HMGA2 regulates acute myeloid leukemia progression and sensitivity to daunorubicin via Wnt/β-catenin signaling

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Abstract. Acute myeloid leukemia (AML) is a malignant disease with an increasing prevalence in adults and children. However, valuable molecular diagnostic research is rare. In the present study, plasmids silencing and overexpressing high-mobility group AT-hook 2 (HMGA2) were respectively transfected in HL60 and NB4 cells. The effects of HMGA2 on AML cell viability, apoptosis, migration and invasion were determined by preforming MTT, flow cytometry, wound scratch and Transwell assays, respectively. Genes associated with apoptosis and Wnt signaling were evaluated by reverse transcription-quantitative (RT-q)-PCR and western blotting. AML cell sensitivity to daunorubicin (DNR) and the regulatory effects of the Wnt signaling pathway via HMGA2 following treatment with the agonist LiCl or antagonist XAV939 were detected by MTT, RT-qPCR and western blot analysis. The results revealed that the expression of HMGA2 was elevated more so in HL60, KG1, U937, Kasumi-1, THP-1 and K562 cells than in NB4 cells. Silencing HMGA2 suppressed cell viability, migration and invasion, enhanced cell apoptosis and sensitivity to DNR, and almost restored the DNR inhibitory function that was promoted by LiCl treatment. In addition, low expression of HMGA2 attenuated X-linked inhibitor of apoptosis and Bcl-2 mRNA and protein levels, and upregulated the expression of Bax and cleaved-caspase-3. Furthermore, silencing HMGA2 not only decreased Wnt and non-phospho-β-catenin expressions, but also partially reversed the increased expressions of these proteins induced by LiCl treatment. On the other hand, overexpression of HMGA2 exhibited the opposite results after transfection in NB4 cells. The results of the present study demonstrated that

HMGA2 played important roles in driving AML progression and chemosensitivity in HL60 and NB4 cells, potentially by activating the Wnt/β-catenin signaling pathway. Therefore, it was suggested that HMGA2 may be a promising molecular marker for AML diagnosis.

Introduction

Leukemia is a malignant disease characterized by the abnormal growth of hematopoietic stem cells. According to the American Cancer Society and the National Cancer Institute in 2016, ~468,000 patients were diagnosed with leukemia in the United States (1). As one of the most common blood tumors in adult leukemia, acute myeloid leukemia (AML), which is characterized by abnormal proliferation and the accumulation of a large number of unusual hematopoietic stem cells in bone marrow, peripheral blood and even in other tissues, could result in the destruction of the hematopoietic system, and the morbidity and mortality of the disease has exhibited an annual increase (1,2). At present, cell-dependent therapy, hepatocyte transplantation, targeted therapy, chemotherapy and radiotherapy have been applied for the treatment of leukemia under different circumstances (3-6). In regard to induction therapy for AML, the main chemotherapy regimen has been the combination of anthracyclines and cytarabine for the past three decades (7). Although a recent multicenter clinical phase III trial revealed that the complete remission rate of patients with AML could be as high as 79%, the overall survival and relapse-free survival were only 20 and 15 months, respectively (8). Therefore, relapse of drug resistance is still a leading cause of mortality in patients with AML, and it is also a major issue in the attempt to maintain longer survival times after initial remission (9).

The etiology of AML is very complex, and current research has indicated that chromosomal abnormalities and reproducible genetic abnormalities were the main mechanisms of morbidity in patients with AML (10-12). High mobility group A2 (HMGA2), a member of the high mobility group protein superfamily, is widely accepted as a new oncogene (13,14). HNGA2 has the physiological functions of inducing gene transcription, integrating retrovirus

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into chromosomes, inducing transformation and promoting the activation of cancer cells; it also plays an important role in maintaining stem cell differentiation potential and self-renewal ability (15-17). Based on the high expression of HMGA2 at the embryonic stage, the association between HMGA2 and stem cells was studied. We previously reported that the expression of HMGA2 was high in embryonic stem cells and its expression gradually decreased with age (18). Meyer *et al* (19) suggested that the level of HMGA2 was increased in the CML-accelerated and CML-blastic phases, when compared with that in the CML-chronic phase. Furthermore, the expression of HMGA2 was negatively correlated to let-7b (19,20). In addition, HMGA2 could accelerate the G2/M phase of cell cycle transformation or induce epithelial-mesenchymal transition to promote tumorigenesis, invasion and metastasis (16,21).

However, the role of HMGA2 in AML and the underlying mechanism are still unclear. Several signaling pathways have been reported to be important in the progression of leukemia including the Wnt/β-catenin, PI3K/Akt/mTOR, NF-κB and Janus kinase/STAT signaling pathways (22-25). The aim of the present study was to investigate the Wnt/β-catenin signaling pathway in regulation of HMGA2 in AML cells.

Materials and methods

Cell culture. The human myeloid leukemia cell lines, NB4, HL60, KG1, U937, Kasumi-1, THP-1 and K562 were purchased from American Type Culture Collection. All cells were cultured at 37°C in 5% CO_2 atmosphere in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.), 100 U/ml penicillin and streptomycin (North China Pharmaceutical Co., Ltd.).

NB4 and HL60 cells were selected to conduct the following experiments. Both cell lines were treated with 10 μg/ml daunorubicin (DNR; Shenzhen Main Luck Pharmaceuticals Inc.) for 24 h at 37°C to evaluate the cell sensitivity to DNR. In addition, NB4 and HL60 cells were treated with 10 μM XAV939 (MedChemExpress USA), or 10 μM XAV939 + 10 μg/ml DNR and 20 mM LiCl (Sigma Aldrich; Merck KGaA), or 20 mM LiCl + 10 μg/ml DNR for 24 h at 37°C to perform mechanism-related experiments.

