

Small interfering-high mobility group A2 attenuates epithelial-mesenchymal transition in thymic cancer cells via the Wnt/ β -catenin pathway

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Received March 30, 2020; Accepted January 29, 2021

DOI: 10.3892/ol.2021.12847

Abstract. Thymus carcinoma is one of the thymic epithelial neoplasms with high metastasis, which does not have any good treatment at present. High mobility group A2 (HMGA2) is highly expressed in a variety of malignant tumors, such as lung cancer, colon cancer and ovarian cancer and is closely related to tumor invasion and metastasis. The present study aimed to investigate the effect and mechanism of HMGA2 on epithelial-mesenchymal transition (EMT) in thymic cancer cells. IU-TAB-1, A549, HCT-116 and 293T cells were screened by testing the protein expression level of HMGA2 through western blotting and subjected to HMGA2 interference [small interfering (si)-HMGA2]. Cell proliferation was evaluated using the Cell Counting Kit-8 assay. Cell migration and invasion were detected using the Transwell assay. Cell apoptosis was examined using flow cytometry and β -catenin expression was observed by immunofluorescence. The levels of E-cadherin, vimentin, Wnt3a, Wnt5a and β -catenin proteins were determined by western blotting. Among the four cell lines tested, IU-TAB-1 cells demonstrated the highest expression of HMGA2 ($P<0.05$) and were hence selected for subsequent experiments. Compared with the control group (untransfected cells), si-HMGA2 resulted in significantly decreased proliferation, migration and invasion of IU-TAB-1 cells, whereas apoptosis was increased ($P<0.05$). The protein expression of vimentin, Wnt3a, Wnt5a and β -catenin was significantly decreased by si-HMGA2 compared with the control group ($P<0.05$), whereas E-cadherin expression was increased ($P<0.05$). After treatment with si-HMGA2 in combination with Wnt/ β -catenin agonists (SKL2001) or inhibitors (XAV-939), EMT was respectively enhanced or inhibited in

IU-TAB-1 cells. Overall, si-HMGA2 may attenuate EMT in thymic cancer cells and the mechanism may be related to the Wnt/ β -catenin pathway.

Introduction

Thymic epithelial tumors are rare malignant tumors of thymic origin that are mainly classified as thymoma, thymic cancer and thymic neuroendocrine tumors (1). Thymic cancer accounts for 20% of thymic epithelial tumors and exhibits a strong metastatic behavior (1). The average age of onset of thymic cancer is ~50 years, with ~50-65% of patients displaying distant metastases on diagnosis in China from 1962-2003 (2). Late and poor prognosis, and intrathoracic and distant metastases are the main causes of death due to thymic cancer (3). Currently, there is no effective treatment for thymic carcinoma, and post-treatment recurrence and tumor metastasis are the most important factors affecting patient prognosis and quality of life (3,4). Hence, the discovery of biomarkers and understanding of the molecular mechanisms of tumor recurrence and metastasis are of great significance for the diagnosis and treatment of thymic cancer.

High mobility group (HMG) proteins are a series of chromatin-related proteins widely present in eukaryotes and consisting of 3 families: HMGA, HMGB and HMGN (5). HMGA2 belongs to the non-histone chromosome HMGA family that exerts its function as a transcription factor by altering the structure of chromatin and the interaction of DNA and target protein (6). HMGA2 is highly expressed in embryonic and immature tissues, but is almost absent in differentiated and mature tissues (7). Studies have found that HMGA2 is also highly expressed in a variety of malignant tumors, such as lung cancer, ovarian cancer and colorectal cancer (8-12), and its enhanced expression is closely related to increased tumor invasiveness and disease prognosis (8-12). Hence, HMGA2 is considered as a potential tumor marker for all types of cancer.

Epithelial-mesenchymal transition (EMT) refers to the loss of polarity, tight junctions and adhesion connections between cells, and it is an effective way for epithelial cells to acquire migration ability and is the phenomenon underlying epithelial cell carcinoma invasion and metastasis (13). EMT is

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Key words: high mobility group A2, epithelial-mesenchymal transition, β -catenin, thymic cancer cells

not only related to normal embryonic development, but is also closely related to tumorigenesis and tumor development (14). Studies have demonstrated that EMT serves a pivotal role in the primary invasion and secondary metastasis of numerous types of cancer, including breast, colon, lung, prostate and liver cancer (15-17). During the occurrence of EMT, epithelial cells lose their polarity and contact with surrounding stromal cells is reduced. Meanwhile, cell migration and motility are enhanced and cells take on a mesenchymal phenotype while losing their epithelial phenotype (13). EMT can be induced by a variety of signaling pathways, such as the transforming growth factor- β , Wnt and Notch pathways. Wnt signaling can induce EMT in tumor cells by inhibiting glycogen synthase kinase 3 β -mediated phosphorylation and β -catenin degradation in the cytoplasm (18,19).

