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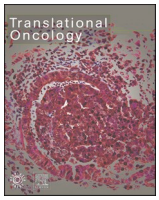

Original Research

Overexpression miR-520a-3p inhibits acute myeloid leukemia progression via targeting MUC1

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ABSTRACT

Background: Acute myeloid leukemia (AML) is one of the familiar malignant tumors in the hematological system. miR-520a-3p is reported to be involved in several cancers' progression. However, miR-520a-3p role in AML remains unclear. In this study, we aimed to clarify the role and potential mechanism of miR-520a-3p in AML.
Methods: Cell viability, proliferation, cycle and apoptosis were detected by MTT assay, colony formation assay, flow cytometry, respectively. The levels of PNCA, Bcl-2, Cleaved caspase 3, Cleaved caspase 9 and β-catenin protein were detected by Western blot. Dual-luciferase reported assay was performed to detect the regulation between miR-520a-3p and MUC1. To verify the effect of miR-520a-3p on tumor proliferation *in vivo*, a non-homogenous transplant model of tumors was established.
Results: miR-520a-3p expression was down-regulated, and MUC1 expression was up-regulated in AML patients. miR-520a-3p overexpression suppressed THP-1 cell proliferation, induced cell cycle G0/G1 inhibition and promoted apoptosis. miR-520a-3p targeted MUC1 and negatively regulated its expression. MUC1 knockdown inhibited THP-1 cell proliferation and promoted apoptosis. miR-520a-3p overexpression inhibited AML tumors growth.
Conclusion: Overexpression miR-520a-3p inhibited AML cell proliferation, and promoted apoptosis via inhibiting MUC1 expression and repressing Wnt/β-catenin pathway activation.

Introduction

Acute leukemia is a diverse group of hematological malignancies, characterized by aberrant proliferation and accumulation of malignantly transformed hematopoietic stem cells in the bone marrow and other hematopoietic tissues [1]. Acute leukemia was separated into two distinct entities, acute lymphoblastic leukemia (ALL), and acute myeloid leukemia (AML), according to their diverse morphologies, prognoses, and preferred treatment protocols [2]. AML is a hematopoietic disorder characterized by numerous cytogenetic and molecular aberrations [3,4]. Consistent with other cancers, AML has a high morbidity and mortality rate. Chemotherapy and allogeneic stem cell transplantation are the main therapeutic strategies of AML [5]. However, the overall five-year survival rate of AML patients is still not ideal [6]. Therefore, it is urgent to investigate the etiology and seek new therapeutic strategies.

MiRNA is an evolutionarily conserved non-coding small RNA of 18–20 bp [7]. It has been reported that miRNA plays a vital role in gene expression by regulating posttranslational translation and may be involved in the regulation of the progression of various diseases [8]. Recent findings suggest that miRNAs are involved in the regulation of leukemia progression including AML and CML. It was reported that miR-152-3p was highly expressed in CML tissues and cells that promoted disease progression [9]. MiR-582-3p overexpression inhibited AML cell proliferation and stagnates cell cycle [10]. MiRNA may be a target of AML therapy. miR-520a-3p is involved in controlling various cancers progression. For example, miR-520a was up-regulated in AML patients, and increased prognostic risk in patients [11]. It has been reported that miR-520a-5p had a therapeutic effect on chronic myeloid leukemia cells by targeting STAT3 and enhanced the anticancer effect of capsaicin [12]. miR-520a-3p could relieve the malignant transformation of NSCLC through the PI3K/Akt pathway [13]. Proliferation of colon cancer cells was inhibited by miR-302c-3p and miR-520a-3p [14]. miR-520a-3p could suppress gastric cancer cells' metastasis [15]. However, the regulatory mechanism of miR-520a-3p has not been reported in AML. In addition, increased evidence indicated that miR-520a-3p regulated various cancers progression by regulating downstream target genes [16]. Furthermore, Starbase predicted that there was a potential binding site between miR-520a-3p and MUC1. However, the role of

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Table 1
Clinical characteristics of AML patients and normal.

Characteristics	Normal (n = 25)	AML (n = 25)	P value
Sex			
Male	13	11	0.775
Female	12	14	
Age (years), mean ± SD	48.8 ± 12.5	49.5 ± 16.4	0.8659
PB blast (%), mean ± SD	40.26 ± 20.1	44.15 ± 23.7	0.5344

Note: AML, Acute myeloid leukemia; *p<0.05.

Table 2
Relationship between miR-520a-3p expression and clinical characteristics in AML.

Characteristics	miR-520a-3p low (n = 12)	high (n = 13)	P value
Sex			0.2377
Male	7	4	
Female	5	9	
Age (years), mean ± SD	48.8 ± 14.2	47.6 ± 17.1	0.851
PB blast (%), mean ± SD	37.53 ± 18.4	42.22 ± 21.3	0.5631
myeloblasts (%), mean ± SD	41.26 ± 13.8	39.47 ± 15.9	0.7673
WBC (/10^9L), mean ± SD	42.67 ± 13.6	55.32 ± 16.5	0.0487*
Hemoglobin (g/L), mean ± SD	9.52 ± 2.3	9.84 ± 2.6	0.7483
Platelet (× /10^9L), mean ± SD	70.7 ± 39.8	66.6 ± 33.2	0.7816
FAB subtypes/n (%)			0.474
M0	1	2	
M1	2	1	
M2	4	3	
M3	0	1	
M4	1	4	
M5	3	1	
M6	1	0	
M7	0	1	
Mutation			0.977
FLT3	2	1	
NPM1	2	2	
TP53	1	1	
CEBPA	2	1	
IDH1	1	3	
IDH2	1	1	
KRAS	1	1	
TET2	1	1	
NRAS	1	1	
RUNX1	0	1	

Note: AML, Acute myeloid leukemia; PB, peripheral blood; WBC, white blood cells; FAB, French–American–British. *p<0.05.

miR-520a-3p and its regulatory mechanism in AML remains unclear.

