

# Bone Marrow Mesenchymal Stem Cells-Derived Exosomal miR-425-5p Inhibits Acute Myeloid Leukemia Cell Proliferation, Apoptosis, Invasion and Migration by Targeting WTAP

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**Introduction:** Acute myeloid leukemia (AML) is a predominant blood malignancy with high mortality and severe morbidity. AML is affected by microRNAs (miRNAs) loaded in exosomes derived from bone marrow mesenchymal stem cells (BM-MSCs). MiR-425-5p has been reported to participate in different cancer models. However, the function of BM-MSCs-derived exosomal miR-425-5p in AML is unclear.

**Methods:** The expression of miR-425-5p was measured by qRT-PCR in clinical AML samples. The immunophenotype of BM-MSCs was analyzed using antibodies against CD44, CD90, and CD105. The exosome was isolated from BM-MSCs. The effect of BM-MSCs-derived exosomal miR-425-5p on AML was analyzed by CCK-8 assay, Edu assay, transwell assay, flow cytometry, AML cells. qRT-PCR, luciferase reporter gene assay and Western blot analysis were also conducted in AML cells.

**Results:** The expression levels of miR-425-5p were decreased in CD34 + CD38-AML cells from primary AML patients compared to that from the bone marrow of healthy cases, and were increased in exosomes from AML patients compared that from healthy cases. Similarly, miR-425-5p was also down-regulated in AML cell lines compared with BM-MSCs. MiR-425-5p was able to express in exosomes from BM-MSCs. CCK-8, Edu, transwell assay and flow cytometry analysis revealed that BM-MSCs-derived exosomal miR-425-5p significantly inhibited cell viability, Edu positive cells, invasion and migration, and induced apoptosis of AML cells. Meanwhile, the expression levels of cleaved PARP and cleaved caspase3 were increased by BM-MSCs-derived exosomal miR-425-5p in cells. MiR-425-5p inhibited the expression of Wilms tumor 1-associated protein (WTAP). Moreover, overexpression of WTAP could reverse the miR-425-5p-induced inhibition effect on AML cell proliferation, apoptosis, migration and invasion.

**Conclusion:** BM-MSCs-derived exosomal miR-425-5p inhibits proliferation, invasion and migration of AML cells and induced apoptosis of AML cells by targeting WTAP. Therapeutically, BM-MSCs-derived exosomal miR-425-5p may serve as a potential target for AML therapy.

**Keywords:** AML, BM-MSCs, exosome, miR-425-5p, WTAP

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## Introduction

Acute myeloid leukemia (AML) is a prevalent destructive cancer characterized by immature leukemic blast cell production and destroyed white blood cell maturation, which can develop into other organs, including gums, skin, and the central nervous

system.<sup>1</sup> AML patients regularly undergo easy bleeding, severe anemia, and infection because of reduced normal blood cells.<sup>2</sup> Hazard factors for AML development contain exposure to carcinogenic agents, cigarette smoking, genetic mutations, chemotherapy, and radiation drugs, such as doxorubicin and cyclophosphamide.<sup>3,4</sup> Even with great improvement in stem cell transplantation and chemotherapy, the survival incidence of AML cases is still unsatisfied due to frequent recurrence and chemoresistance.<sup>5</sup> In recent years, anthracycline and cytarabine combined with intensive chemotherapy (IC) induction regimen, and marrow transplant, have become the principal therapy choices for AML patients, but the efficacy was generally restricted by high recurrence rates and poor prognosis.<sup>6,7</sup> Therefore, it is of great importance to illustrate the mechanisms underlying AML progression to address these challenges.

Bone marrow mesenchymal stem cells (BM-MSCs) are a group of cells with differentiating potentials, which are able to differentiate into myoblasts, adipocytes, chondrocytes and osteoblasts.<sup>8</sup> BM-MSCs play essential roles in modulating the hematopoietic capacity of bone marrow at physiological conditions and have been recognized as the foundation of the hematopoietic stem cell context.<sup>9</sup> AML is featured by hematopoietic insufficiency, and BM-MSCs from AML patients present notable proliferation deficiency and reduced osteogenic differentiation ability.<sup>10</sup> Studies have demonstrated the possible function of BM-MSCs transplantation for blood malignancy, including AML.<sup>11,12</sup> In addition to the role of BM-MSCs, the bioactive substrates and paracrine agents delivered by BM-MSCs also have therapeutic and supportive impacts.<sup>13,14</sup>

Exosomes are nano-sized particles that can transport vesicles of biological loads, such as mRNAs, long non-coding RNAs (lncRNAs), proteins, and microRNAs (miRNAs), leading to the phenotypic effect in receiving cells.<sup>15</sup> Exosomes are identified as crucial regulators of BM-MSCs activity.<sup>10</sup> MiRNAs are small ncRNAs with 20–25 nucleotides in length, which regulate different targets that are involved in diverse physiological and pathological processes, such as cell apoptosis, differentiation, proliferation, invasion, metastasis, and tumorigenesis.<sup>16</sup> Moreover, exosomes can shuttle certain miRNAs and exert critical functions in long-distance cell communication and cancer development.<sup>17</sup> It has been well recognized that exosomes can promote cancer progression by regulating the expression of miRNAs in tumors and surrounding cells.<sup>18,19</sup> Meanwhile, the BM-MSCs-derived

exosomal miRNAs are involved in regulating AML pathogenesis.<sup>20</sup> Furthermore, miR-425-5p is a well-studied miRNA in multiple cancer models, including prostate cancer, pancreatic cancer, and gastric cancer.<sup>21–23</sup> And it has been reported that miR-425-5p is down-regulated in exosomes from clinical AML samples.<sup>20</sup> However, the role of BM-MSCs-derived exosomal miR-425-5p in the development of AML remains elusive.

Wilms tumor 1 (WT1)-associated protein (WTAP) is a nuclear protein known as its particular interaction with WT1.<sup>24</sup> WTAP also has a tight connection with malignant tumors. For instance, WTAP is up-regulated in cholangiocarcinoma, particularly in patients with vascular invasion or lymph node metastasis.<sup>25</sup> It was also reported that the expression levels of WTAP were closely correlated with tumor-node-metastasis stages.<sup>25</sup> Besides, WTAP plays a crucial role in the invasion and migration of cholangiocarcinoma cells.<sup>25</sup> Distinctly, WTAP is up-regulated in glioblastoma and is correlated with the prognosis of glioblastoma.<sup>26</sup> The oncogenic role of WTAP has also been observed in AML.<sup>27</sup> Overexpression of WTAP inhibits differentiation and enhances proliferation of AML cells.<sup>27</sup> However, the correlation of WTAP with BM-MSCs-derived exosomal miR-425-5p in the regulation of AML remains unclear.

In this study, we aimed to investigate the role and the underlying mechanism of BM-MSCs-derived exosomal miR-425-5p in AML. Our data showed that miR-425-5p was downregulated in the exosomes from AML patients and AML cell lines. BM-MSCs-derived exosomal miR-425-5p significantly inhibited cell proliferation, invasion and migration, and induced apoptosis of AML cells. MiR-425-5p inhibited the expression of WTAP by targeting its 3'-untranslated regions (3'-UTR). Overexpression of WTAP reversed the effect of miR-425-5p on AML. Thus, we discovered a novel function of AML in inhibiting AML development by regulating WTAP.