Cell transfection. HL60 and NB4 cells, with or without drug treatments, were respectively seeded in 6-well plates ($1.0x10^5$) for 24 h at 37°C before transfection. Silencing HMGA2 [small interfering RNA (siRNA/si-) HMGA2; forward, 5'-AGA UUGAGAUUGAAAGUGCCU-3' and reverse, 5'-GCA CUUUCAAUCUCAAUCUCU-3'], overexpressing HMGA2 (HMGA2) and negative control (NC) plasmids (5 μg/well of each plasmid) were synthetized by Invitrogen (Thermo Fisher Scientific, Inc.). Lipofectamine 2000™ (Invitrogen; Thermo Fisher Scientific, Inc.) was applied to determine transient transfection according to manufacturer's protocol. siHMGA2, siNC, HMGA2 or NC and Lipofectamine 2000™ were respectively added to Opti-Minimum Essential Medium (MEM; Gibco; Thermo Fisher Scientific, Inc.) medium. The Lipofectamine/siRNA or Lipofectamine/overexpressing RNA mixtures were cultured at 20°C for 10 min and then

Opti-MEM RPMI-1640 medium was added. After 6 h of culture, the media was changed back to RPMI-1640 medium containing 10% FBS. After 24 h culture, cells were used in the subsequent experiments.

Cell viability. Cell viability was determined using a 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT; Beyotime Institute of Biotechnology) assay. After transfection or treatment with drugs for 24, 48 or 72 h, $5x10^3$ cells per well were seeded into 96-well plates and cultured at 37°C with 5% CO_2. Subsequently, 10 μl MTT was added into each well containing culture medium for a further 1 h. Then, 100 μl DMSO was added to dissolve crystals once the media was removed. The optical density values were detected using a Microplate Reader (Thermo Fisher Scientific, Inc.) at 490 nm.

Wound scratch and Transwell assays. Cells ($5x10^4$) were seeded in 12-well plates and incubated at 37°C for 24 h. A sterile pipette tip (10 μl) was used to draw a wound in the center of the plate. The plates were gently washed 3 times with PBS. Then, the cells were cultured in serum-free medium for 0 or 48 h. The scratch area was measured using ImageJ software version 1.8.0 (National Institutes of Health).

The invasion activity of cells was detected using a 24-well transwell chamber coated with Matrigel (Corning, Inc.). After 24 h of transfection treatment, the cells were resuspended in serum-free medium and $1x10^5$ cells were added into the coated upper chamber. RPMI-1640 medium containing 10% FBS was added to the lower chamber and the cells were incubated for 48 h at 37°C in an environment with a 5% CO_2. The cells were fixed with 4% formaldehyde for 20 min at 25°C and stained with 1% crystal violet for a further 15 min at 37°C. The number of invading cells was counted at x200 magnification using a light microscope.

Flow cytometry. Transfected cells ($5x10^5$) were digested with 0.25% trypsin and centrifugated at 1,000 x g for 5 min at 37°C. The apoptosis assay was performed using Annexin V-FITC. The cells ($5x10^5$ cells/well) were washed twice using washing buffer, and the suspension was cultured with the Annexin V-FITC and propidium iodide apoptosis kit [cat. no. 70-AP101-60; MultiSciences (Lianke) Biotech Co., Ltd.] in the dark at 25°C for 20 min according to the manufacturer's instructions. Binding buffer was subsequently added to each well. A flow cytometer was used to detect samples within 1 h and BD CellQuest™ Pro Software version 1.2 was used for analysis (BD Biosciences).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA in cultured cells or cells treated with drugs or plasmids was extracted with TRIzol regent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. The Superscript II first-strand cDNA synthesis System (Invitrogen; Thermo Fisher Scientific, Inc.) was used to perform RT. RT-qPCR was carried out using the SYBR Fast qPCR Mix (Invitrogen; Thermo Fisher Scientific, Inc.) for HMGA2, X-linked inhibitor of apoptosis (XIAP), Bcl-2 and Bax. GAPDH was used as an internal control. The thermocycling conditions of qPCR were as follows: For HMGA2 and

GAPDH, 95°C for 3 min, 95°C for 1 min followed by 30 cycles of 60°C for 30 sec and 72°C for 30 sec; for XIAP, 95°C for 3 min, 95°C for 30 sec followed by 35 cycles of 58°C for 30 sec and 72°C for 30 sec; and for Bcl-2 and Bax, 95°C for 5 min, 95°C for 10 sec followed by 40 cycles of 60°C for 34 sec. Primers were purchased commercially (Invitrogen; Thermo Fisher Scientific, Inc.) and the sequences are listed in Table I. The expression levels of the above genes were determined using the $2^{-\Delta\Delta Cq}$ method (26).

Western blot analysis. RIPA lysis buffer (Thermo Fisher Scientific, Inc.) was used to extract total protein from the cultured cells. Subsequently, protein concentration was determined using an Enhanced BCA Protein Assay kit (Beyotime Institute of Biotechnology). The proteins (20 µg/lane) were subjected to 12% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore). Then 5% milk PBS with 0.1% Triton X-100 was applied to block the membranes at room temperature for 2 h, which were then incubated with the following: Anti-HMGA2 antibody (cat. no. ab97276; 1:2,000; Abcam), anti-XIAP antibody (cat. no. ab21278; 1:1,000; Abcam), anti-Bcl-2 antibody (cat. no. ab32124; 1:1,000; Abcam), anti-cleaved caspase-3 antibody (cat. no. ab2302; 1:1,000; Abcam), anti-Wnt antibody (cat. no. ab28472; 1:1,000; Abcam), anti-non-phospho (Np)-β-catenin antibody (cat. no. 8814; 1:1,000; Cell Signaling Technology, Inc.) and anti-GAPDH antibody (cat. no. ab9485; 1:2,500; Abcam) overnight at 4°C. The membranes were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (1:2,000; cat. no. SA00001-2; ProteinTech Group, Inc.) at 4°C for 1 h after washing with PBST (containing 0.05% Tween-20) three times. Protein bands were detected with ECL (Thermo Fisher Scientific, Inc.) and visualized using Quantity One software version 4.6.2 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analysis was detected by GraphPad Prism version 6.0 software (GraphPad Software, Inc.). All data were presented as the mean ± standard deviation from three independent experiments. Differences were analyzed using one-way analysis of variance following Tukey's post hoc test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression and transfection efficiency of HMGA2 in AML cells. The 7 AML cell lines, including NB4, HL60, KG1, U937, Kasumi-1, THP-1 and K562, were analyzed to determine the expression of HMGA2. As shown in Fig. 1A, the HL60, KG1, U937, Kasumi-1, THP-1 and K562 cell lines had higher expressions of HMGA2 than NB4 cells. Therefore, NB4, which had a relatively low expression of HMGA2, was selected for the HMGA2 overexpression experiments, and HL60 was selected for the siHMGA2 transfection experiments. RT-qPCR (Fig. 1B) and western blot analysis (Fig. 1C and D) revealed a decreased expression of HMGA2 (P<0.01) in siHMGA2 HL60 cells and an elevated expression of HMGA2 (P<0.01) in NB4 cells, thereby indicating successful transfection.