Mucin-1 (MUC1) is an ectopic epithelial glycoprotein highly expressed in AML cell lines and patient tissues [17,18]. MUC1 is consisted by two functional regions, N-end subunit (MUC1-N) and C-end subunit (MUC1-C), both located on the cell surface [19]. MUC1 mainly realizes its biological function through N-terminal subunit (MUC1- N). The specific mechanism is that the MUC1-C subunit interacts with receptor tyrosine kinases located on the cell membrane of the nucleus, and interacts with transcription factors such as NF-κB and transformation-related β-catenin [20,21]. β-catenin is a component of adhesion junction of mammalian epithelial cells, which connects adhesion molecules to cells through α-catenin [22]. Researches showed that that Wnt/ β-catenin pathway was closely related to AML progression. The findings of Jiang et al showed that Wnt/β-catenin pathway was necessary for the development of leukemic stem cells, and the inactivation of Wnt/ β-catenin pathway exerted anti-leukemia activity [23,24]. Ji et al reported that CD82 promoted the survival of childhood AML cells by activating Wnt/ β-catenin pathway [25]. Moreover, there was evidence that MUC1 was involved in the regulation of Wnt/ β-catenin pathway [21]. MUC1 promoted levels of β-catenin in cell plasma and nuclei, while inhibition of MUC1 inhibited the activation of the Wnt/β-catenin pathway [26]. In this paper, whether MUC1 regulates Wnt/β-catenin in AML remains to be further explored.

Summary, we aimed to clarify the regulatory roles of miR-520a-3p in AML, and the possible mechanism of MUC1 to regulate AML progress. These findings indicated that miR-520a-3p overexpression inhibited AML cell progression and suppressed MCU1 expression. The role of miR-520a-3p in AML was confirmed, and new knowledge was provided for AML treatment strategy.

Materials and methods

Patients and tissue specimen

AML blood samples (25 samples) and normal blood samples (healthy control group, 25 samples) were collected from Huizhou Municipal Central Hospital during July 2019 to August 2020. Normal blood samples were collected from healthy blood donors. Before sample collection, these patients did not receive chemotherapy or radiotherapy and there were no infections or various cancers, indicting the patient did not suffer from multiple cancers.

Moreover, clinical characteristics of AML patients and healthy blood donors was analyzed in Table 1, and relationship between miR-520a-3p expression and clinical characteristics in AML was analyzed in Table 2. All patients signed written informed consent. All experimental programs were approved by the Huizhou Municipal Central Hospital ethics committee, and the experimental procedure was carried out according to the principles of the Helsinki declaration. Peripheral blood mononuclear cells (PBMCs) used in this study were separated based on previous reports [27].

Cell culture

THP-1 cells were purchased from ATCC (Rockefeller, Maryland, USA). THP-1 cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum and 1% P/S. All cells were cultured in a wet incubator containing 5% CO_2 at 37°C.

Cell transfection

According to the instructions, mimics NC (negative control of miR-520a-3p mimics), miR-520a-3p mimics (simulated the high-level expression of miR-520a-3p in cells), sh-NC (negative control of sh-MUC1), sh-MUC1 (inhibited MUC1 expression by forming small non-coding RNA molecules with hairpin structure), inhibitor NC (negative control of miR-520a-3p inhibitor), miR-520a-3p inhibitor (inhibited miR-520a-3p expression) were transfected into THP-1 cells by using Lipofectamine™ 3000 (Takara, Dalian, China). Following 48 h transfection, THP-1 cells were applied to subsequent experiments. All plasmids used in this study were synthesized by Invitrogen (Carlsbad, CA)

MTT assay

The cell viability was detected by the MTT assay. 1×10^4 THP-1 cells were inoculated in 96-well plates for 24 h and incubated with 20 μL MTT reagent which dissolved in phosphate buffer (PBS) (Roche, Basel, Switzerland, 5 mg/ml) at 37 °C for 4 h. The culture medium was removed and 150 μL dimethyl sulfoxide (DMSO, Roche, Basel, Switzerland) was added. A microplate reader (Olympus, Tokyo, Japan) was used to evaluate cell viability with absorbance of 490 nm.

Colony formation assay

Cell proliferation was detected by colony formation assay. In detail, THP-1 cells (1×10^3 cell/per well) were added to a 6-well cell culture plate. After 7–10 days, the cells were fixed at 10–15 min with 4% formaldehyde, and the cells were dyed in the crystal violet (Abcam, Cambridge, UK) for 15 min at room temperature, and finally there were

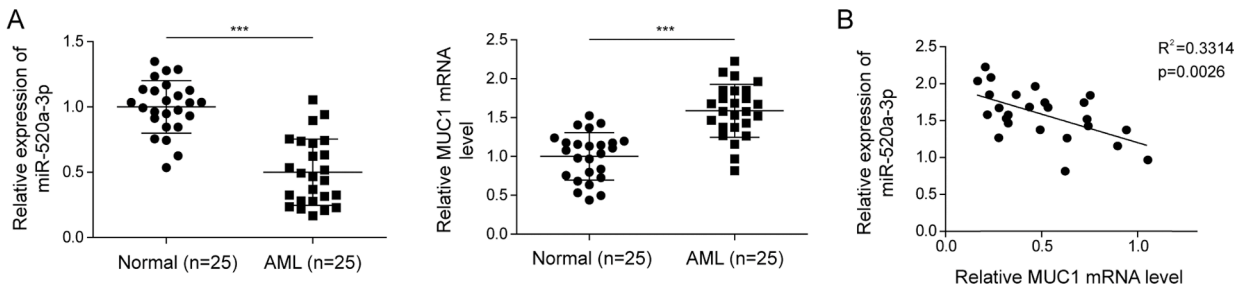

Fig. 1. The levels of miR-520a-3p and MUC1 in AML patients. A. The levels of miR-520a-3p and MUC1 were detected by qRT-PCR in AML patients ($n = 25$) and normal volunteers ($n = 25$) which served as the negative control. B. Correlation analysis between miR-520a-3p and MUC1. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

5 cell fields were selected for counting.