## Materials and Methods

### AML Clinical Samples

A total of 20 bone marrow (BM) samples from primary AML patients and healthy volunteers were obtained from the Third Affiliated Hospital of Sun Yat-Sen University. The samples were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  before use. All patients and healthy volunteers signed the written consent form. Informed consent from the patients and healthy volunteers. Approval from

**Table 1** The Information of Patients

Variables	Total (n = 20)
Sex	
Male	12
Female	8
Age (year)	57±7.6
Lab data <sup>a</sup>	
WBC (/μL)	27,120 (930–428, 120)
Hb (g/dL)	7.3 (4.5–13.0)
Platelet (×1000/μL)	35 (5–396)
Blast (/μL)	14,259 (0–398, 392)
LDH (U/L)	1129 (349–8116)
FAB	
M0	0
M1	5
M2	4
M4	3
M5	5
M6	3

**Note:** <sup>a</sup>median (range).

the Ethics Committee of The Third Affiliated Hospital of Sun Yat-Sen University Compliance with the Declaration of Helsinki. The information of patients was shown in Table 1.

## Patient Specimens, CD34 + CD38<sup>+</sup> cells and AML Cell Line Separation

Human cord blood (hCB) specimens were collected after elective caesarean delivery following full-term pregnancy at Beijing Union Hospital. All experimental procedures were approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University. Each participant signed the informed consent. CD38 and CD34 MicroBead kits (Miltenyi, Germany) were used to separate CD34 + CD38<sup>+</sup> cells from hCB and bone marrow.<sup>28</sup>

## Identification of BM-MSCs

The human BM-MSCs were derived from normal subjects and purchased from BeNa Culture Collection (Beijing, China). BM-MSCs were maintained in high glucose Dulbecco's modified Eagle's medium (DMEM, Gibco, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco), 4 mM L-glutamine and sodium pyruvate at 37 °C with 5% CO<sub>2</sub>. For identification of BM-MSCs, the morphology of BM-MSCs at passage 3 was observed under a microscope. The immunophenotype of BM-MSCs was analyzed using antibodies against CD44, CD90, and

CD105 (ebioscience, Thermo Fisher Scientific, CA, USA) using a FACScalibur flow cytometer (Becton Dickinson, Bedford, MA, USA) as previously described.<sup>14</sup>

## Exosome Isolation and Analysis

The culture medium and plasma were centrifuged at 3000x g for 15 min to drop cells and cellular debris. Next, Exoquick exosome precipitation solution (System Biosciences, USA) was used to isolate exosomes. The characteristics of exosomes were analyzed by transmission electron microscopy (TEM) as previously described.<sup>29</sup>

## Cell Culture and Treatment

The KG-1A, NB4, MV411, and THP-1 cell lines were obtained from American Type Tissue Culture Collection. The human BM-MSCs were purchased from BeNa Culture Collection (Beijing, China). The KG-1A, NB4, MV411, THP-1 cells and BM-MSCs were cultured in DMEM medium (Gibco, USA) containing 100 units/mL penicillin (Gibco, USA), 0.1 mg/mL streptomycin (Gibco, USA), and 10% fetal bovine serum (Gibco, USA) at 37 °C with 5% CO<sub>2</sub>. The control mimic, miR-425-5p mimic, pcDNA3.1 and pcDNA3.1-WTAP overexpression vectors were obtained from GenePharma (China). Transfection of cells was performed using Liposome 3000 (Invitrogen, USA) according to the manufacturer's instructions. To evaluate the effect of BM-MSCs-derived exosomal miR-425-5p on AML, the exosomes were extracted from BM-MSCs cells treated with control mimic or miR-425-5p mimic, and KG-1A and THP-1 cells were further treated with exosomes for further analysis.

## Colony Formation Assay

Cell proliferation was evaluated by colony formation assay. About 1×10<sup>3</sup> cells were plated in 6 wells and incubated in DMEM at 37 °C. After 2 weeks, cells were cleaned with PBS Buffer, precipitated in methanol for 30 min, and dyed with 1% crystal violet dye. The number of colonies was then calculated.

## Edu Assay

Cell proliferation was evaluated by EdU assay using EdU detecting kit (RiboBio, China). Briefly, KG-1A and THP-1 cells were cultured with EdU for 2 h, followed by fixing using 4% paraformaldehyde at room temperature for 30 min. Then, cells were permeabilized with 0.4% Triton X-100 for 10 min and stained with staining cocktail of EdU at room temperature for 30 min in the dark. Next, the

nuclear of the cells was stained with Hoechst at room temperature for 30 min. Images were analyzed using a fluorescence microscope.<sup>30</sup>

## Transwell Assay

Migration and invasion of AML cells were measured using Transwell assay with a Transwell plate (Corning, USA) following the manufacturer's instructions. Briefly, the upper chamber was plated with about  $1 \times 10^5$  cells, then solidified with 4% paraformaldehyde and dyed with crystal violet. The invaded and migrated cells were recorded and calculated.<sup>30</sup>

## Cell Apoptosis Assay

About  $2 \times 10^5$  cells were plated on 6-well dishes. Cell apoptosis was measured using the Annexin V-FITC Apoptosis Detection Kit (CST, USA) following the manufacturer's instructions. Briefly, about  $2 \times 10^6$  cells were collected and washed by binding buffer and dyed at 25 °C, followed by flow cytometry analysis.<sup>31</sup>

## Quantitative Reverse Transcription-PCR (qRT-PCR)

Total RNAs were extracted from the tissues and cells using TRIZOL (Invitrogen, USA). The first-strand cDNA was synthesized using Stand cDNA Synthesis Kit (Thermo, USA). The qRT-PCR was carried out using SYBR Real-time PCR I kit (Takara, Japan). The standard control for mRNA and miRNA was GAPDH and U6, respectively. Quantitative determination of the RNA levels was conducted by SYBR GreenPremix Ex Taq™ II Kit (TaKaRa, Japan). The experiments were independently repeated for three times. The primer sequences were as follows:

miR-425-5p F: 5'-GTGCCGAAAGACACGATCACTCCCG-3'

miR-21-5p R: 5'-CCAGTGCAGGGTCCGAGGT-3'

WTAP F: 5'-TCCGACTAGCAACCAAGGAA-3'

WTAP R: 5'-ATCTCAGTTGGGCAACGCTC-3'

GAPDH F: 5'-AAGAAGGTGGTGAAGCAGGC-3'

GAPDH R: 5'-TCCACCACCCAGTTGCTGTA-3'

U6 F: 5'-GCTTCGGCAGCACATATACTAA-3'

U6 R: 5'-AACGCTTCACGAATTTGCGT-3'

## Luciferase Reporter Gene Assay

Luciferase reporter gene assay was conducted using a Dual-luciferase Reporter Assay System (Promega,