Table I. Primers used in reverse transcription-quantitative PCR.

Gene	Primer	Sequence (5'-3')
HMGA2	Forward	AGTCCCTCTAAAGCAGCTCAAAAG
	Reverse	GCCATTTCCTAGGTCTGCCTC
XIAP	Forward	ATGACTTTTAACAGTTTTGAAGG
	Reverse	GCTCGTGCCAGTGTTGATGCTG
Bcl-2	Forward	GGATTGTGGCCTTCTTTGAG
	Reverse	TACCCAGCCTCCGTTATCCT
Bax	Forward	CCGATTCATCTACCCTGCTG
	Reverse	TGAGCAATTCCAGAGGCAGT
GAPDH	Forward	AGCCACATCGCTCAGACAC
	Reverse	GCCCAATACGACCAAATCC

HMGA2, high-mobility group AT-hook 2; XIAP, X-linked inhibitor of apoptosis.

HMGA2 regulates the proliferation, apoptosis, migration and invasion of AML HL60 and NB4 cells. As aforementioned, the present study selected two AML cell lines, which either possessed a high or low expression of HMGA2, and were subsequently transfected with either silencing or overexpression HMGA2 plasmids. The results revealed that silencing HMGA2 decreased HL60 cell viability in 24 h; however, no significant difference in comparison to control was identified. However, siHMGA2 significantly inhibited cell proliferation from 48 h, compared with the control (P<0.01; Fig. 2A). In addition, in NB4 cell, overexpressing HMGA2 promoted cell viability starting from 48 h (P<0.05; Fig. 2B). In regard to cell apoptosis, the Annexin-V-FITC assay revealed that silencing HMGA2 significantly induced cell apoptosis (P<0.01) and overexpression of HMGA2 produced the opposite result (P<0.05; Fig. 2C and D). These results were in agreement with the expression levels of the apoptosis-related genes in AML cells. Silencing HMGA2 significantly suppressed the mRNA and protein expressions of XIAP (P<0.01) and Bcl-2 (P<0.01), and significantly increased Bax and cleaved caspase-3 the mRNA and protein levels (P<0.01; Fig. 2E-G). In addition, significantly increased expression levels of XIAP and Bcl-2, and significantly reduced expression levels of Bax and cleaved caspase-3 were found in the NB4 cells overexpressing HMGA2 (P<0.01; Fig. 2E-G). Subsequently, the effect of HMGA2 on cell migration (Fig. 3A) and invasion (Fig. 3B) was investigated. As excepted, silencing HMGA2 significantly inhibited cell migration (P<0.01; Fig. 3C) and invasion (P<0.01; Fig. 3D). By contrast, overexpression of HMGA2 contributed to the promotion of cell migration and invasion (P<0.01).

Effect of HMGA2 on cell sensitivity to DNR in AML HL60 and NB4 cells. DNR in combination with Cytarabine is widely accepted as a classical AML induction mitigation scheme (27). In addition, Idarubicin has been approved by the US Food and Drug Administration for the combination chemotherapy for AML since 1990 (28). DNR has a certain inhibitory effect on AML, which was confirmed in the present study as presented
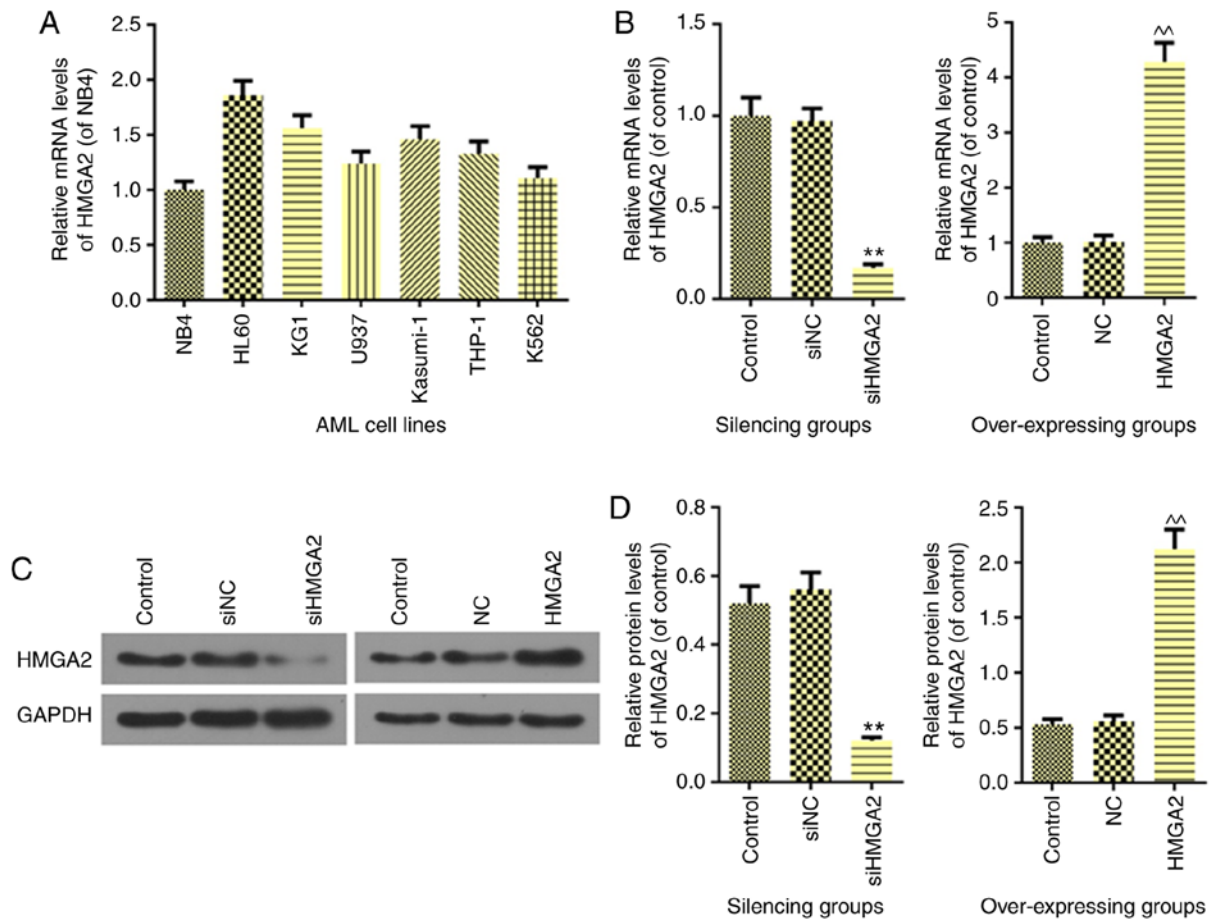
Figure 1. Expression and transfection efficiency of HMGA2 in acute myelocytic leukemia cells. (A) Seven acute myelocytic leukemia cell lines (NB4, HL60, KG1, U937, Kasumi-1, THP-1 and K562) were used to determine the mRNA expression of HMGA2 via RT-qPCR. siHMGA2 was successfully transfected into HL60 cells and plasmids overexpressing HMGA2 were successfully transfected into NB4 cells. The transfection efficiency of HMGA2 in HL60 (left panel) and NB4 (right panel) cells was determined via (B) RT-qPCR and (C and D) western blot analysis. GAPDH was used as the internal control. Data were presented as the mean ± standard deviation from three independent experiments. **P<0.01 vs. HL60 cell control; $^{^^}$P<0.01 vs. NB4 cell control. HMGA2, high-mobility group AT-hook 2 overexpression group; RT-qPCR, reverse transcription-quantitative PCR; si-, small interfering RNA; NC, negative control.