Flow cytometry (FCM) analysis

Flow cytometry was performed to assess cells apoptosis. To simplify, THP-1 cells were inoculated in 96-well plates for 24 h, 10 μL FITC and 5 μL PI (Beyotime, Nanjing, China) were added to THP-1 cells and incubated at 37 °C for 2 h. In this process, Annexin V probe labeled with FITC was able to bind to phosphoester serine with eversion. The proportion of early (Annexin V+/p-) and late (Annexin V+/p+) apoptotic cells was analyzed by flow cytometry (BD, San Jose, CA, USA).

Dual-luciferase reporter assay

The target gene of miR-520a-3p was predicted by using Starbase (http://starbase.sysu.edu.cn/). Furthermore, according to the previous description, the physical interaction between miR-520a-3p and MUC1 was confirmed by the dual-luciferase reporter assay [28]. In particular, the 3′-UTR fragment of MUC1 containing the miR-520a-3p binding site and its mutant (mut) fragment were subcloned to pGL3 luciferase reporter vectors (Promega, Madison, WI, USA) to construct MUC1-wt and MUC1-mut. THP-1 cells were co-transfected with the MUC1-wt, MUC1-mut, miR-520a-3p mimics and mimics NC by Lipofectamine™3000 (Takara, Dalian, China). After 48 h transfection, luciferase activity was detected.

Murine xenograft model

Fifteen BALB/c male mice (6 weeks old) were purchased from the National Laboratory Animal Center (Taipei, Taiwan, R.O.C.). In the experiment of subcutaneous tumor implantation, 1×10^6 THP-1 cells transfected with miR-520a-3p mimics or mimics NC were re-suspended in 100 μL 1 × PBS and subcutaneously injected into the left side of nude mice ($n = 5$ in each group). The tumor volume was monitored once a week. On 28 days, the mice were relieved by intraperitoneal injection of excess pentobarbital. The tumors were further removed. All animal test procedures were approved by the Huizhou Municipal Central Hospital ethics committee.

Immunohistochemistry (IHC) assay

To explore the effect of miR-520a-3p on the proliferation of AML *in vivo*, we detected the level of Ki67 by IHC. Ki67 is an indicator of cell proliferation. The higher the value of Ki67, the higher the malignant degree of tumor cells [29]. Tumor tissue sections were fixed with formaldehyde, and paraffin was embedded. The prepared tumor tissue sections were incubated in Ki67 antibodies (Abcam, Cambridge, UK, 1:500). Horseradish peroxidase anti rabbit immunoglobulin G staining and diaminobenzidine staining were carried out. The mouse was incubated with a secondary antibody and stained with envision G2/AP rabbit/mouse (permanent red) (Dako, Glostrup, Denmark) and hemoxylin reverse staining.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Trizol reagent (Carlsbad Province, U.S.A.) was used to extracted total RNA of THP-1 cells and PBMCs and RNA concentration was measured by spectrophotometry. RT-reagent kit (Carlsbad, USA) was used to synthesize the cDNA. qRT-PCR implementations used SYBR Green PCR Master Mix (Invitrogen, Carlsbad, USA), as previously described [30]. The level of mRNA was compared with β-actin, and the level of miRNA was compared with U6. The relative expression was calculated by $2^{-\Delta\Delta Ct}$ methods. Primer was designed and synthesized by Ruibo Biotechnology Co., Ltd (Guangzhou, China). The primers applied in this study were as follows:

miR-520a-3p forward: 5'- AACCTCCCAAAGTGCTTCCCTTT -3',
miR-520a-3p reverse: 5'- CAGTGCAGGGTCCGAGGT -3';
MUC1 forward: 5'- GCCACTTCTGCCAACTTGTA -3',
MUC1 reverse: 5'- TGAGCTTCCACACACTGAGA -3';
U6 forward: 5'- CTCGCTTCGGCAGCACA -3',
U6 reverse: 5'- AACGCTTCACGAATTTGCGT -3';
β-actin forward: 5'- TGGCACCACACCTTCTACAA -3',
β-actin reverse: 5'- CCAGAGGCGTACAGGGATAG -3'.

Western blot

Cell lysate (Beyotime, Nanjing, China) was used to isolate total protein of THP-1 cells. Western blotting was performed according to the method described earlier [21]. Briefly, the same amount of protein (30 micrograms) was separated by 10% SDS-PAGE gel and transferred to fluorinated polyethylene (PVDF) membrane (Invitrogen, Carlsbad, USA). 5% bovine serum albumin was sealed for 2 h and cultured at 4 °C for one night. HRP was used for 2 h at room temperature to enhance the chemical amplification reagent. All the antibodies used in this study were purchased from Abcam (Cambridge, UK), including PNCA (1 μg/ml, ab29), Bcl-2 (1:1000, ab32124), Cleaved caspase 3 (1:500, ab32042), Cleaved caspase 9 (1:500, ab2324) and β-catenin (1:1000, ab32572). Taking GAPDH (1:2000, ab8245) as the internal reference. Image J software (National Institutes of Health, Bethesda, MD, USA) was used to analyze target bands.

Statistical analysis

Statistical data are expressed as mean ± standard deviation (SD) and all data were analyzed by SPSS software (IBM, NY, USA). All the experiments had to be independently replicated at least for three times. After the analysis conforms to the normal distribution, Student t test was compared between two groups and One-way ANOVA followed the Tukey post test used for multiple group comparisons. Pearson linear correlation analysis was used to analyze the correlation between miR-520a-3p and MUC1 expression. All data of cell experiments were analyzed using Mann-Whitney U test for comparing two groups and Kruskal Wallace for non-parametric ANOVA of three or more groups. $P<0.05$ was statistically significant.