The Wnt/ β -catenin signaling pathway participates in regulating embryonic development and plays an indispensable role in tumor generation and development (19). A study has demonstrated that the expression of factors associated with Wnt/ β -catenin in ovarian epithelial carcinoma tissues is significantly higher compared with that in benign ovarian tumor tissues (20). Huang *et al* (21) observed that the expression of Wnt/ β -catenin in nasopharyngeal tissues of patients with nasopharyngeal carcinoma was significantly higher compared with that in normal control tissues. The aforementioned finding suggests that the occurrence and development of nasopharyngeal carcinoma is highly associated with abnormal Wnt/ β -catenin signaling. To the best of our knowledge, few studies have investigated the association between HMGA2, WNT signaling and EMT in thymic cancer. Studies have reported that HMGA2 can affect the EMT of gastric cancer, tongue cancer and retinoblastoma by regulating the Wnt/ β -catenin signaling pathway (22-24). Our previous study found that with the increase in clinical staging of thymic cancer, the expression of β -catenin gradually increased, suggesting that Wnt/ β -catenin is closely related to thymic cancer (25). Hence, it was speculated that the abnormal expression of HMGA2 may activate the Wnt/ β -catenin pathway and promote tumorigenesis. The present study aimed to investigate the effect and mechanism of HMGA2 on EMT in thymic cancer cells. HMGA2 interference vectors [small interfering (si)-HMGA2] were constructed to study the effect of HMGA2 on EMT in thymic cancer cells. Further molecular experiments were performed to verify whether these effects were achieved via the Wnt/ β -catenin signaling pathway. The findings of the present study will provide some insights for the treatment of thymic cancer.

Materials and methods

Cell culture and transfection. IU-TAB-1, a thymic cancer cell line (cat. no. T8001), was obtained from Applied Biological Materials Inc. Cell lines 293T, A549 and HCT-116 (normal control cell lines) were kindly provided by the Stem Cell Bank of the Chinese Academy of Medical Sciences. IU-TAB-1, 293T, A549 and HCT 116 cells were cultured in RPMI 1640 (cat. no. SH30022.01B; Hyclone; Cytiva), Prigrow II (cat. no. TM002; Applied Biological Materials Inc.), F-12K (cat. no. 21127-022; Gibco; Thermo Fisher Scientific, Inc.) or McCoy's 5A (cat. no. 16600-082; Gibco; Thermo Fisher

Scientific Inc.) medium, respectively, supplemented with 10% fetal bovine serum (FBS; cat. no. 10270-106; Gibco; Thermo Fisher Scientific Inc.) in an atmosphere containing 5% CO₂ and 95% air at 37°C. The medium was replaced every 24 h and the cells were subcultured or cryopreserved in liquid nitrogen at -196.56°C when the confluence reached 70-80%.

The full-length cDNA of HMGA2 (NM_001300918.1) was obtained from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/search/all/?term=NM_001300918.1). IU-TAB-1 cells were transfected with HMGA2 siRNA using Lipofectamine[®] 2000 reagent (cat. no. 13778030; Invitrogen; Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. Briefly, 100 pmol siRNA and 5 μ l Lipofectamine[®] RNAiMAX were added to 250 μ l Opti-MEM at 4°C for 20 min, respectively, and then the above mixture was incubated at 37°C in a 5% CO₂ incubator for 48 h. The cell experiments were divided into 5 groups: i) Control (untransfected cells); ii) HMGA2-siRNA1; iii) HMGA2-siRNA2; iv) HMGA2-siRNA3; and v) non-targeting negative control. The transfection efficiency was detected by reverse transcription-quantitative PCR (RT-qPCR). Wnt signaling antagonist (XAV-939; cat. no. HY-15147; 4 μ M) and agonist (SKL2001; cat. no. HY-101085; 30 μ M) were purchased from MCE. XAV-939 and SKL2001 were added after the cells were stably transfected for 48 h and cultured in a constant temperature incubator at 37°C for 48 h.

Cell Counting Kit-8 assay. IU-TAB-1 cells were seeded in a 96-well plate at a density of 5x10³ cells/ml in Prigrow II medium containing 10% FBS. Following 48 h of treatment, 10 μ l Cell Counting Kit-8 (CCK-8) solution (cat. no. C1706; Bioswamp Wuhan Biele Biotechnology, Co., Ltd.) was added to each well and the cells were cultured at 37°C for 4 h. Cell proliferation was examined by measuring the optical density at 450 nm using a plate reader (Multiskan FC; Thermo Fisher Scientific Inc.).