in Fig. 4A (P<0.01). In addition, silencing HMGA2 contributed to the inhibitory effect on cell viability induced by DNR (P<0.05; Fig. 4A). Nevertheless, overexpression of HMGA2 could significantly reverse the DNR-induced reduction of cell proliferation (P<0.01; Fig. 4A). Furthermore, the results demonstrated that the mRNA and protein levels of HMGA2 were not altered when comparing the NC and NC + DNR groups, indicating that DNR did not affect HMGA2 expression (P>0.05). Compared with siNC + DNR, siHMGA2 in combination with DNR significantly downregulated the expression of HMGA2 both at the mRNA and protein levels (P<0.01; Fig. 4B-D). However, in NB4 cells, the effect of DNR or HMGA2 overexpression + DNR on the expression of HMGA2 was opposite to that observed in HL60 cell (P<0.01; Fig. 4B-D).

HMGA2 affects the DNR-induced inhibitory effect of Wnt/β-catenin signaling in AML HL60 and NB4 cells. An increasing body of evidence has indicated that abnormal activation of Wnt/β-catenin signaling contributes to the progression of tumors (29-31). Therefore, the present study investigated whether HMGA2 regulated this signaling

pathway. Western blot analysis revealed that DNR could inhibit the protein expressions of Wnt and Np-β-catenin (P<0.05; Fig. 4E-G), but its inhibitory effect was not more significant than siHMGA2 treatment (P<0.01). Notably, when siHMGA2 was combined with DNR, the Wnt and Np-β-catenin protein levels were significantly reduced (P<0.01), indicating that AML cells treated with this combination had suppressed Wnt/β-catenin signaling. On the other hand, overexpression of HMGA2 not only increased the protein expressions of Wnt (P<0.01) and Np-β-catenin (P<0.05), but also attenuated the inhibitory effects of DNR on the Wnt and Np-β-catenin levels (P<0.05; Fig. 4E and G). For the experiments involving the agonist and antagonist of Wnt/β-catenin signaling, LiCl and XAV939 were employed, respectively. As expected, the agonist LiCl significantly promoted the expressions of Wnt and Np-β-catenin (P<0.01; Fig. 5A and B), and the antagonist XAV939 significantly inhibited Wnt/β-catenin signaling activation (P<0.01; Fig. 5C and D). The results further revealed that DNR partially suppressed the promotional effects of LiCl on the expressions of Wnt and Np-β-catenin (P<0.01; Fig. 5A and B). In addition, siHMGA2 could further enhance the inhibitory effect
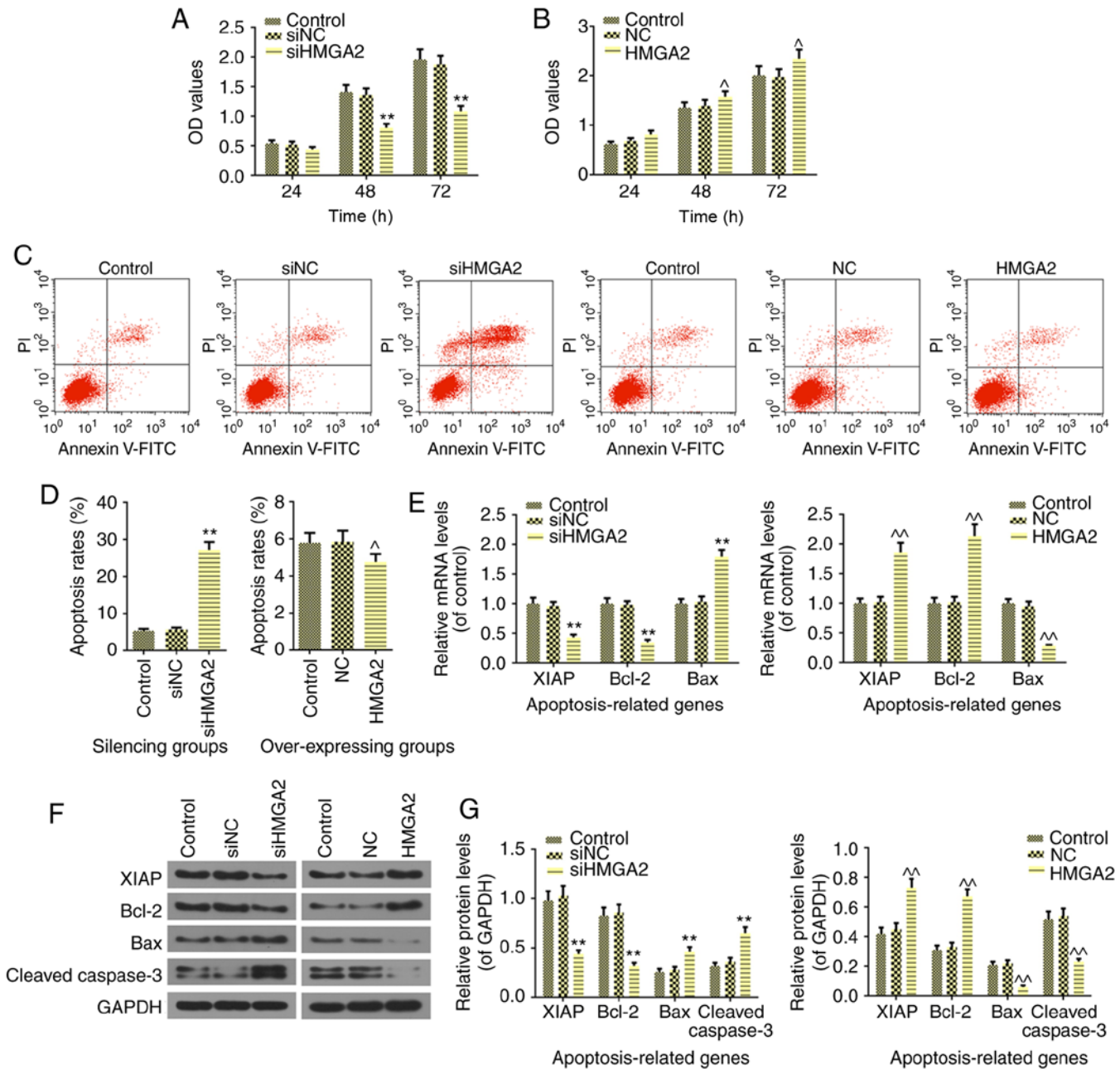
Figure 2. HMGA2 regulates the proliferation and apoptosis of acute myelocytic leukemia HL60 and NB4 cells. (A) The effect of silencing HMGA2 on cell viability was evaluated by MTT assay in HL60 cells at 24, 48 and 72 h. (B) The effect of overexpressing HMGA2 on cell viability was determined by MTT assay in NB4 cells at 24, 48 and 72 h. (C and D) The effect of HMGA2 on cell apoptosis was determined by flow cytometry in HL60 and NB4 cell. (E) The effects of HMGA2 on the mRNA expressions of XIAP, Bcl-2 and Bax were determined by reverse transcription-quantitative PCR in HL60 and NB4 cells. (F and G) The effects of HMGA2 on the protein expressions of XIAP, Bcl-2, Bax and cleaved caspase-3 were determined by western blotting in HL60 and NB4 cells. GAPDH was used as an internal control. Data were presented as the mean ± standard deviation from three independent experiments. **P<0.05 vs. HL60 cell control; $^{\wedge}$P<0.05 and $^{\wedge\wedge}$P<0.01 vs. NB4 cell control. HMGA2, high-mobility group AT-hook 2 overexpression group; si-, small interfering RNA; NC, negative control; XIAP, X-linked inhibitor of apoptosis.