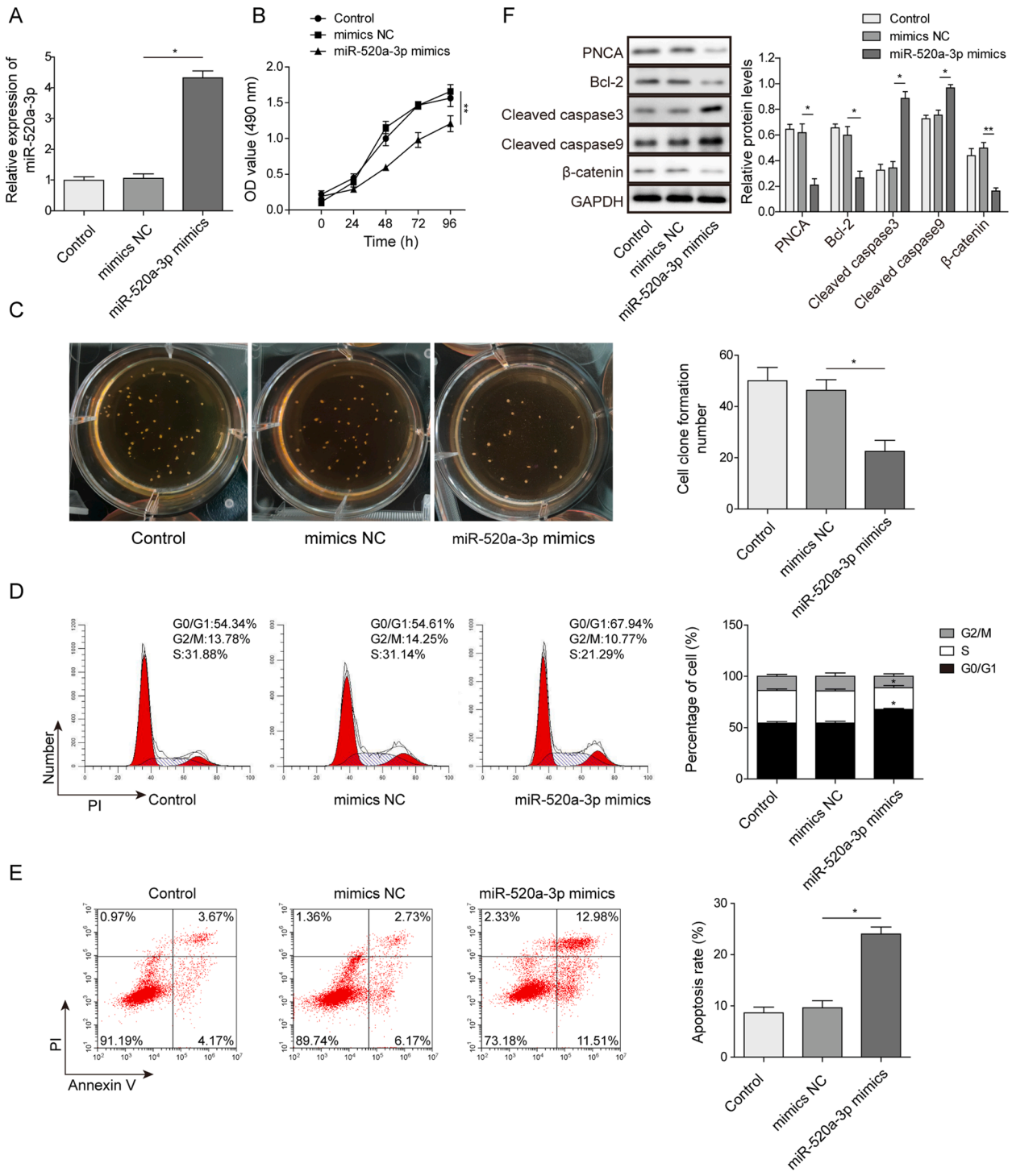

Fig. 2. miR-520a-3p overexpression inhibited THP-1 cell proliferation and accelerated cell apoptosis. miR-520a-3p mimics and mimics NC were transfected into THP-1 cells. A. miR-520a-3p expression was detected by qRT-PCR. B. the cell viability was determined by MTT assay. C. Cell proliferation was detected by colony formation assay. D-E. Cell cycle and apoptosis were analyzed by flow cytometry. F. Western blot was used to detect the protein levels of PNCA, Cleaved caspase 3, Cleaved caspase 9, Bcl-2 and β-catenin. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Results

miR-520a-3p and MUC1 expression in AML patients

For investigating the miR-320a-3p effect and its downstream mechanism on AML, the expression of miR-520a-3p was assessed in AML patients ($n = 25$) and normal volunteers ($n = 25$) using qRT-PCR. The results indicated that miR-520a-3p expression was down-regulated in AML patients, but MUC1 expression was significantly up-regulated in AML patients (Fig. 1A). Moreover, Pearson correlation analysis showed that there was a negative correlation between miR-520a-3p and MUC1 in AML patients (Fig. 1B). Furthermore, we analyzed the clinical characteristics of AML blood patients and healthy blood donors. Results showed that there was no significant difference in sex, age and peripheral blood count between AML blood patients and healthy blood donors (Table 1). Subsequently, we further analyzed the relationship between

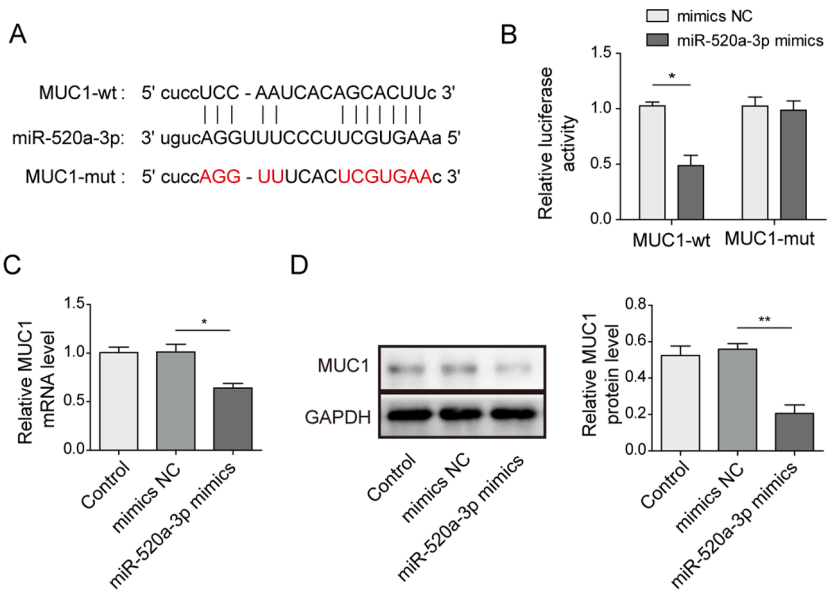

Fig. 3. miR-520a-3p negatively regulated MUC1 expression. A. Starbase software was used to predict the downstream targets of miR-520a-3p. B. The direct binding relationship between miR-520a-3p and MUC1 was confirmed by double luciferase reporter gene assay. C. MUC1 expression in THP-1 cells was detected by qRT-PCR. D. MUC1 expression in THP-1 cells was detected by Western blot. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