Transwell migration and invasion assays. IU-TAB-1 cells were cultured in serum-free Prigrow II medium for 24 h and resuspended in Prigrow II medium containing 1% FBS. Subsequently, the cells were seeded into Transwell chambers at 1x10⁵ cells/ml, while 0.75 ml of Prigrow II medium containing 10% FBS was added into the lower chambers. The plate was incubated in 5% CO₂ at 37°C for 48 h. Subsequently, 1 ml of 4% formaldehyde solution was added to each well, and the plate was incubated at 4°C for 10 min for immobilization. Following 30 min of incubation at room temperature with 0.5% crystal violet solution (cat. no. C1701; Bioswamp Wuhan Biele Biotechnology Co. Ltd.), the cells were observed under a fluorescent microscope (magnification, x200).

For cell invasion assay, the upper chambers were pre-coated with 80 μ l of Matrigel (cat. no. 356234; BD Biosciences). The chambers were incubated at 37°C for 30 min for gel formation and hydrated in 1% FBS for 4 h before use. In the lower chambers, 750 μ l Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS was added. Subsequently, IU-TAB-1 cells were added to the upper chambers at a density of 1x10⁵ cells/well and incubated for 48 h at 4°C. Next, 1 ml of 4% paraformaldehyde (cat. no. 10010018; Sinopharm

Table I. Primer sequences used for reverse transcription-quantitative PCR.

Primer	Sequence (5'-3')
HMGA2-F	TTCAGCCCAGGGACAA
HMGA2-R	CCAGGCAAGGCAACAT
GAPDH-F	CCACTCCTCCACCTTTG
GAPDH-R	CACCACCCTGTTGCTGT

F, forward; R, reverse; HMGA2, high mobility group A2.

Chemical Reagent, Co. Ltd.) and 1 ml of 0.5% crystal violet (cat. no. C1701; Bioswamp Wuhan Bienle Biotechnology Co. Ltd.) were added at room temperature, and the invaded cells were counted under a fluorescent microscope.

Flow cytometry. Both early and late stages of apoptosis rate of IU-TAB-1 cells was analyzed using flow cytometry according to the manufacturer's instructions. IU-TAB-1 cells (1×10^6 /ml) were cultured for 24 h in 37°C and harvested. Subsequently, 1 ml of pre-cooled PBS was added and the cells were centrifuged at 1,000 x g. Then, 10 μ l of Annexin V-FITC (cat. no. 556547; BD Biosciences) and 10 μ l of PI (cat. no. 556547; BD Biosciences) were added. The data were analyzed using CytExpert software v.2.0 (Beckman Coulter, Inc.).

Immunofluorescence. IU-TAB-1 cells (1×10^6) were fixed in 4% paraformaldehyde for 30 min at room temperature. After washing twice with pre-cooled phosphate-buffered saline (PBS), the cells were permeabilized in 5% Triton X-100 (cat. no. CB1701; Bioswamp Wuhan Bienle Biotechnology Co. Ltd.) for 20 min and blocked with 5% bovine serum albumin at 37°C for 1 h. The cells were then incubated with antibodies against β -catenin (1:200; cat. no. MAB37201; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.) overnight at 4°C, followed by incubation with Alexa Fluor 594-conjugated Goat Anti-Rabbit (1:200; cat. no. PAB160018; Bioswamp Wuhan Bienle Biotechnology Co. Ltd.) for 30 min at room temperature and counterstained with 4',6-diamidino-2-phenylindole to identify the nuclei at 4°C for 5 min. Images were captured with a fluorescence microscope (DMIL LED; Leica Microsystems GmbH).

RT-qPCR. Total RNA was extracted from the cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific Inc.) according to the manufacturer's instructions. cDNA was synthesized using a reverse transcriptase kit (cat. no. 639505; Takara Bio Inc.). qPCR was performed with a CFX-Connect 96 real-time system (Bio-Rad Laboratories Inc.) using the SYBR Green PCR kit (cat. no. KM4101; KAPA Biosystems; Roche Diagnostics). qPCR was performed in duplicate and the thermocycling conditions were as follows: 95°C for 3 min for denaturation; 39 cycles of denaturation at 95°C for 5 sec, 56°C for 10 sec and 72°C for 25 sec; and 65°C for 5 sec and 95°C for 50 sec for annealing and extension. The results were analyzed by the $2^{-\Delta\Delta C_q}$ method (26). GAPDH was used as the reference gene. The primers were designed and configured by Wuhan

Tianyi Huayu gene Biotechnology Co. Ltd. and are listed in Table I.