USA). Briefly, KG-1A and THP-1 cells were treated with the control mimic or miR-425-5p mimic, the vector containing WTAP and WTAP mutant fragment were transfected in cells using Lipofectamine 3000 (Invitrogen, USA), followed by the analysis of luciferase activities. Renilla was used as a normalized control.<sup>30</sup>

## Western Blot Analysis

Total proteins were extracted from cells or mice tissues with RIPA buffer (CST, USA). Protein concentrations were measured using the BCA Protein Quantification Kit (Abbkine, USA). The same amount of protein samples were divided by SDS-PAGE (12% polyacrylamide gels), followed by transferring to PVDF membranes (Millipore, USA). The membranes were blocked with 5% milk and incubated with the primary antibodies for WTAP (1:1000) (Abcam, USA), Casp101 (1:1000) (Abcam, USA), CD63 (1:1000) (Abcam, USA), Grp78 (1:1000) (Abcam, USA), caspase3 (1:1000) (Abcam, USA), PARP (1:1000) (Abcam, USA), and  $\beta$ -actin (1:1000) (Abcam, USA) at 4 °C overnight, in which  $\beta$ -actin served as the control. Then, the corresponding second antibodies (1:1000) (Abcam, USA) were used for hatching the membranes at room temperature for 1 h, followed by visualization using an Odyssey CLx Infrared Imaging System.

## Statistical Analysis

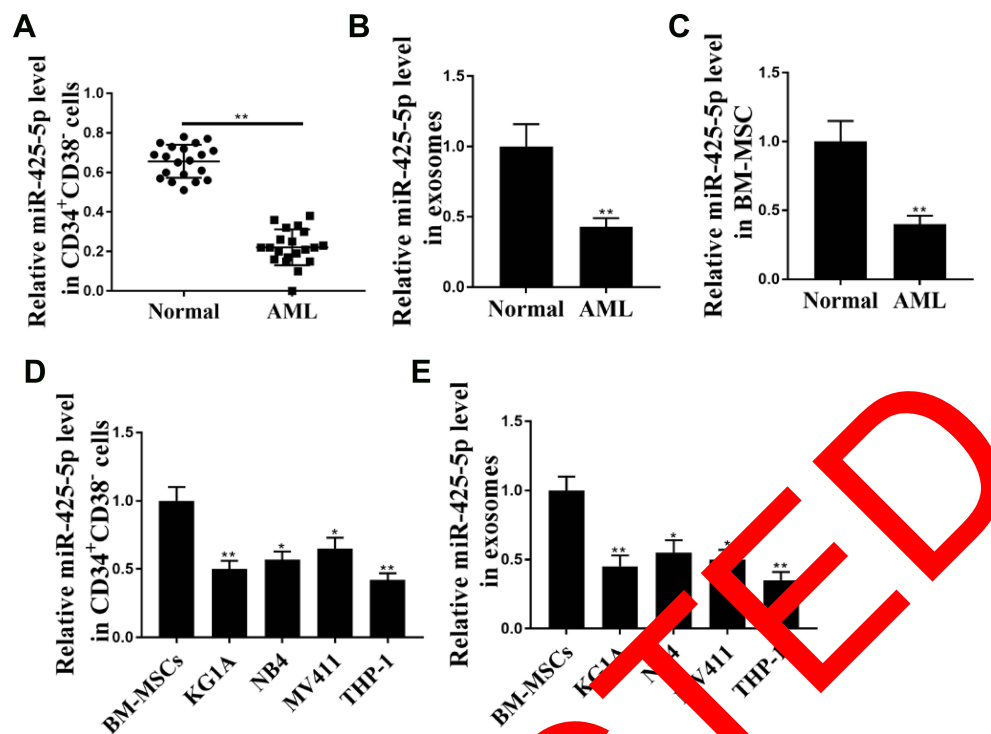
Data were presented as mean  $\pm$  standard deviation (SD). All statistical analyses were conducted using Prism software v.7 (GraphPad, La Jolla, CA, USA). The unpaired Student's *t*-test was used to compare two groups, and the one-way ANOVA was used to compare multiple groups.  $P < 0.05$  was considered as statistically significant.

## Results

### The Expression Levels of miR-425-5p are Decreased in the Exosome of AML Patients

To assess the potential correlation of miR-425-5p with AML, the expression of miR-425-5p in AML patients was detected. It showed that the expression levels of miR-425-5p were significantly decreased in the CD34<sup>+</sup>CD38<sup>+</sup> AML cells from primary AML patients ( $n = 20$ ) compared to that from bone marrow of healthy cases ( $n = 20$ ) ( $P < 0.01$ , Figure 1A). Moreover, the exosomes were extracted, and the expression levels of miR-425-5p were reduced in primary AML patients ( $n = 20$ ) compared





**Figure 1** The expression levels of miR-425-5p are decreased in the exosome of AML patients. **(A)** The expression levels of miR-425-5p were measured by qPCR assays in the CD34<sup>+</sup>CD38<sup>−</sup> AML cells from primary AML patients (n = 20) and bone marrow of healthy cases (n = 20). **(B)** The expression levels of miR-425-5p were tested by qPCR assays in the exosomes from primary AML patients (n = 20) and normal cases. **(C)** The expression levels of miR-425-5p were evaluated by qPCR assays in the BM-MSCs from primary AML patients (n = 20) and normal cases. **(D)** The expression levels of miR-425-5p were assessed by qPCR assays in the KG-1a, NB4, MV411, THP-1 cells, and BM-MSCs. **(E)** The expression levels of miR-425-5p were tested by qPCR assays in the exosomes from KG-1a, NB4, MV411, THP-1 cells, and BM-MSCs. Data are presented as mean ± SD. Statistic significant differences were indicated: \**P* < 0.05, \*\**P* < 0.01.

with that in the normal cases (n = 20) (*P* < 0.01, **Figure 1B**), indicating that miR-425-5p may be correlated with the clinical development of AML. Importantly, the expression levels of miR-425-5p were also decreased in BM-MSCs from AML patients compared with that from the controls (*P* < 0.01, **Figure 1C**). Meanwhile, miR-425-5p was also down-regulated in the AML cell lines, including KG-1A, NB4, MV411, and THP-1 cells, compared with BM-MSCs (*P* < 0.01, **Figure 1D**). Similarly, the expression levels of miR-425-5p were decreased in the exosomes from KG-1A, NB4, MV411, and THP-1 cells compared to that from BM-MSCs (*P* < 0.01, **Figure 1E**). Taken together, these data suggest that the expression of miR-425-5p is inhibited in the exosomes of AML patients and cells lines.