the miR-520a-3p expression and the clinical characteristics of patients based on the median of miR-520a-3p expression. As shown in Table 2, the high level of miR-520a-3p was associated with WBC in patients, while there was no statistic difference with other clinical features (sex, age, PB blast, myeloblasts, hemoglobin, platelet, FAB subtypes/n and mutation). Taken together, miR-520a-3p and MUC1 could participate in AML progression.

miR-520a-3p overexpression inhibited THP-1 cell proliferation and promoted apoptosis

To further explore miR-520a-3p role in AML progression, miR-520a-3p was overexpressed in THP-1 cells (Fig. 2A). The results of MTT and colony formation assays showed that miR-520a-3p overexpression significantly inhibited THP-1 cell viability and proliferation (Fig. 2B and C). Consistently, miR-520a-3p overexpression stagnated THP-1 cell cycle at the stage of G0/G1 (Fig. 2D). Apoptosis analysis revealed that miR-520a-3p overexpression facilitated THP-1 cells apoptosis (Fig. 2E). miR-520a-3p overexpression inhibited proliferative protein PNCA expression while promoted apoptosis related proteins expression including Cleaved caspase 3, Cleaved caspase 9, and suppressed anti-apoptotic protein Bcl-2 expression (Fig. 2F). Moreover, miR-520a-3p overexpression inhibited β-catenin expression (Fig. 2F), indicating miR-520a-3p overexpression might inhibit Wnt/β-catenin pathway. Collectively, miR-520a-3p overexpression inhibited THP-1 cells proliferation and accelerated apoptosis via suppressed the activation of Wnt/β-catenin pathway.

miR-520a-3p negatively regulated MUC1 expression

To determine the downstream regulatory factors of miR-520a-3p in AML, Starbase software was used to predict the downstream target of miR-520a-3p. As shown in Fig. 3A, there was a binding site between miR-520a-3p and 3'-UTR of MUC1. Subsequently, dual-luciferase reporter assay analysis revealed that the luciferase activity of MUC1-wt reported gene was inhibited by co-transfection of miR-520a-3p mimics, but the co-transfection of miR-520a-3p mimics did not affect the luciferase activity of MUC1-mut reported gene (Fig. 3B). In addition, MUC1 expression in THP-1 cells was significantly suppressed by miR-520a-3p overexpression (Fig. 3C and D). Taken together, miR-520a-3p targeted MUC1 and negatively regulated its expression in THP-1 cells.

MUC1 knockdown inhibited THP-1 cell proliferation and accelerated apoptosis

To examine the effect of MUC1 on AML progression, we knocked down MUC1 expression in THP-1 cells. Results showed that the expression of MUC1 was significantly decreased after transfection of sh-MUC1 (Fig. 4A and B). Subsequently, the vitality and proliferation of THP-1 cells were inhibited by MUC1 knockdown (Fig. 4C and D). Furthermore, MUC1 knockdown arrested the THP-1 cell cycle in the G0/G1 phase, and significantly accelerated THP-1 cell apoptosis (Fig. 4E and F). Finally, MUC1 knockdown increased Cleaved caspase 3, Cleaved caspase 9 proteins expression, and inhibited PNCA, Bcl-2 and β-catenin proteins expression (Fig. 4G). Taken together, MUC1 knockdown repressed THP-1 cell proliferation and accelerated cell apoptosis via inhibiting the activation of Wnt/β-catenin pathway.

MUC1 knockdown weakened the promoting effect of miR-520a-3p knockdown in THP-1 cells

To further explore whether miR-520a-3p has an effect on AML progression by regulating MUC1 expression, miR-520a-3p was silenced alone in THP-1 cells and a function rescue experiment was set up, miR-520a-3p inhibitor + sh-NC group and miR-520a-3p inhibitor + sh-MUC1 group. Results showed that miR-520a-3p knockdown inhibited miR-520a-3p expression, but accelerated MUC1 expression in THP-1 cells. Whereas the promotion of MUC1 expression induced by miR-520a-3p knockdown was alleviated by MUC1 knockdown (Fig. 5A and B). Subsequently, THP-1 cell viability and proliferation was accelerated by miR-520a-3p knockdown, while MUC1 knockdown antagonized the promoted effect (Fig. 5C and D). miR-520a-3p knockdown reduced the number of G0/G1 cells, phase S cells increase, which was reversed by MUC1 knockdown (Fig. 5E). Consistently, THP-1 cell apoptosis was inhibited by miR-520a-3p knockdown, while MUC1 knockdown alleviated the inhibited effect (Fig. 5F). miR-520a-3p knockdown promoted proliferative-related protein PNCA expression, while it inhibited apoptosis-related proteins expression, and promoted anti-apoptosis-related protein expression. While the effect was neutralized by MUC1 knockdown. Particularly, miR-520a-3p knockdown accelerated