Western blotting. Following 48 h of treatment, the IU-TAB-1 (1×10^6 /ml) cells were washed with cold PBS and lysed using a lysis buffer (cat. no. 180006; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.), and the proteins were quantified by the bicinchoninic acid assay kit (cat. no. 180007; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.). The proteins (20 μ g protein per lane) were separated on a 12% gel using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membranes were blocked with a buffer containing 5% skimmed milk in PBS with 0.05% Tween-20 for 2 h at room temperature and incubated overnight at 4°C with primary antibodies against HMGA2 (1:1,000; cat. no. PAB40807; Bioswamp Wuhan Bienle Biotechnology Co. Ltd.), Wnt3a (1:1,000; cat. no. PAB30170; Bioswamp Wuhan Bienle Biotechnology Co. Ltd.), Wnt5a (1:1,000 cat. no. PAB37965; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.), β -catenin (1:1,000; cat. no. MAB37201; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.), E-cadherin (1:1,000, cat. no. PAB33542; Bioswamp Wuhan Bienle Biotechnology Co. Ltd.), vimentin (1:1,000; cat. no. PAB40646; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.) and GAPDH (1:2,000; cat. no. PAB36264; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.). Following 3 washes with PBS/10% Tween-20, the membranes were incubated with horseradish peroxidase-conjugated secondary goat rabbit IgG (1:20,000; cat. no. PAB160011; Bioswamp Wuhan Bienle Biotechnology Co., Ltd.) for 2 h at room temperature. Protein bands were visualized by enhanced chemiluminescence color detection (Tanon-5200; Tanon Science and Technology Co., Ltd.) and analyzed using GIS software v.4.2 (Tanon Science and Technology Co., Ltd.).

Statistical analysis. All data are presented as the mean \pm standard deviation. Statistical analysis was performed by one-way analysis of variance followed by the post-hoc Tukey's post-hoc test using SPSS 19.0 software (IBM Corp.). All figures were prepared using GraphPad Prism 5.0 software (Graph Pad Software Inc.). $P < 0.05$ was considered to indicate statistical significance and all statistical analyses were based on 3 independent experiments.

Results

HMGA2 expression in IU-TAB-1, A549, HCT-116 and 293T cells. As shown in Fig. 1, compared with that in 293T cells, the protein expression of HMGA2 was significantly increased in IU-TAB-1, A549 and HCT-116 cells ($P < 0.05$), with IU-TAB-1 cells demonstrating the highest expression. Hence, IU-TAB-1 cells were used for subsequent experimentation.

Controls for interference expression. mRNA expression of HMGA2 in the control, HMGA2-siRNA1, HMGA2-siRNA2, HMGA2-siRNA3 and negative control groups was observed to confirm HMGA2 inhibition by the interference vector (Fig. 2). The results confirmed that all 3 HMGA2 siRNAs significantly downregulated the expression of HMGA2 compared with the control and negative control IU-TAB-1 cells ($P < 0.05$; Fig. 2).

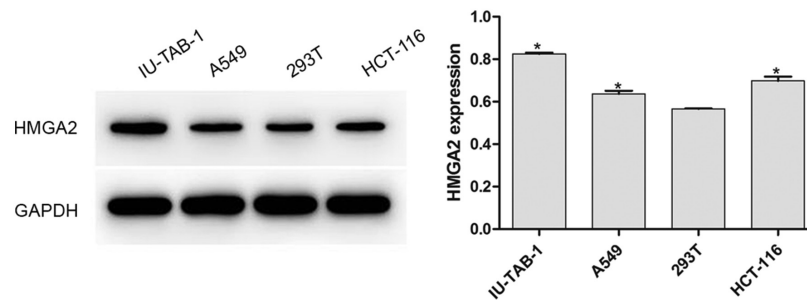


Figure 1. HMGA2 protein expression assessed by western blotting in IU-TAB-1, A549, HCT-116 and 293T cells. Western blotting (A) representative image and (B) quantification of HMGA2 expression (n=3). *P<0.05 vs. 293T cells. HMGA2, high mobility group A2.

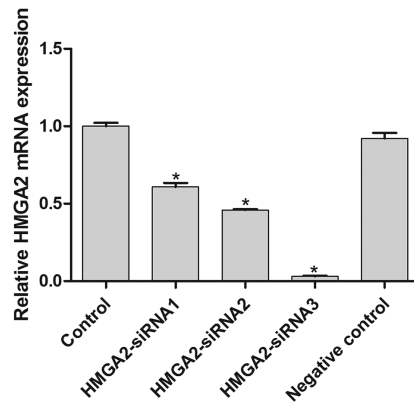


Figure 2. Transfection efficiency of HMGA2 siRNA transfected in IU-TAB-1 cells. Statistical analysis of HMGA2 detected by RT-qPCR (n=3). *P<0.05 vs. control. The full-length cDNA of HMGA2 (NM_001300918.1) was obtained from the NCBI database and IU-TAB-1 cells were transfected with HMGA2 siRNA. HMGA2, high mobility group A2; RT-q, reverse transcription-quantitative; si, small interfering; control, untransfected IU-TAB-1 cells.

