The expression of miR-155 in bone marrow samples of AML was detected by QRT-PCR. (C) The expression of miR-155 in AML cells was detected by QRT-PCR. (D) Double luciferase assays were used to verify the binding of MAGI2-AS3 and miR-155. (E,F) RNA immunoprecipitation assays and RNA pull-down assays were used to detect the binding of MAGI2-AS3 and miR-155. (G) The levels of miR-155 in HL-60 cells with low and high expression of MAGI2-AS3 were detected by QRT-PCR. (H) A Pearson correlation analysis was conducted to detect the correlation between the expression of MAGI2-AS3 and miR-155 in AML bone marrow samples. *P<0.05; **P<0.01; ***P<0.001. MiRNA, microRNAs; AML, acute myeloid leukemia; QRT-PCR, quantitative reverse transcription PCR; NC, normal control; IgG, immunoglobulin G; Ago2, argonaute 2.
Figure 4 ZNF238 was a direct target of miR-155. (A) The bioinformatics analysis showed that there were conservative binding sites between ZNF238 and miR-155. (B) Double luciferase assays were used to verify the binding of MAGI2-AS3 and miR-155. (C) The levels of ZNF238 mRNA in HL-60 cells with low and high expression of miR-155 were detected by QRT-PCR. (D,E) A Pearson correlation analysis was conducted to detect the correlation between the expression of ZNF238 and miR-155 or MAGI2-AS3 in AML bone marrow samples. (F) The expression of ZNF238 in bone marrow samples of AML was detected by QRT-PCR. (G) The expression of ZNF238 in AML cells was detected by QRT-PCR. (H) CCK-8 assays were used to detect the proliferation of HL-60 cells. (I) Flow cytometry was used to detect the apoptosis of HL-60 cells. (J) CCK-8 assays were used to detect the sensitivity of HL-60 cells to doxorubicin. *P<0.05; **P<0.01; ***P<0.001. QRT-PCR, quantitative reverse transcription PCR; AML, acute myeloid leukemia; CCK-8, Cell Counting Kit-8; NC, normal control.
Silencing MAGI2-AS3 facilitated the survival of AML myeloid cells and enhanced their resistance to ADM by upregulating miR-155

To explore the effects of the MAGI2-AS3/miR-155/ZNF238 signal axis on the development of AML and chemosensitivity, we conducted a series of molecular biology experiments with HL-60 cells. Silencing MAGI2-AS3 caused a drop in the expression of MAGI2-AS3 and ZNF238, but an elevation in the expression of miR-155. Additionally, when miR-155 was lowly expressed, the expression of ZNF238 was upregulated (all P<0.05; see Figure 5A-5C). The CCK-8 analysis showed that the cellular survival rate increased at 24, 48, and 72 h after silencing MAGI2-AS3, but decreased after transfection with miR-155 inhibitors (P<0.05; see Figure 5D). Further, the flow cytometry results revealed that the apoptosis of HL-60 cells was reduced after the silencing of MAGI2-AS3, but increased after transfection with miR-155 inhibitors (P<0.05; see Figure 5E).

The immunoblotting outcomes revealed that the expression of caspase-3 and Bax in HL-60 cells was reduced before the silencing of MAGI2-AS3, but elevated before transfection with miR-155 suppressors (P<0.05; see Figure 5F). Next, the HL-60 cells were exposed to 50 μg/mL of doxorubicin. The outcomes of the CCK-8 analysis revealed that the susceptibility of HL-60 cells to ADM decreased after the low expression of MAGI2-AS3, while the sensitivity of HL-60 cells to ADM increased after treatment with miR-155 inhibitors (P<0.05; see Figure 5G). The expression of drug resistance proteins MRP1 and P-GP were upregulated after silencing MAGI2-AS3, which was saved by the low expression of miR-155 (P<0.05; see Figure 5H). Thus, silencing MAGI2-AS3 promotes the vitality of AML bone marrow cells and weakens their sensitivity to doxorubicin by upregulating the expression of miR-155.

Discussion

At present, the tolerance of AML patients to chemotherapy and radiotherapy has led to a bottleneck in traditional therapy, which has become one of the reasons for the high mortality of patients (24). Thus, it is particularly important to identify the molecular mechanism of AML resistance for disease diagnosis and treatment, and to overcome the problem of drug resistance in AML treatment.

Non-coding RNA plays a considerable role in AML. The role of non-coding RNA in AML has attracted much attention (25). It is essential to determine the function of lncRNA and miRNA in the pathogenesis of AML. In our study, we found a negative correlation between MAGI2-AS3 and the occurrence of AML. The expression of MAGI2-AS3 was decreased in AML spinal cord tissue and AML cells. The low expression of MAGI2-AS3 accelerated the viability of AML cells, and inhibited apoptosis and sensitivity to chemotherapeutic drugs. Our results suggest that the downregulation of MAGI2-AS3 may induce AML progression (see Figure 6) by regulating the miR-155/ZNF238 axis. These findings may provide new ideas for the clinical treatment of and molecular research on AML.