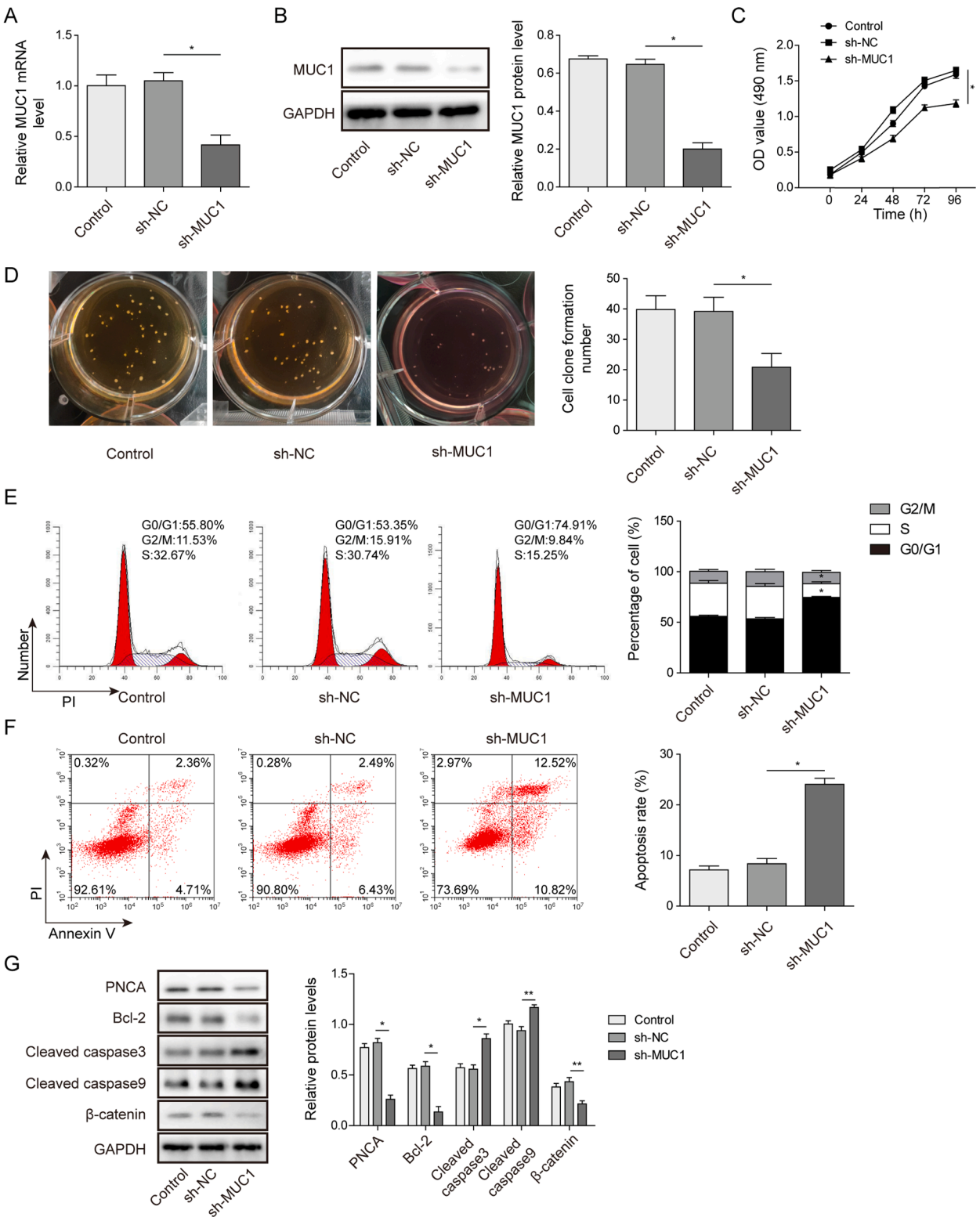

Fig. 4. MUC1 knockdown inhibited THP-1 cell proliferation and accelerated apoptosis. sh-NC and sh-MUC1 were transfected into THP-1 cells. A-B. MUC1 expression was detected by qRT-PCR and Western blot. C. the cell viability was determined by MTT assay. D. Cell proliferation was detected by colony formation assay. E-F. Cell cycle and apoptosis were analyzed by flow cytometry. G. Western blot was used to detect the protein levels of PNCA, Cleaved caspase 3, Cleaved caspase 9, Bcl-2 and β-catenin. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

β-catenin expression, while it was antagonized by MUC1 knockdown (Fig. 5G). Taken together, MUC1 knockdown weakened the promoting effect of miR-520a-3p knockdown in THP-1 cells.

miR-520a-3p overexpression inhibited tumor growth in nude mice

To further investigate the effect of miR-520a-3p on AML tumor growth, we injected THP-1 cells transfected with mimics NC and miR-