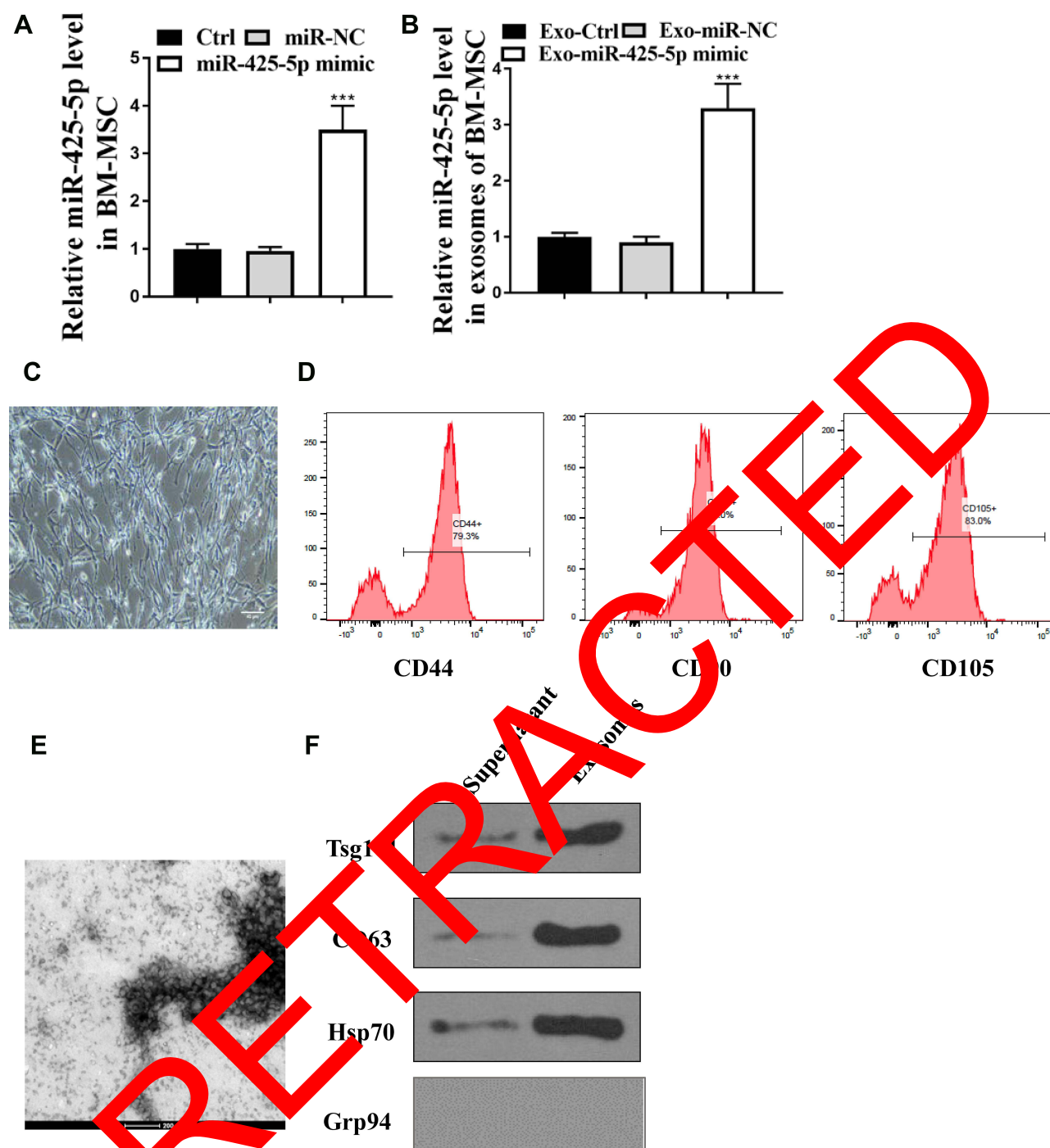
### miR-425-5p is Expressed in the Exosomes from BM-MSCs

Then, whether miR-425-5p was loaded in the exosome of BM-MSCs was explored. BM-MSCs were derived from normal subjects. For identification of BM-MSCs, the

morphology of BM-MSCs at passage 3 was observed under a microscope (**Figure 2C**). The immunophenotype of BM-MSCs was analysed using antibodies against CD44, CD90, and CD105 (**Figure 2D**). BM-MSCs were treated with miR-425-5p mimic and the treatment efficiency was validated (*P* < 0.001, **Figure 2A**). The expression of miR-425-5p was inhibited in the exosomes from miR-425-5p mimic-treated BM-MSCs (*P* < 0.001, **Figure 2B**). In addition, the characteristic of the exosome was identified by transmission electron microscopy (TEM) (**Figure 2E**). The expression of the exosome markers, such as CD63 and Tsg101, was enriched in the exosomes (**Figure 2F**). Together, these results indicate that miR-425-5p is expressed in the exosomes from BM-MSCs.

### BM-MSCs-Derived Exosomal miR-425-5p Inhibits Viability and Induces Apoptosis of AML Cells

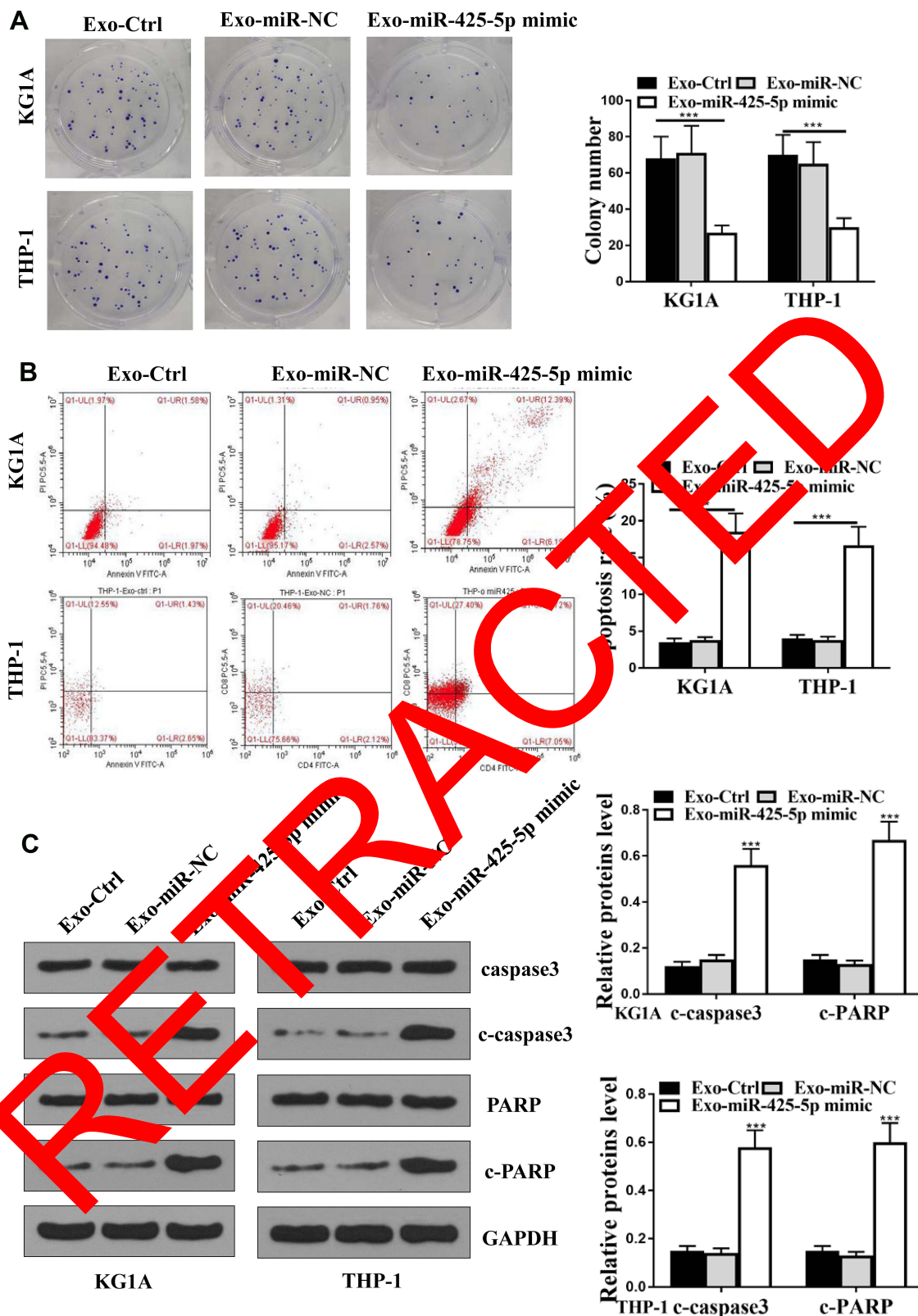
Since miR-425-5p was incorporated in the exosomes from BM-MSCs, we assessed the effect of BM-MSCs-derived exosomal miR-425-5p on AML. The exosomes were