HMGA2-siRNA3 induced the lowest HMGA2 expression among the 3 siRNAs and was hence used to silence HMGA2 in the subsequent experiments.

si-HMGA2 attenuates EMT in IU-TAB-1 cells. To observe the effect of HMGA2 on cell proliferation, migration and invasion, CCK-8 and Transwell assays were performed (Fig. 3A-C). Compared with the control, si-HMGA2 significantly suppressed cell proliferation, migration and invasion (P<0.05; Fig. 3A, B and D). Flow cytometry revealed that si-HMGA2 increased the apoptotic rate of IU-TAB-1 cells compared with control and negative control cells (Fig. 3D). The expression of EMT-related proteins (E-cadherin, vimentin, Wnt3a, Wnt5a, and β -catenin) was further assessed (Fig. 3E). The protein expression of vimentin, Wnt3a, Wnt5a and β -catenin were significantly decreased by si-HMGA2 (P<0.05; Fig. 3E), whereas that of E-cadherin was increased significantly compared with the control group (P<0.05; Fig. 3E), which indicated that HMGA2 silencing inhibited EMT in IU-TAB-1 cells.

si-HMGA2 attenuates EMT in IU-TAB-1 cells via the Wnt/ β -catenin pathway. Immunofluorescence was conducted to observe the protein expression of β -catenin. Fig. 4A reveals that si-HMGA2 induced lower expression of β -catenin compared with that in the control and negative control

groups. Proteins associated with the Wnt/ β -catenin pathway were subsequently assessed using western blotting, which demonstrated that si-HMGA2 significantly downregulated the expression of Wnt3a, Wnt5a, vimentin and β -catenin compared with the control group (P<0.05; Fig. 4B), and upregulated the levels of E-cadherin, suggesting that si-HMGA2 inhibited the activation of Wnt/ β -catenin signaling. To further study whether si-HMGA2 affects EMT in IU-TAB-1 cells by regulating the Wnt/ β -catenin pathway, Wnt/ β -catenin agonists or inhibitors were applied (Fig. 4B and C). Compared with the control and NC, the migration, invasion and expression of vimentin of cells treated with Wnt/ β -catenin agonists were increased (P<0.05; Fig. 4B and C), whereas E-cadherin expression was significantly decreased (P<0.05; Fig. 4B and C). On the other hand, the combination of si-HMGA2 with Wnt/ β -catenin inhibitors suppressed the migration, invasion, and expression of vimentin of IU-TAB-1 cells (P<0.05; Fig. 4B and C), whereas E-cadherin expression was increased significantly compared to the control and NC group (P<0.05; Fig. 4B and C).

Discussion

The human HMGA2 gene is located on the chromosome band 12q14-15 and contains at least 5 exons distributed in the ≥ 160 -kb genomic region (6). HMGA2 is almost undetectable in healthy adult tissues; however, HMGA2 upregulation has been detected in breast cancer (27), sarcoma (28), pancreatic cancer (29) and non-small cell lung cancer (30) tissues. Whether HMGA2 is also upregulated in thymic cancer has not been studied to the best of our knowledge. IU-TAB-1, A549 and HCT-116 are common tumor cell lines (31-33). Studies have demonstrated that HMGA2 is highly expressed in a variety of malignant tumors, including lung, colon and ovarian cancer (8-12), and its enhanced expression is closely related to enhanced tumor aggressiveness and disease prognosis (8-12). Hence, we speculated that the occurrence and development of thymic cancer is also related to the abnormally high expression of HMGA2. Hence, in the present study, A549, HCT-116 and 293T cells were used as controls. The results of the present study demonstrated that HMGA2 expression in IU-TAB-1 cells was significantly higher compared with that in A549 and HCT-116 cells, which suggested that HMGA2 expression was significantly increased in thymic cancer cells.