Thousands of intergenic lncRNA have been identified by genome-wide sequencing and have been shown to play a vital role in a variety of diseases. MAGI2-AS3 is a newly identified type of lncRNA in human malignant tumors, and has a fundamental effect on gene regulation in many diseases. For example, MAGI2-AS3 promotes the progression of gastric cancer by upregulating ZEB1 in combination with miR-141/200a (12). Additionally, in Alzheimer’s disease, the upregulation of MAGI2-AS3 promotes neurotoxicity and neuroinflammation by downregulating miR-374b-5p (26). Conversely, the expression of MAGI2-AS3 in leukemic stem cells has been shown to be downregulated (13). Similarly, in the present study, we found that the level of MAGI2-AS3 was decreased in AML bone marrow samples and cells, and the low expression of MAGI2-AS3 was associated with a poor prognosis. Further, our functional experiments confirmed that MAGI2-AS3 promoted the proliferation of AML bone marrow cells, and inhibited apoptosis and sensitivity to doxorubicin. Thus, the newly identified lncRNA of MAGI2-AS3 may be involved in the development of AML.

Recent studies have shown that the mutual regulation between lncRNA and miRNA plays a considerable role in the process of AML. Yang et al. showed that the high expression of lncRNA SNHG16 promotes the progression of AML by downregulating miR-183-5p and upregulating FOXO1 (27). Additionally, Zhao et al. confirmed that lncRNA-H19 promotes AML cell activity and inhibits apoptosis by targeting miR-29a-3p (28). Similarly, LINC00265 upregulation attenuates the apoptosis of AML cells via the miR-485/IRF2 axis, which in turn inhibits the progression of AML (29). In the present study, through the bioinformatics analysis, miR-155 was predicted to be the target of MAGI2-AS3. According to previous research, miR-155 is involved in a variety of diseases; for example,
miR-155 is highly expressed in glioma cells, and the overexpression of miR-155 promotes the proliferation, migration, and invasion of glioma cells by activating PI3K/AKT signaling (30). Additionally, a study has shown that MAGI2-AS3 targeting miR-155 promotes non-small cell lung cancer cells proliferation, migration, and invasion (11).
In recent years, the role of miR-155 in AML has been widely examined, and studies have shown that there is a positive correlation between miR-155 and the occurrence of AML (31,32). This study confirmed that MAGI2-AS3 regulated AML by regulating miR-155, and confirmed the negative correlation between MAGI2-AS3 and miR-155. Likewise, it was confirmed that the low expression of miR-155 could partially reverse the role of the low expression of MAGI2-AS3 in promoting AML.

ZNF238 is a kind of C2H2 zinc finger protein, which can regulate gene expression as a transcriptional activator or transcriptional inhibitor. It has been reported that ZNF238 can inhibit tumor growth in brain tumors and is considered a tumor suppressor (33). A research has shown that miRNA recognizes the 3’ untranslated region of the target gene mRNA by base complementary pairing, inhibits the translation or degradation of target mRNAs and regulates gene expression. For example, miR-206 can induce rhabdomyosarcoma cell differentiation by activating RUNX1 and ZNF238 (34). Additionally, in addition, in leukemia, the expression of miR-155 is up-regulated in leukemia. The up-regulation of miR-155 is related to the poor prognosis of leukemia, and promotes the proliferation of acute lymphocytes by targeting ZNF238 (22). In the present study, the expression of ZNF238 was found to be decreased in AML cells, ZNF238 was confirmed to be the target of miR-155, and miR-155 was shown to regulate the survival of AML cells via the negative regulation of ZNF238. Moreover, further experiments confirmed that MAGI2-AS3 regulated the proliferation and drug resistance of AML cells by adsorbing miR-155 to regulate the expression of ZNF238 in vivo.

In short, this study showed the important role of the MAGI2-AS3/miR-155/ZNF238 axis. We found that MAGI2-AS3 gene knockout increased the miR-155 of AML cells, decreased ZNF238, promoted the proliferation of AML cells, and inhibited the apoptosis and drug resistance of AML cells. However, this study had some limitations, and the question of whether other downstream miRNAs related to MAGI2-AS3 also participate in AML requires further study. Additionally, previous study has shown that in breast cancer, MAGI2-AS3 inhibits breast cancer progression by upregulating MAGI2 DAN methylation and inhibiting the Wnt/β-Catenin pathway (34). However, the question of whether MAGI2-AS3 can regulate related signal pathways in AML needs to be further studied.

In sum, MAGI2-AS3 can be used as a therapeutic target for AML and provides some reference for clinical treatment.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://atm.amegroups.com/article/view/10.21037/atm-21-6853/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). All the bone marrow patients signed informed consent forms before the collection of the samples. The research plan was approved by the ethics board of The First Affiliated Hospital of China Medical University (No. 20170325).

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