**Figure 2** miR-425-5p is expressed in the exosomes from BM-MSCs. **(A and B)** BM-MSCs were treated with control mimic or miR-425-5p mimic. **(A)** The expression of miR-425-5p was measured by qPCR in the BM-MSCs. **(B)** The expression of miR-425-5p was measured by qPCR in the exosomes from BM-MSCs. **(C)** The morphology of BM-MSCs at passage 3 was observed under a microscope (scale bar: 200  $\mu$ m). **(D)** The immunophenotype of BM-MSCs was analyzed using antibodies against CD44, CD90, and CD105 by flow cytometry. **(E)** The characteristics of exosomes were analyzed by the transmission electron microscopy (TEM) in the BM-MSCs. **(F)** The expression of CD63, CD81, Tsg101, Grp94, and  $\beta$ -actin was assessed by Western blot analysis in the exosomes from BM-MSCs. Data are presented as mean  $\pm$  SD. Statistic significant differences were indicated: \*\*\* $P < 0.001$ .

extracted from BM-MSCs treated with control mimic or miR-425-5p mimic, and KG-1A and THP-1 cells were further treated with the exosomes. Colony formation assay results showed that miR-425-5p mimic significantly inhibited cell proliferation of KG-1A and THP-1 cells ( $P <$

0.05, **Figure 3A**). Meanwhile, cell apoptosis of KG-1A and THP-1 cells was enhanced by miR-425-5p mimic ( $P < 0.001$ , **Figure 3B**). Similarly, the expression levels of cleaved PARP and cleaved caspase3 were significantly increased by miR-425-5p mimic in KG-1A and THP-1



**Figure 3** BM-MSCs-derived exosomal miR-425-5p inhibits viability and induces apoptosis of AML cells. (A–C) The exosomes were extracted from the BM-MSCs cells treated with control mimic or miR-425-5p mimic, and the KG-1A and THP-1 cells were further treated with the exosomes. (A) The cell proliferation was measured by colony formation assay in the cells. (B) The cell apoptosis was measure by flow cytometry analysis in the cells. (C) The expression of PARP, cleaved PARP (c-PARP), caspase3, cleaved caspase3 (c-caspase3), and  $\beta$ -actin was assessed by Western blot analysis in the cells. The results of Western blot analysis were quantified by ImageJ software. Data are presented as mean  $\pm$  SD. Statistic significant differences were indicated: \*\*\* $P < 0.001$ .

cells ( $P < 0.001$ , Figure 3C). These results indicate that BM-MSCs-derived exosomal miR-425-5p inhibits viability and induces apoptosis of AML cells.

## BM-MSCs-Derived Exosomal miR-425-5p Inhibits Proliferation, Invasion, and Migration of AML Cells

Next, we further assessed the role of BM-MSCs-derived exosomal miR-425-5p in modulating proliferation, invasion, and migration of AML cells. The exosomes were extracted from BM-MSCs treated with control mimic or miR-425-5p mimic, and KG-1A and THP-1 cells were further treated with the exosomes. Edu assay results showed that cell proliferation was inhibited by miR-425-5p mimic in KG-1A and THP-1 cells ( $P < 0.01$ , Figure 4A). Meanwhile, invasion and migration of KG-1A and THP-1 cells were reduced by miR-425-5p mimic ( $P < 0.01$ , Figure 4B). Together, these data indicate that BM-MSCs-derived exosomal miR-425-5p inhibits proliferation, invasion and migration of KG-1A and THP-1 cells.

## miR-425-5p Targets WTAP in AML Cells

Then, we aimed to investigate the mechanism underlying the miR-425-5p-mediated AML. The miR-425-5p-targeted site in WTAP 3'-UTR was identified by bioinformatics analysis using Targetscan ([http://www.targetscan.org/vert\\_72/](http://www.targetscan.org/vert_72/)) (Figure 5A). Notably, miR-425-5p mimic treatment inhibited luciferase activities of the wild type WTAP but did not affect WTAP with the mutated miR-425-5p-binding site ( $P < 0.01$ , Figure 5B). Exosomes were extracted from BM-MSCs treated with the control mimic or miR-425-5p mimic, and KG-1A and THP-1 cells were further treated with the exosomes. The expression of WTAP was inhibited by miR-425-5p in the cells ( $P < 0.01$ , Figure 5C). Similarly, miR-21-5p mimic could decrease the expression levels of WTAP in the cells ( $P < 0.01$ , Figure 5D). The expression levels of WTAP were increased in CD34<sup>+</sup>CD38<sup>−</sup> AML cells from primary AML patients ( $n = 20$ ) compared to that from bone marrow of healthy cases ( $n = 20$ ) ( $P < 0.001$ , Figure 5E). The WTAP was up-regulated in KG-1A, NB4, MV411, and THP-1 cells compared with that in BM-MSCs ( $P < 0.001$ , Figure 5F). Taken together, these results indicate that miR-425-5p targets WTAP in AML cells.