E-cadherin loss is a prominent feature of cellular EMT (34). Decreased E-cadherin levels can lead to reduced cell adhesion and allow cells to acquire characteristics that enable invasion

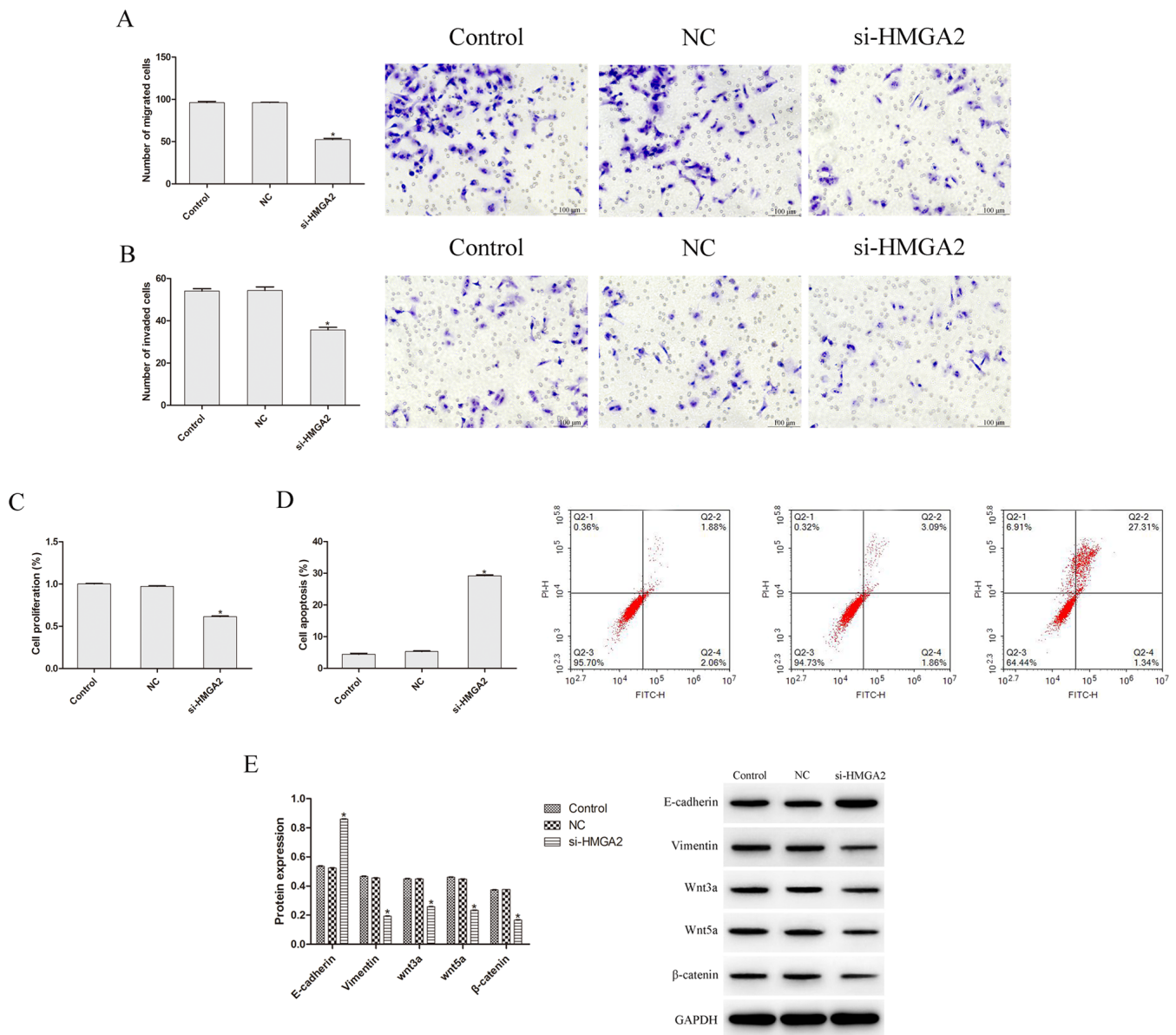


Figure 3. Effects of si-HMGA2 on EMT in IU-TAB-1 cells. (A-C) si-HMGA2 inhibited the proliferation, migration and invasion of IU-TAB-1 cells (magnification, x200) (n=3). *P<0.05 vs. control. (D) Cell apoptosis was detected by flow cytometry (n=3). *P<0.05 vs. control. (E) EMT-related proteins were detected by western blotting (n=3). *P<0.05 vs. control. HMGA2, high mobility group A2; si, small interfering; EMT, epithelial-mesenchymal transition; PI, propidium iodide; NC, negative control; control, untransfected IU-TAB-1 cells.