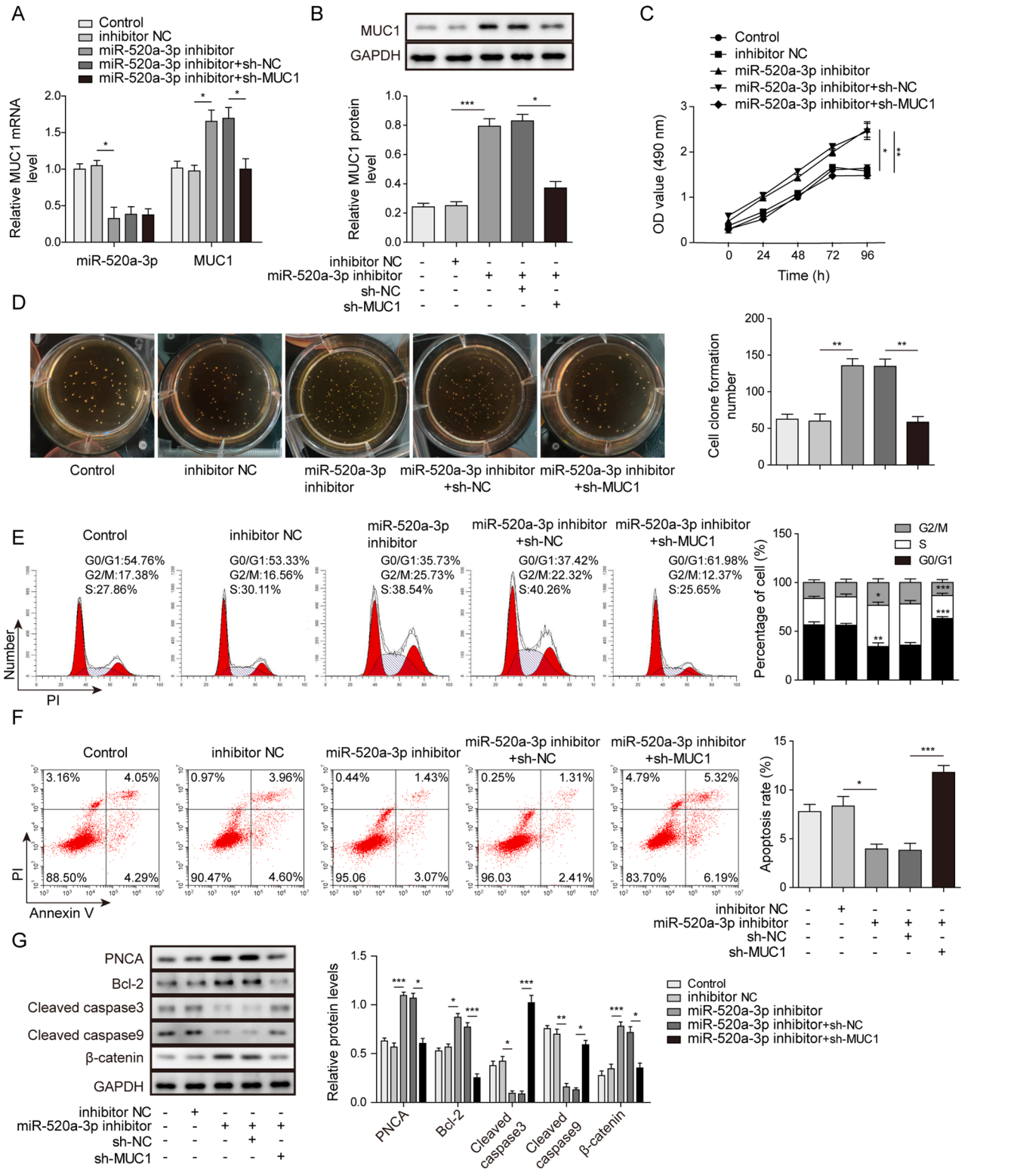

Fig. 5. MUC1 knockdown weakened the promoting effect of miR-520a-3p knockdown in THP-1 cells. Inhibitor NC, miR-520a-3p inhibitor, miR-520a-3p inhibitor + sh-NC and miR-520a-3p inhibitor + sh-MUC1 were transfected into THP-1 cells. A-B. The levels of miR-520a-3p and MUC1 were detected by qRT-PCR and Western blot. C. The cell viability was determined by MTT assay. D. Cell proliferation was detected by colony formation assay. E-F. Cell cycle and apoptosis were analyzed by flow cytometry. G. Western blot was used to detect the protein levels of PNCA, Cleaved caspase 3, Cleaved caspase 9, Bcl-2 and β-catenin. *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

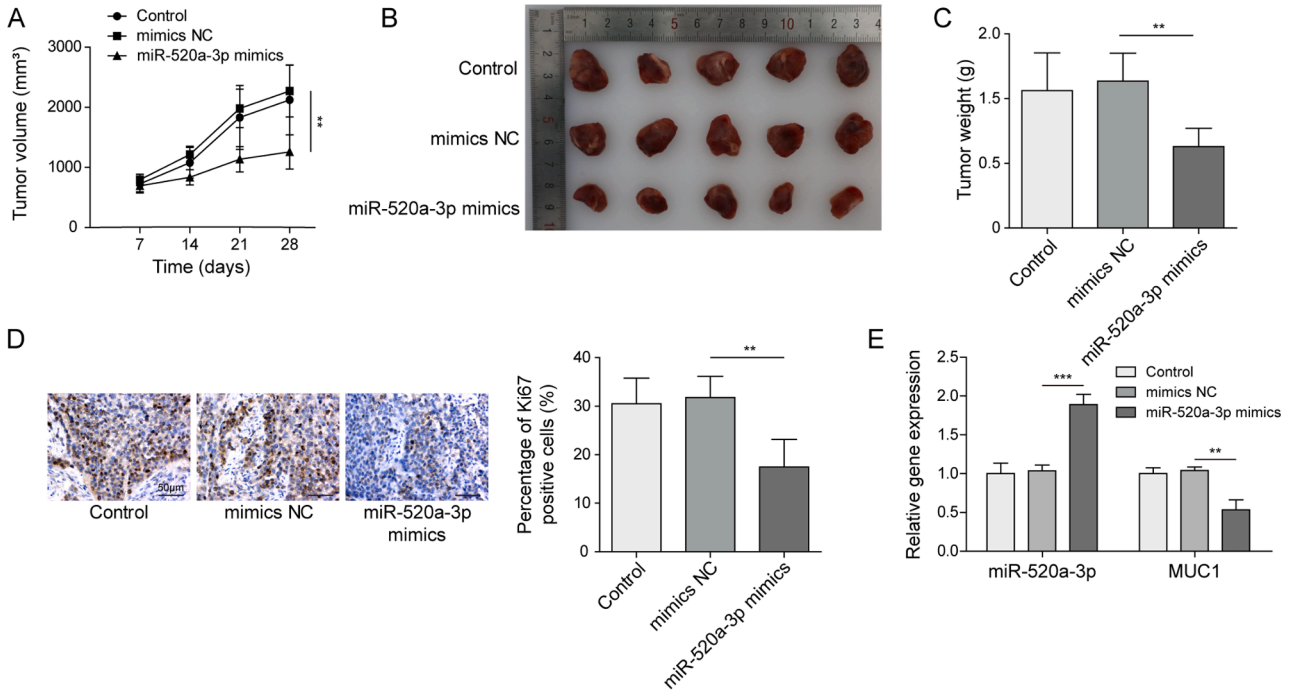

Fig. 6. miR-520a-3p overexpression inhibited tumor growth in nude mice. We established a subcutaneous tumor model in nude mice by injecting THP-1 cells transfected with miR-520a-3p mimics and mimics NC into nude mice. A-B. Statistical analysis of tumor volume ($n = 5$). C. Statistical analysis of tumor weight ($n = 5$). D. the expression of Ki67 in tumor tissue was detected by immunohistochemistry ($n = 5$). E. qRT-PCR was used to detect the expressions of miR-520a-3p and MUC1 ($n = 5$). *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

520a-3p mimics subcutaneously into nude mice to establish a subcutaneous tumor model. Statistical analysis of tumors volume and weight indicated that the volume and weight of AML tumor in miR-520a-3p overexpressed nude mice were lower than that in mimics NC and normal nude mice (Fig. 6A–C). IHC analysis indicated that Ki67 positive cells rate was significantly decreased in tumor tissues of miR-520a-3p overexpression nude mice (Fig. 6D). Furthermore, miR-520a-3p expression was upregulated and MUC1 expression was downregulated after miR-520a-3p overexpression (Fig. 6E). Taken together, miR-520a-3p overexpression inhibited tumor growth in nude mice.