of DNR, which was partially reversed by LiCl (Wnt, P<0.05; Np-β-catenin, P<0.01). As shown in Fig. 5C and D, XAV939 in combination with DNR had the strongest inhibitory effect on Wnt and Np-β-catenin expressions, when compared with treatment alone (P<0.01). Overexpression of HMGA2 could significantly upregulate the Wnt and Np-β-catenin protein levels in DNR treated cells, compared with DNR only treatment (P<0.05) and the effect of HMGA2 overexpression in turn was partially reversed by the combination of XAV939 and DNR (P<0.01; Fig. 5C and D). In addition, LiCl could

induce cell proliferation (P<0.05; Fig. 5E) as expected and XAV939 significantly inhibited cell viability (P<0.01; Fig. 5F). DNR could also significantly reverse the changes in the cell viability of the cells treated with LiCl (P<0.01) or XAV939 (P<0.05). Furthermore, silencing HMGA2 suppressed the increase in cell viability in DNR-treated HL60 cells, which was partially reversed by LiCl (P<0.05). Similarly, overexpressing HMGA2 increased cell viability in DNR-induced NB4 cells, and this effect was partially reversed by XAV939 treatment (P<0.01).
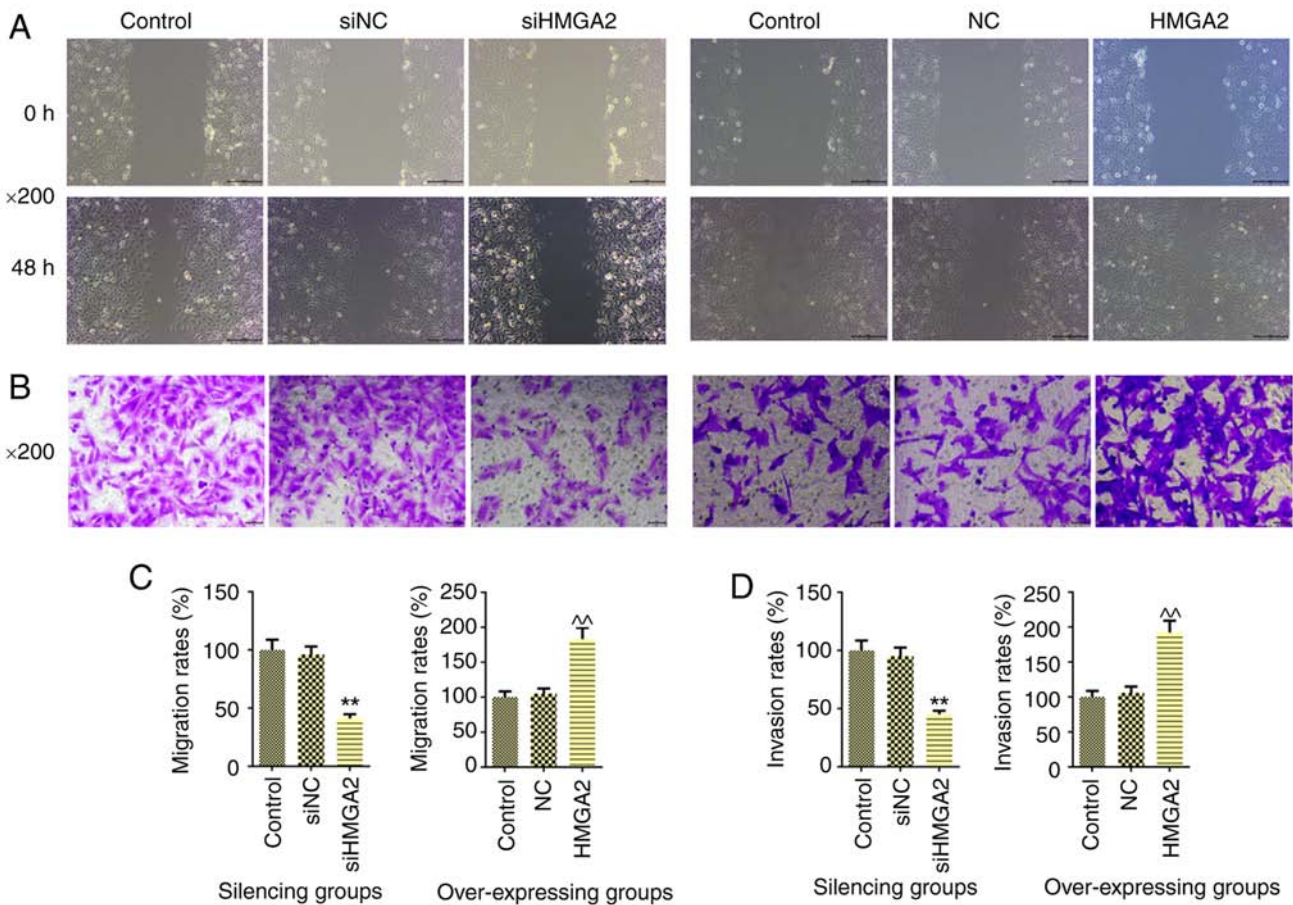
Figure 3. HMGA2 regulates the migration and invasion of AML HL60 and NB4 cells. (A) Cell migration was detected by wound scratch assay and observed at 0 and 48 h using an optical microscope (magnification, x200). (B) Cell invasion was evaluated via a Transwell assay and observed after 48 h using an optical microscope (magnification, x200). (C) The effect of HMGA2 on AML cell migration in HL60 and NB4 cells. (D) The effect of HMGA2 on AML cell invasion in HL60 and NB4 cells. Data were presented as the mean ± standard deviation from three independent experiments. $^{**}P<0.05$ vs. HL60 cell control; $^{\wedge\wedge}P<0.01$ vs. NB4 cell control. AML, acute myelocytic leukemia; HMGA2, high-mobility group AT-hook 2 overexpression group; si-, small interfering RNA; NC, negative control.