and metastasis (35). Studies have reported mutations in the E-cadherin gene or downregulation of E-cadherin expression in lung, breast, gastric and other epithelial cancer types (36,37). Vimentin is one of the main components of the medium fibers of fibroblasts (38). When E-cadherin is lost, the expression of vimentin and N-cadherin increases and cells acquire an interstitial phenotype (39). The present study demonstrated that HMGA2 silencing upregulated the expression of E-cadherin and downregulated that of vimentin, which suggested that inhibition of HMGA2 suppressed EMT in thymic cancer cells. Cell infiltration is an important feature of malignant tumors, wherein EMT serves an important role (13). Cells undergoing EMT can grow on and penetrate Matrigel, revealing that EMT may be an important factor for tumor cells to break through the basement membrane (40). In clinical treatment, tumor cells metastasize to other sites through blood vessels and lymphatic

vessels, representing further tumor deterioration and a poor clinical prognosis. E-cadherin expression is inversely related to the degree of tumor differentiation and lymph node metastasis (39). After injection of E-cadherin-deficient tumor cells into nude mice, carcinogenicity and metastasis were significantly enhanced (41). In the present study, HMGA2 silencing inhibited the proliferation, migration and invasion of thymic cancer cells, while promoting cell apoptosis. In the present study, further evaluation of the expression of EMT-associated proteins demonstrated that when HMGA2 was suppressed, E-cadherin was upregulated and vimentin was downregulated, which suggested that inhibition of HMGA2 suppressed EMT in thymic cancer cells.

β -catenin is the core molecule of the Wnt pathway and its accumulation in the cytoplasm is the key to Wnt/ β -catenin activation (42). When Wnt signaling is activated, the