Discussion

The high morbidity and mortality of acute myeloid leukemia necessitates the search for more effective therapeutic strategies. Understanding the pathogenesis of AML will help discover new therapeutic strategies [31,32]. In this study, we found that miR-520a-3p repressed cell proliferation, promoted apoptosis, and suppressed AML tumor progression, at least partly via targeting MUC1/Wnt/β-catenin axis in AML.

Emerging evidence shows miRNAs act as an effective biomarker or therapeutic target for a variety of diseases [8], and various miRNAs are shown to play a vital regulatory role in AML progression [33]. MiRNAs exert antileukemic activity, while others exert leukemic promoting activity. Chen et al. 's study showed that miR-9 had effect on growth and survival of tumor cells by targeting Hes1 [34]. Yuan et al. research indicated that miRNA-650 exerted its anti-leukemia activity by targeting Gfi1 to inhibit the proliferation of AML cells [35]. Therefore, miRNAs as the biomarker of AML is a double-edged sword. Furthermore, miR-520a-3p plays an inhibitory role in colorectal cancer, thyroid cancer, osteosarcoma and other cancers progression [36–38]. Interestingly, it was reported that miR-520a was up-regulated in AML patients [11]. MiR-520a-5p displayed a therapeutic effect upon chronic myelogenous leukemia cells by targeting STAT3 and enhanced the anticarcinogenic role of capsaicin [12]. According to miRBase analysis, there are two kinds of miR-520a: miR-520a-3p and miR-520a-5p. Both 3p and 5p of miRNA are processed and modified by the same precursor miRNA, so it is difficult to analyze whether the expression of 3p and 5p is consistent, and the modification mechanism of 3p and 5p may be different. At present, the study of miR-520a-3p in AML has not been reported yet. In this study, it was shown that miR-520a-3p expression was downregulated in AML patients, and miR-520a-3p overexpression inhibited THP-1 cell proliferation, promoted apoptosis, and inhibited tumor growth of nude mouse. It is suggested that miR-520a-3p plays a negative role in AML progression and can be used as an effective target for AML therapy. Furthermore, the number of samples in the study is small, the source of samples is inconsistent, the diet of patients, geographical differences and other factors may lead to different results.

MUC1 is a key oncoprotein, which is closely related to leukocyte self-renewal and anti-apoptosis [39]. MUC1 is encoded by a gene at position 21 of the long arm of chromosome 1, and in tumor cells, this gene is altered, resulting in abnormal expression of MUC1 [40]. Our results showed that MCC1 was highly expressed in AML patients, consistent with previous studies. Previous studies have shown that MUC1 contains a SAGGSSLS base sequence that binds to β-catenin, and that the combination of MUC1 and β-catenin promotes the nuclear metastasis of tumors [41]. Moreover, it has been reported that there was a close relationship between Wnt/β-catenin pathway and the progression of AML. It was reported that Wnt/β-catenin pathway was necessary for AML development [42]. The results showed that the knockdown of MUC1 gene inhibited β-catenin proteins expression in AML, which in turn inhibited AML cells proliferation and promoted apoptosis. This may be achieved by affecting downstream cell cycle proteins and inhibiting Wnt/β-catenin pathway. Previous studies have also suggested that MUC1 may be directly related to the transcription factor β-catenin, thereby regulating metastasis in multiple cancers [39]. Furthermore, miRNAs are proved involved in the regulation of disease processes by targeting MUC1. Deng et al. 's research indicated miR-206 inhibited gastric cancer cell metastasis by targeting MUC1 [43]. In this study, we found that miR-520a-3p was targeted at MUC1 and negatively regulated

MUC1 expression. MUC1 gene knockout weakened miR-520a-3p gene knockout effect in THP-1 cells. miR-520a-3p negatively regulated MUC1 expression in THP-1 cells, inhibiting the activation of the Wnt/β-catenin pathway. Furthermore, whether Wnt/β-catenin affected the MUC1 function in THP-1 cells remained to be further explored in the future.

Summary, our findings illustrated that miR-520a-3p overexpression inhibited cell proliferation, promoted apoptosis and inhibited tumor growth, at least partly by targeting MUC1/Wnt/β-catenin axis in AML. Our study may provide a novel insight into developing a therapeutic strategy for AML. Overall, the complexities and mysteries of miRNAs in AML still exist. This study may help us understand how miRNAs affect this deadly leukemia. Only a better understanding of these mechanisms can better utilize miRNA therapeutics to improve the treatment of clinical diseases.

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Ethics approval

All patients were informed before the inclusion, and the agreement was obtained. All experimental programs were approved by the Huizhou Municipal Central Hospital ethics committee, and the experimental procedure was carried out according to the principles of the Helsinki declaration.

All animal test procedures were approved by the Huizhou Municipal Central Hospital ethics committee.

CRediT authorship contribution statement

Xiao-Yu Chen: Conceptualization, Visualization, Formal analysis, Supervision, Writing – original draft, Writing – review & editing. **Xiao-Hua Qin:** Funding acquisition, Writing – review & editing. **Xiao-Ling Xie:** Writing – original draft, Writing – review & editing. **Cai-Xiang Liao:** Methodology, Writing – review & editing. **Dong-Ting Liu:** Methodology, Writing – review & editing. **Guo-Wei Li:** Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

There are no conflicts of interest to declare.

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