Discussion

The present study investigated the HMGA2 levels in several AML cell lines, amongst which the NB4 cell line had relatively reduced expression of HMGA2, and HL60 cells had the greatest expression of HMGA2. Subsequently, these two cell lines were selected to be used in the following experiments. To the best of our knowledge, previous studies have only reported that HMGA2 has a high expression in a large number of malignant tumors including thyroid, ovarian, prostate, gallbladder and bladder cancers, and gastric adenocarcinoma and esophageal squamous cell carcinoma (32-35). In addition, it has been reported that elevated HMGA2 levels were detected in AML (36-39) and Nyquist *et al* (39) demonstrated that t(12;13)(q14;q31) led to HMGA2 upregulation in AML. Through transfection with siHMGA2 in HL60 cells and overexpression HMGA2 in NB4 cells, the present study revealed that silencing HMGA2 could inhibit cell proliferation, migration and invasion as well as induce cell apoptosis. The present *in vitro* experiments were in agreement with the results obtained by Tan *et al* (38) who reported that reduced expression of HMGA2 in AML cells also suppressed cell proliferation. In addition, a marked reduction in XIAP and Bcl-2 expression levels and upregulation of Bax and cleaved

caspase-3 levels occurred in following siHMGA2 transfection in HL60 cells. It has been well established that XIAP is the most potent endogenous caspase inhibitor in the IAP family, which is the only endogenous protein capable of acting on both the initiation and effect of caspases (40,41). If XIAP is activated, the junction region of its baculoviral IAP repeat 1 (BIR1) and BIR2 domains can bind to the active sites of the effectual caspase-3,7 to competitively inhibit the activity of caspase-3,7 (42). Saraei *et al* (43) also suggested that XIAP could be putative in resensitizing tumor necrosis factor-related apoptosis-inducing ligand in leukemia.