## miR-425-5p Inhibits AML by Targeting WTAP

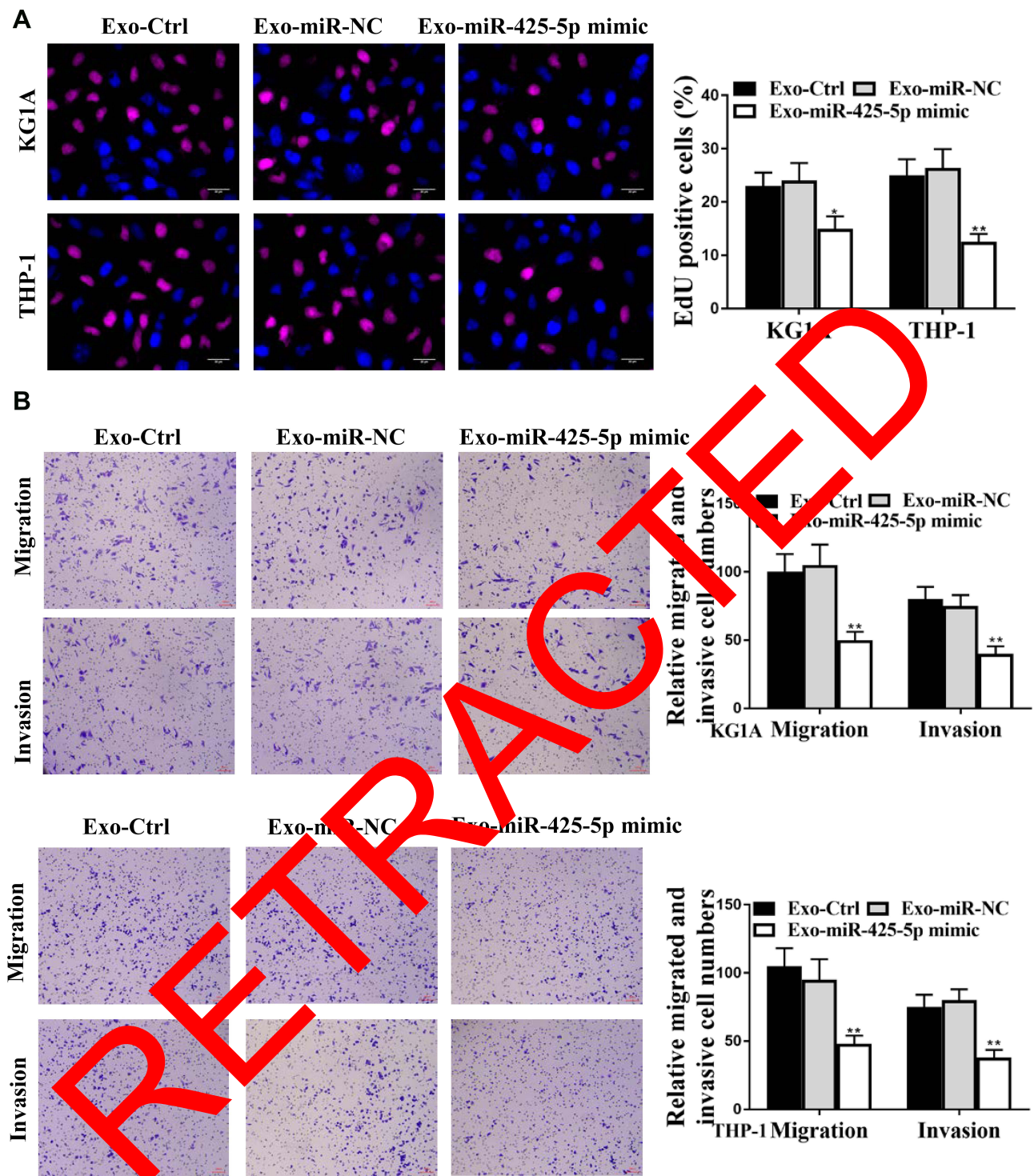
Next, we explored whether miR-425-5p inhibits AML by targeting WTAP. The efficiency of overexpression of WTAP was confirmed in KG-1A cells ( $P < 0.01$ , Figure 6A). Colony formation assay revealed that overexpression of WTAP could rescue miR-425-5p mimic-inhibited cell proliferation in the cells ( $P < 0.01$ , Figure 6B). Meanwhile, the miR-425-5p mimic-induced apoptosis of KG-1A cells was blocked by overexpression of WTAP ( $P < 0.01$ , Figure 6C). Edu assay results showed that overexpression of WTAP could enhance proliferation decreased by miR-425-5p mimic in KG-1A cells ( $P < 0.01$ , Figure 6D). Moreover, overexpression of WTAP rescued the miR-425-5p mimic-inhibited invasion and migration of KG-1A cells ( $P < 0.05$ , Figure 6E). Together, these data indicate that miR-425-5p inhibits AML by targeting WTAP.

## Discussion

AML is the predominant type of blood cancer with severe morbidity and high mortality.<sup>32</sup> The mechanism of the progression of AML remains unclear, limiting the development of biomarkers of diagnosis, prognosis and effective therapies for AML patients.<sup>33</sup> During the past decade, BM-MSCs miRNAs have been demonstrated to play crucial roles in the modulation of AML. In this study, we aimed to investigate the role of BM-MSCs-derived exosomal miR-425-5p in the development of AML. We observed abnormal expression of miR-425-5p in AML patients and AML cell lines. BM-MSCs-derived exosomal miR-425-5p attenuated cell proliferation, invasion and migration, and induced apoptosis of AML cells by inhibiting the expression of WTAP by targeting the 3'-UTR of WTAP. Our findings demonstrated that BM-MSCs-derived exosomal miR-425-5p inhibited AML by regulating WTAP.

It has been reported that aberrantly expressed exosomal miRNAs participate in the development of AML. For example, exosomal miRNA-4532 of AML cells regulates regular hematopoiesis in hematopoietic stem cells through stimulating the LDOC1-related STAT3 signaling.<sup>34</sup> High plasma exosomal miR-125b levels serve as the possible biomarker for the bad prognosis of intermediate-risk AML.<sup>35</sup> Serum exosome-derived miRNA-532 is a new prognostic biomarker for patients with AML.<sup>36</sup> Elevated plasma extracellular vesicle-derived miR-10b foretells the