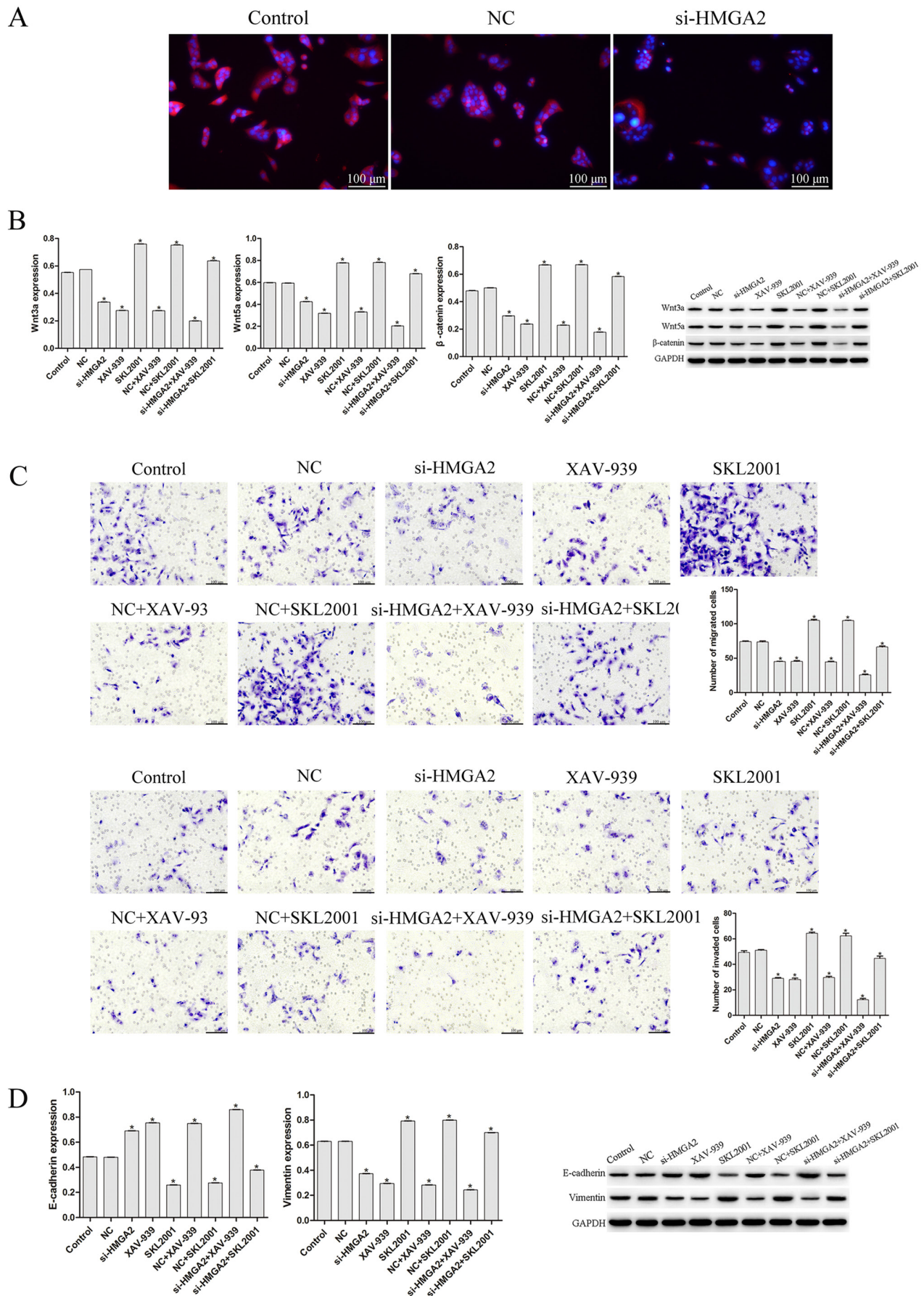


Figure 4. si-HMGA2 attenuates EMT in IU-TAB-1 cells via the Wnt/ β -catenin pathway. (A) β -catenin expression was observed by immunofluorescence (scale bar, 100 μ m). (B) Protein expression of Wnt3a, Wnt5a, β -catenin, E-cadherin and vimentin was measured by western blotting. (C) Effect of si-HMGA2 in combination with Wnt/ β -catenin pathway agonists (SKL2001) or inhibitors (XAV-939) on IU-TAB-1 cell migration and invasion (n=3). *P<0.05 vs. control. HMGA2, high mobility group A2; si, small interfering; EMT, epithelial-mesenchymal transition; NC, negative control; Control, IU-TAB-1 cells.

Wnt protein binds to the extracellular domain of frizzled protein. β -catenin cannot be degraded, and a large amount of free β -catenin accumulates in the cytoplasm, enters the nucleus and combines with the transcription factor T cytokine/lymphocyte enhancing factor to regulate cell proliferation and apoptosis (43). Qin *et al* (44) demonstrated that the expression of β -catenin in SNK-6 and YTS cell lines was significantly higher compared with that in normal natural killer cells and was significantly higher in natural-killer/T cell lymphoma tissues compared with reactive hyperplasia of lymph nodes. Ebert *et al* (45) demonstrated that β -catenin expression was increased in gastric cancer tissues, and a β -catenin gene mutation was detected compared to the normal gastric tissue. Shi and Yin (46) studied the expression of β -catenin and hepatocyte nuclear factor-1 α in hepatocellular carcinoma tissues and their effects on the prognosis of hepatocellular carcinoma. The results of the aforementioned study revealed that the clinical prognosis of patients with abnormal β -catenin expression was significantly worse compared with that of patients with normal β -catenin expression, which suggested that abnormal expression of β -catenin was related to the development of hepatocellular carcinoma (46). The findings of the present study revealed that when the expression of HMGA2 was silenced, β -catenin was downregulated, suggesting that HMGA2 inhibition reduced β -catenin accumulation of thymic cancer cells. The present study further examined the effect of si-HMGA2 on the Wnt/ β -catenin signaling pathway and demonstrated that HMGA2 silencing inhibited Wnt/ β -catenin signaling. This finding indicated that the effect of HMGA2 on EMT in thymic cancer cells may be achieved by regulating the activity of Wnt/ β -catenin signaling. Inhibiting HMGA2 activity inhibited the EMT activity of HMGA2 thymogenic cancer cells in the present study. In the present study, cells were treated with si-HMGA2 in combination with Wnt/ β -catenin agonists (SKL2001) or inhibitors (XAV-939), and the changes in EMT in thymic cancer cells were observed. In the present study, cell migration and invasion and vimentin expression were significantly enhanced by Wnt/ β -catenin agonists compared with that in control cells, while the expression of E-cadherin was significantly decreased. Correspondingly, Wnt/ β -catenin inhibitors demonstrated the opposite effect. These findings demonstrated that HMGA2 inhibition suppressed Wnt/ β -catenin activation and inhibited EMT in thymic cancer cells, providing a potential therapeutic strategy for the clinical treatment of thymic cancer.

The present study had several limitations. Firstly, E-cadherin is the only *bona fide* Wnt target investigated in the present study, hence the association between HMGA-2 and Wnt signaling requires further investigation. T cell factor/lymphoid enhancer factor activity should be detected in future studies. Secondly, the use of just one thymic cell line is a limitation of the present study. More cell lines and clinical samples should be investigated in future studies to verify the findings of the present study.

In conclusion, HMGA2 may be a key protein that regulates EMT in thymic cancer cells. In the present study, inhibition of HMGA2 significantly attenuated cell proliferation, migration and invasion, and promoted apoptosis, and this mechanism may be related to Wnt/ β -catenin signaling.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The data used in this study are available from the corresponding author upon request.

Authors' contributions

ST and JC performed the experiments, collected and analyzed the data. ST drafted the manuscript. JC revised the manuscript for important intellectual content. ST and JC confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

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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