The present study is, to the best of our knowledge, not the first to determine the levels of HMGA2 in AML cells, but is the first to study the effect of it on DNR in regard to AML cell sensitivity. DNR, as an anthracycline-based chemotherapy drug, is also a cycle nonspecific agent with strong anti-tumor properties. Currently, almost all first-line standard regimens contain DNR (44). Quiney *et al* (45) reported that there were some patients with DNR resistance in the clinic. The present results revealed that silencing HMGA2 could enhance the inhibition of AML cells by DNR (10 μg/ml), while overexpressing HMGA2 presented the opposite result in comparison with that produced by silencing HMGA2. Previous studies have demonstrated that targeting HMGA2 could regulate
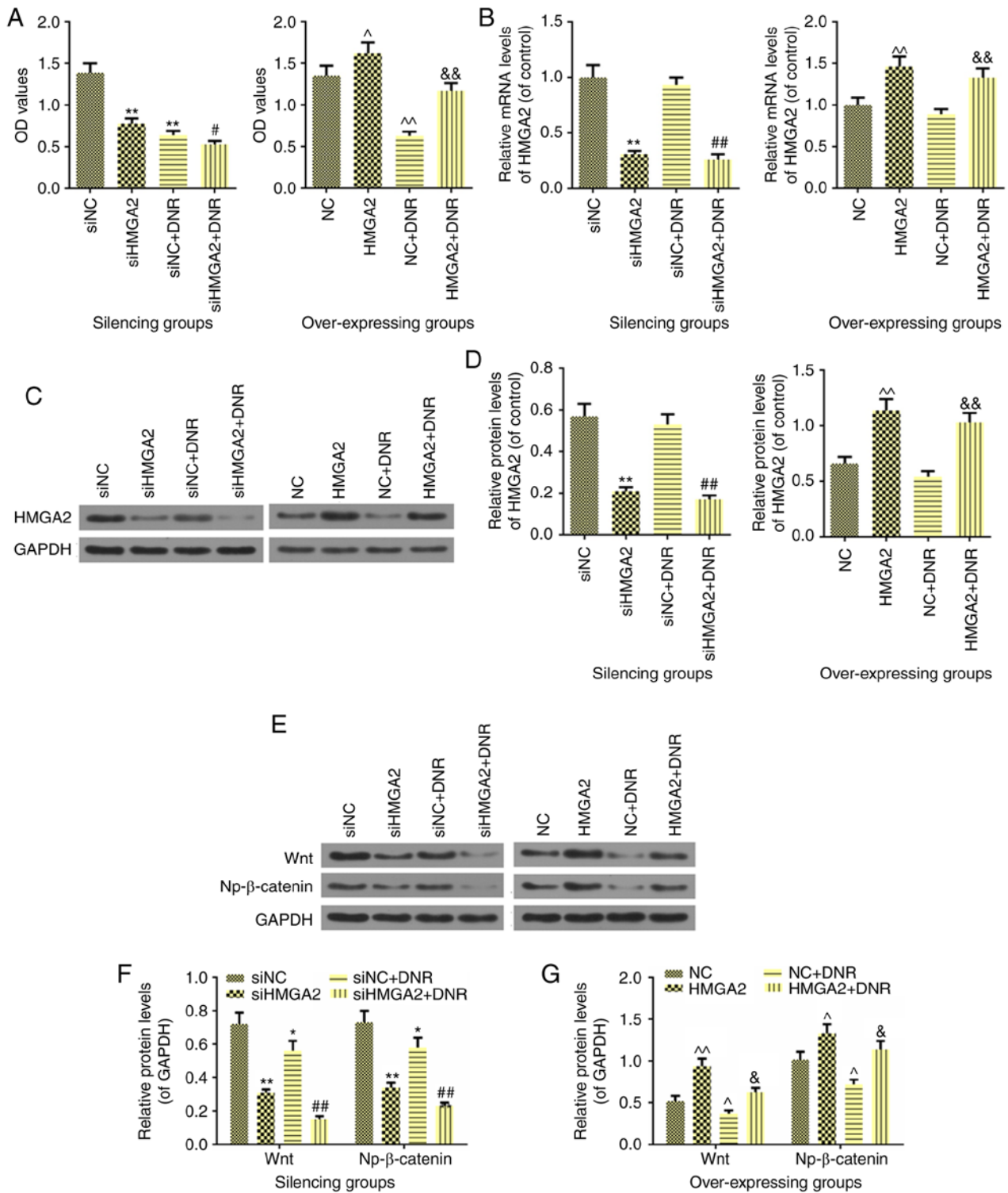
Figure 4. Effects of HMGA2 on cell sensitivity to DNR and the expressions of Wnt and Np-β-catenin in AML HL60 and NB4 cells. (A) The effect of HMGA2 on cell sensitivity to DNR as determined by MTT assay. (B) The mRNA level of HMGA2 was detected by reverse transcription-quantitative PCR to observe the effect of silencing or overexpressing HMGA2 on DNR in AML HL60 and NB4 cells. (C and D) The protein level of HMGA2 was detected by western blotting to observe the effect of silencing or overexpressing HMGA2 on DNR in AML HL60 and NB4 cells. (E) The effects of HMGA2 on the expressions of Wnt and Np-β-catenin, which were inhibited by DNR, were detected by western blot analysis in AML HL60 and NB4 cells. (F) Relative protein levels of Wnt and Np-β-catenin, which were regulated by silencing HMGA2 in HL60 cell. (G) Relative protein levels of Wnt and Np-β-catenin, which were regulated by overexpressing HMGA2 in NB4 cell. GAPDH was used as an internal control. Data were presented as the mean ± standard deviation from three independent experiments. *P<0.05 and **P<0.01 vs. of HL60 cell siNC; $^{\wedge}$P<0.05 and $^{\wedge\wedge}$P<0.01 vs. NB4 cell NC; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. siNC+DNR; $^{\&}$P<0.05 and $^{\&\&}$P<0.01 vs. NC+DNR. DNR, daunorubicin; AML, acute myelocytic leukemia; HMGA2, high-mobility group AT-hook 2 overexpression group; si-, small interfering RNA; NC, negative control; Np-β-catenin, non-phospho-β-catenin.