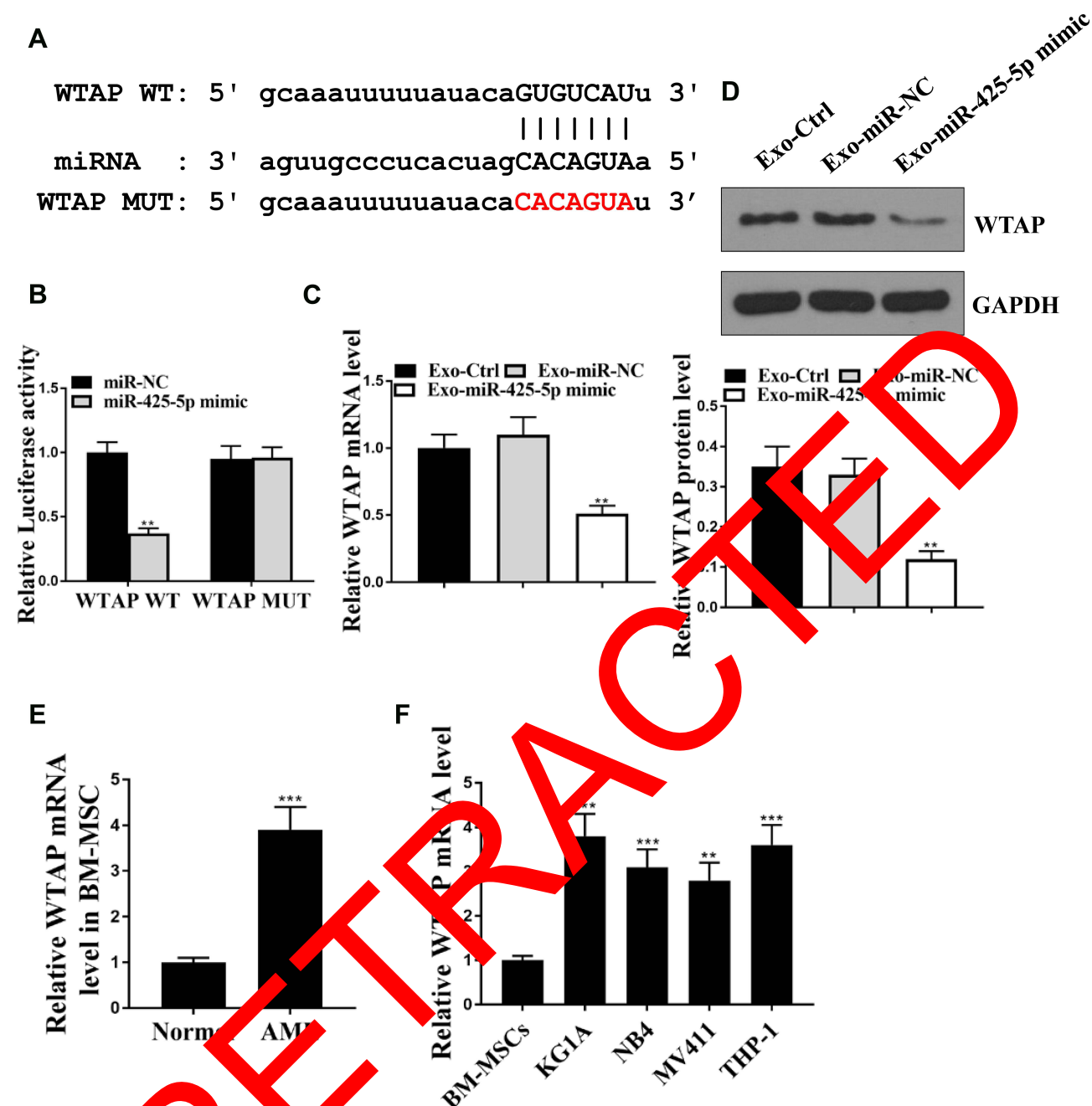




**Figure 4** Exosomal miR-21-5p inhibits apoptosis of ovarian cancer cells. **(A and B)** The exosomes were extracted from the BM-MSCs cells treated with control mimic or miR-425-5p mimic, and the KG-1A and THP-1 cells were further treated with the exosomes. **(A)** The cell proliferation was measured by Edu assay in the cells. **(B)** The invasion and migration were assessed by transwell assays in the cells. Data are presented as mean  $\pm$  SD. Statistic significant differences were indicated: \* $P < 0.05$ , \*\* $P < 0.01$ .

poor prognosis of AML patients.<sup>37</sup> Exosomal miR-486 controls hypoxia-related erythroid differentiation by regulating Sirt1 in erythroleukemia cells.<sup>38</sup> Moreover, as a well-studied miRNA, miR-425-5p is abnormally expressed in multiple cancer models and contributes to

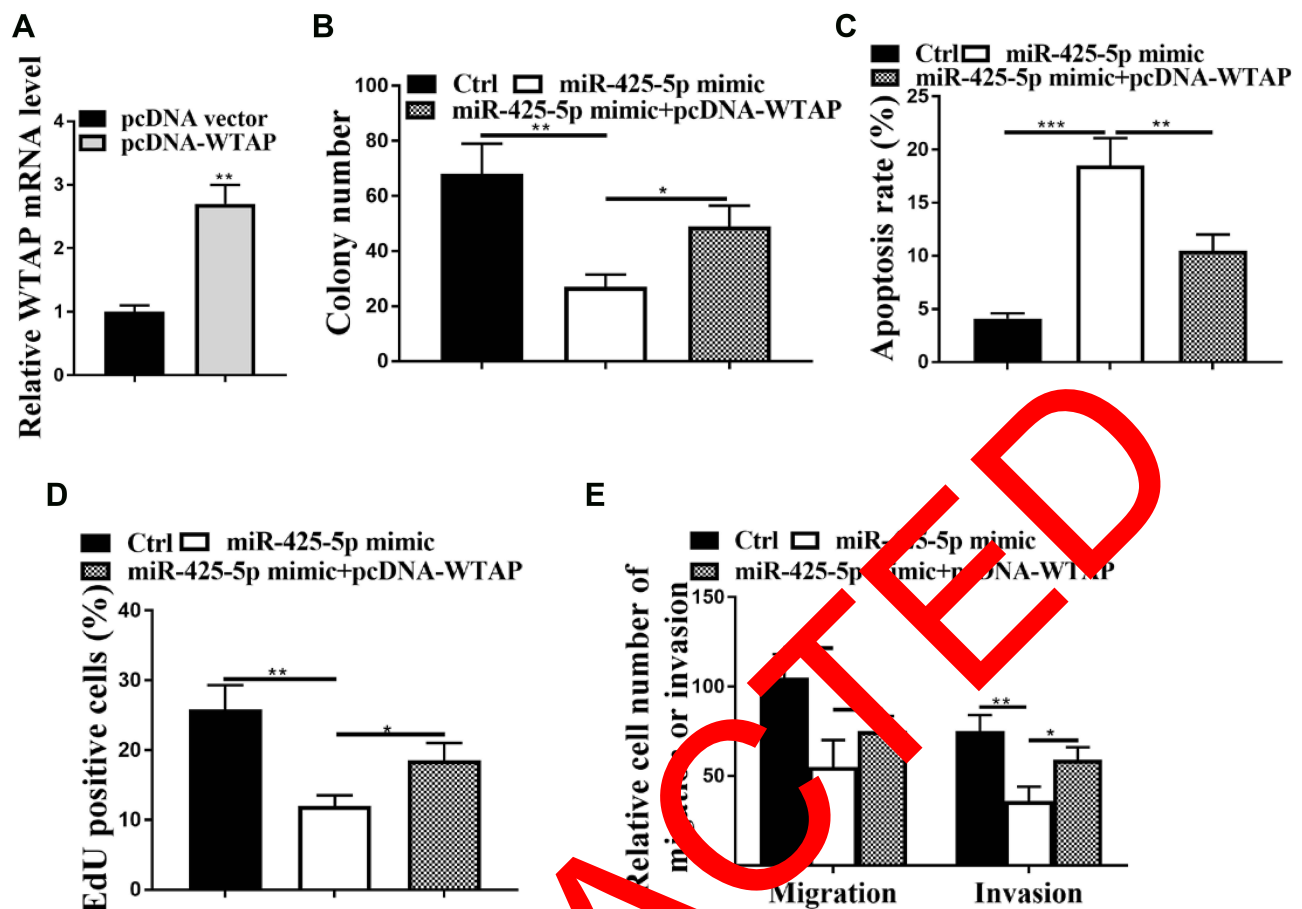
cancer development. For instance, the clinic significance of miR-425-5p and its association with apoptosis and proliferation of gastric cancer cells has been reported.<sup>39</sup> Down-regulation of miR-425-5p represses cervical cancer progression by regulating AIFM1.<sup>40</sup> MiR-425-5p inhibits