chemoresistance in several types of cancers, such as colorectal cancer in which HMGA2 could increase the chemoresistance to 5-fluorouracil by activating disheveled segment polarity protein 2/Wnt signaling (46).
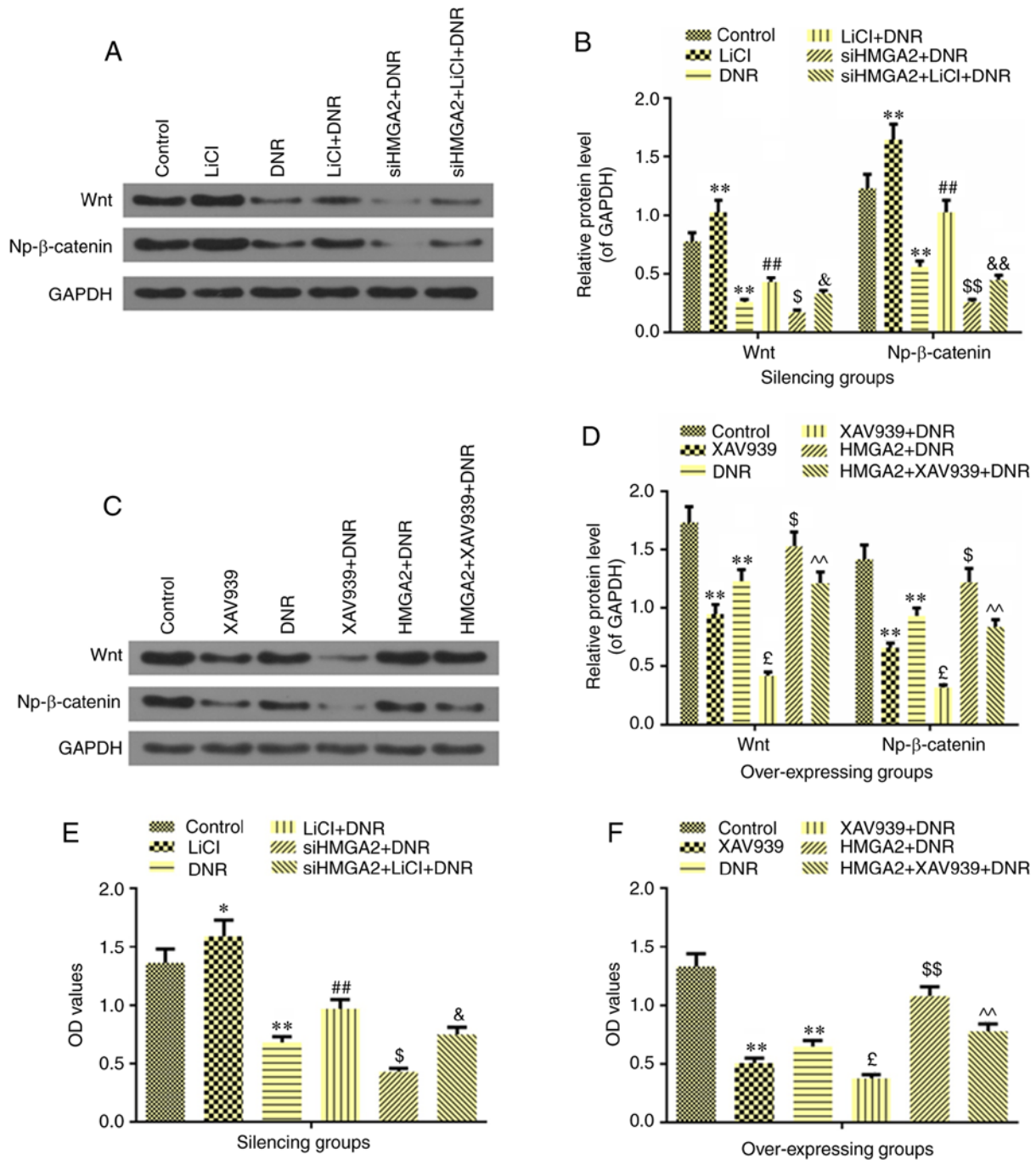
Figure 5. Effects of HMGA2 on the Wnt/β-catenin signaling pathway and cell viability following treatment with LiCl, XAV939 or DNR in AML HL60 and NB4 cells. (A) The effects of silencing HMGA2 on the protein expressions of Wnt and Np-β-catenin in AML HL60 cells treated with LiCl or DNR were determined by western blot analysis. (B) Relative protein levels in AML HL60 cells after silencing HMGA2. (C) The effects of overexpressing HMGA2 on the protein expressions of Wnt and Np-β-catenin in AML NB4 cells treated with XAV939 or DNR were determined by western blot analysis. (D) Relative protein levels in AML NB4 cells after HMGA2 overexpression. (E) The effects of silencing HMGA2 on the cell viability in AML HL60 cells treated with LiCl or DNR as determined by MTT assay. (F) The effects of overexpressing HMGA2 on the cell viability in AML NB4 cells treated with XAV9393 or DNR were evaluated by MTT assay. GAPDH was used as an internal control. Data were presented as the mean ± standard deviation from three independent experiments. *P<0.05 and **P<0.01 vs. Control; $^{##}$P<0.01 vs. LiCl; $^$$P<0.05 and $^{$$}$P<0.01 vs. DNR; $^&$P<0.05 and $^{&&}$P<0.01 vs. LiCl+DNR; $^£$P<0.05 vs. XAV939; $^{^^}$P<0.01 vs. XAV939+DNR. DNR, daunorubicin; AML, acute myelocytic leukemia; HMGA2, high-mobility group AT-hook 2 overexpression group; si-, small interfering RNA; NC, negative control; Np-β-catenin, non-phospho-β-catenin.

The molecular mechanism of the role of HMGA2 in the genesis and development of AML is not clearly defined. A previous study has suggested that HMGA2 could promote the growth of AML cells by regulating the Akt signaling (38). Tan *et al* (47) subsequently demonstrated that silencing HMGA2 induced the terminal differentiation of myeloid

leukemia primary blasts and cell lines. Ohshima *et al* (48) suggested that HMGA2 and the let-7 family were negatively regulated and were correlated with the invasiveness of gastric cancer. This negative regulatory effects contributed to tumorigenesis via the regulation of some molecular signaling pathways such as the growth factor signaling

pathway and Ras signaling pathway (48). Watanabe *et al* (49) believed that the upregulation of HMGA2 expression activated the Ras signaling pathway, leading to the development of pancreatic cancer. The Wnt signaling pathway not only played a key role in regulating embryonic development, but also its abnormal activation was closely associated with the progression of tumors (50-53). Therefore, the present study ultimately indicated that the Wnt/β-catenin pathway was the underlying mechanism. β-catenin cannot be degraded in the presence of Wnt signaling. Thus, a large number of free β-catenins accumulate in the cytoplasm and enter the nucleus in order to bind to the transcription factor T cytokine/lymphocyte enhancer, which initiates a series of downstream target molecules such as c-myc and cyclin D1 expression, thereby participating in cell proliferation and apoptosis (54). The present study revealed that silencing HMGA2 markedly inhibited Wnt and Np-β-catenin (active) protein levels of Wnt signaling and enhanced protein sensitivities to DNR; moreover, the activity of the Wnt signaling agonist LiCl was partially reversed in a previous study (55). To the best of our knowledge, the present study is the first to investigate the effect of HMGA2 on the regulation of Wnt/β-catenin in AML cells. However, in gastric cancer, Zha *et al* (56) had already confirmed that HMGA2 was conducive to EMT by activating Wnt/β-catenin signaling. Similarly, Wend *et al* (57) suggested that the Wnt10B/β-catenin signaling was closely associated with HMGA2 and promoted metastatic triple-negative breast cancer cell proliferation.

In conclusion, the present study demonstrated that HMGA2 played important roles in driving AML progression in HL60 and NB4 cells, potentially through the activation of the Wnt/β-catenin signaling pathway. In addition, it was revealed that HMGA2 could regulate AML cell sensitivity to DNR. As such HMGA2 may be a promising molecular marker for AML diagnosis.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SY made substantial contributions to the conception and design of the study. YG, QH, GW, SC, MZ and YW acquired, analyzed and interpreted the data. SY and YG drafted the article and revised it critically for important intellectual content. All authors gave final approval of the version to be published. All authors agree to be held accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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