**Figure 5** miR-425-5p targets WTAP in AML cells. **(A)** The interaction of miR-425-5p and WTAP 3'-UTR was identified by bioinformatic analysis using Targetscan (<http://www.targetscan.org/vert-7.2/>). **(B)** KG-1A and THP-1 cells were treated with miR-425-5p mimic or the control mimic. The luciferase activities of wild type WTAP (WTAP WT) and WTAP with the miR-425-5p-binding site mutant (WTAP MUT) were determined by luciferase reporter gene assays in the cells. **(C and D)** The exosomes were extracted from the BM-MSCs treated with miR-425-5p mimic or the control mimic, and the KG-1A and THP-1 cells were further treated with the exosomes. **(C)** The mRNA expression of WTAP was measured by qPCR assays in the cells. **(D)** The protein expression of WTAP was tested by Western blot analysis in the cells. The results of Western blot analysis were quantified by ImageJ software. **(E)** The expression levels of WTAP were measured by qPCR assays in the CD34<sup>+</sup>CD38<sup>-</sup> AML cells from primary AML patients (n = 20) and bone marrow of healthy cases (n = 20). **(F)** The expression levels of miR-425-5p were assessed by qPCR assays in the KG-1A, NB4, MV411, THP-1 cells, and BM-MSCs. Data are presented as mean  $\pm$  SD. Statistic significant differences were indicated: \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

the expressions of TUG1 and lncRNA MALAT1, and represses osteosarcoma development by regulating the Wnt/ $\beta$ -catenin signaling.<sup>41</sup> Prader-Willi region non-protein coding RNA 1 suppresses gastric cancer progression by targeting miR-425-5p.<sup>42</sup> MiR-425-5p modulates

chemoresistance by regulating programmed cell death 10 in colorectal cancer cells.<sup>43</sup> MiR-425-5p is involved in the lncRNA LINC-PINT/PTCH1/SHH axis-regulated chemoresistance and laryngeal carcinoma cell stemness.<sup>44</sup> Meanwhile, miRNA profiling showed that miR-425-5p is



**Figure 6** miR-425-5p inhibits AML progression by targeting WTAP. (A) The expression of WTAP was measured by qPCR in the KG-1A cells transfected with pcDNA3.1 or pcDNA3.1-WTAP overexpression vector. (B-E) The KG-1A cells were treated with control mimic, miR-425-5p mimic, or co-treated with miR-425-5p mimic and pcDNA3.1-WTAP overexpression vector. (B) The cell proliferation was tested by colony formation assay in the cells. (C) The cell apoptosis was measured by flow cytometry analysis in the cells. (D) The cell proliferation was analyzed by EdU assay in the cells. (E) The invasion and migration were assessed by transwell assays in the cells. Data are presented as mean  $\pm$  SD. Statistic significant differences were indicated: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

reduced in exosomes from clinical AML samples.<sup>20</sup> Furthermore, BM-MSCs play critical roles in the regulation of AML. It has been reported that AML converts BM-MSCs in the leukemia-permitted microenvironment by exosomes secretion.<sup>45</sup> Human BM-MSCs-derived exosomal miR-22-3p inhibits proliferation of AML cells by regulating IRF2 and INPP4B.<sup>46</sup> MiR-7977 represses the Hippo-YAP signaling in BM-MSCs during AML progression.<sup>47</sup> In this study, we found that the expression levels of miR-425-5p were decreased in CD34+CD38- AML cells from primary AML patients compared to that from the bone marrow of healthy cases. The expression levels of miR-425-5p were reduced in the exosomes from primary AML patients compared to those from normal cases. It implies that the abnormally expressed miR-425-5p in the exosomes may be involved in the progression of ovarian cancer in a clinical context, serving as a potential biomarker of ovarian cancer. Meanwhile, miR-425-5p was

down-regulated in the AML cell lines compared with BM-MSCs. We also found that miR-425-5p was expressed in the exosomes from normal subject derived BM-MSCs, and BM-MSCs-derived exosomal miR-425-5p inhibited proliferation, invasion, and migration and induced apoptosis of AML cells. These data present a novel function of BM-MSCs-derived exosomal miR-425-5p in AML progression, providing valuable evidence for the fundamental role of BM-MSCs-derived exosomal miRNAs in the development of AML. However, the effect of BM-MSCs-derived exosomal miR-425-5p on the proliferation invasion, migration, and apoptosis of AML cells was only investigated in vitro, and the lacking of in vivo evidence may limit the profound role of BM-MSCs-derived exosomal miR-425-5p in the regulation of AML progression. It needs to be further investigated in future study.

WTAP has been shown to function as an essential regulator in the development of AML. For example,



WTAP promotes hepatocellular carcinoma progression by m6A-HuR-related epigenetic inhibiting of ETS1.<sup>48</sup> WTAP serves as a prognosis biomarker for ovarian cancer and controls the ovarian cancer cell progression.<sup>49</sup> WTAP forms a complex with BCL6 through Hsp90 and exerts an oncogenic function in diffuse large B-cell lymphoma.<sup>50</sup> The up-regulation of WTAP is associated with poor prognosis of gastric cancer through regulating tumor-associated T lymphocyte infiltration.<sup>51</sup> WTAP increases chemoresistance to gemcitabine and metastasis in pancreatic cancer by up-regulating Fak mRNA.<sup>52</sup> WTAP enhances the proliferation of renal cell carcinoma cells by stabilizing CDK2.<sup>53</sup> Moreover, several studies have identified the malignant role of WTAP in AML progression. It has been reported that WTAP serves as a new oncogenic factor in AML.<sup>27</sup> WTAP is up-regulated in AML samples and contributes to AML progression by modulating m6A methylation.<sup>54</sup> Our findings further demonstrated that miR-425-5p could target WTAP in AML cells and inhibited AML progression by targeting WTAP. These data identify the new downstream target WTAP of miR-425-5p, identifying the correlation of WTAP with miR-425-5p in AML. Previous studies have identified the role of miR-425-5p and WTAP in cancer cells, but there were few studies investigating the relationship between the two. Based on this, the novelty of this work is recognized. The chemoresistance is a severe clinical challenge of AML, leading to relapse of AML patients. The function of miR-425-5p in the modulation of chemotherapy resistance of AML cells will need to be further explored in the future.

## Conclusion

In conclusion, we discovered that BM-MSCs-derived exosomal miR-425-5p inhibited proliferation, invasion and migration of AML cells and induced apoptosis of AML cells by targeting WTAP. Our finding provides new insights into the mechanism by which BM-MSCs-derived exosomal miR-425-5p attenuates the AML development, enhancing understanding of the correlation of BM-MSCs-derived exosomal miRNA with AML. BM-MSCs-derived exosomal miR-425-5p may serve as a potential target for AML therapy.

## Disclosure

The authors declare no conflicts of interest for this work.

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