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High Glucose Promotes Epithelial-Mesenchymal Transition, Migration and Invasion in A20 Murine Diffuse Large B-Cell Lymphoma Cells Through Increased Expression of High Mobility Group AT-Hook 2 (HMGA2)

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Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: Patients with type 2 diabetes mellitus have been reported to be at increased risk of developing non-Hodgkin's lymphoma (NHL). Diffuse large B-cell lymphoma (DLBCL) is the most common type of high-grade NHL. This study aimed to investigate the effects of high glucose on cell migration, invasion and epithelial-mesenchymal transition (EMT), and the expression of high mobility group AT-hook 2 (HMGA2) in A20 murine DLBCL cells.

Material/Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot were used to analyze the expression of HMGA2 at the gene and protein level and EMT markers in the A20 murine DLBCL cell line. A transwell assay evaluated cell migration and invasion of A20 cells. Short-interfering RNA (siRNA) was used to knockdown HMGA2 expression.

Results: High glucose levels upregulated the expression of HMGA2, induced phenotypic changes of EMT, and increased cell migration and invasion in A20 cells. Knockdown of HMGA2 by siRNA effectively inhibited EMT induced by high glucose in A20 cells by directly regulating the Wnt/ β -catenin signaling pathway.

Conclusions: In the A20 murine DLBCL cell line, high glucose upregulated the expression of HMGA2 to induce EMT and promote cell migration and invasion through the Wnt/ β -catenin signaling pathway.

MeSH Keywords: Epithelial-Mesenchymal Transition • High Mobility Group Proteins • Hyperglycemia • Lymphoma, Large B-Cell, Diffuse

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Background

Diffuse large B-cell lymphoma (DLBCL) is the most common type of high-grade non-Hodgkin's lymphoma (NHL) the incidence has been rising [1]. Type 2 diabetes mellitus (T2DM) is a risk factor for several malignant tumors [2,3]. Also, patients with T2DM have been shown to have an increased incidence of NHL, suggesting that hyperglycemia might have a role in the development of lymphoma [4]. However, the molecular mechanisms involved in the association between hyperglycemia and DLBCL remains unknown.

Recent studies have investigated the role of epithelial-mesenchymal transition (EMT) in tumor progression, invasion, and metastasis [5,6]. During EMT, cells lose their adherence junctions, undergo morphological changes, and acquire mesenchymal features. EMT results in the upregulation of mesenchymal markers, including N-cadherin, and down-regulation of key adherence proteins, including E-cadherin, which enhance the invasive and migratory properties of tumor cells [7]. High glucose has been reported to induce EMT and to promote invasion in colon cancer, pancreatic cancer, and breast cancer cells [8–10]. However, it remains unclear whether hyperglycemia has a role in regulating EMT in DLBCL cells.

High mobility group protein A2 (HMGA2) is a key transcription regulator that belongs to the HMGA family that contains the functional sequences called AT-hooks. HMGA2 is abundantly expressed in a variety of tumors, and recent studies have suggested an important role for HMGA2 in the progression of EMT as well as cancer invasion and metastasis [11]. HMGA2 regulates the action of transcription factors responsible for EMT by binding to the specific AT-rich sequences in DNA and altering the structure of chromatin [12,13]. Also, HMGA2 is involved in high glucose-induced EMT in colorectal cancer cells [8]. However, the functional role of HMGA2 during the process of EMT in DLBCL remains unknown.

Therefore, this study aimed to investigate the effects of high glucose on cell migration, invasion and epithelial-mesenchymal transition, and the expression of high mobility group AT-hook 2 (HMGA2) in A20 murine DLBCL cells.

Material and Methods

Cell culture

A20 murine diffuse large B-cell lymphoma (DLBCL) cells (ATCC, Manassas, VA, USA) were routinely cultured in complete RPMI-1640 medium (Gibco, Thermofisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), and maintained at 37°C in a humidified atmosphere containing

5% CO₂. A20 cells were passaged when they were 80–90% confluent. For glucose stimulation experiments, cells were cultured in increasing concentrations of glucose at 5 mM, 25 mM, and 30 mM (Sigma-Aldrich, St. Louis MO, USA).

Knockdown of HMGA2 by short-interfering RNA (siRNA)

Knockdown of HMGA2 in A20 cells was conducted using siRNA. Cells were transfected with a murine HMGA2-targeting siRNA (siHMGA2) or a corresponding negative control siRNA purchased from RiBoBio Co. (RiBoBio, Guangzhou, China) using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). Transient transfection of the siRNAs was performed at a final concentration of 50 nM according to the manufacturer's instructions. At 48 hours after transfection, cells were harvested for further analysis and the knockdown efficiency was assessed by quantitative real-time PCR (qRT-PCR) and Western blot.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from A20 cells using the TRIzol RNA extraction reagent following the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The RNA quality was determined using a NanoDrop ND-2000 spectrophotometer (Thermofisher Scientific, Waltham, MA, USA). A specific M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) was used for reverse transcription reactions into cDNA. For the subsequent qRT-PCR assay, a Roche LightCycler 480 system (Roche, Basel, Switzerland) was used with a SYBR Premix system (Takara, Minato-ku, Tokyo, Japan), according to the manufacturer's instructions. The gene-specific primer sequences are shown in Table 1. Relative quantification of the target genes was conducted in accordance with the comparative 2^{-ΔΔCt} method, and β-actin or GAPDH was used as the internal control. Each experiment was performed in triplicate.

Western blot

A20 cells were harvested and total protein was extracted by using a specific RIPA Buffer (Beyotime, Shanghai, China), and protein concentration was determined using the BCA protein kit (Beyotime, Shanghai, China), according to the manufacturer's instructions. Equal amounts of protein (30–50 μg) were then loaded into a 10–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels (Bio-Rad, Hercules, CA, USA), and transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore, Burlington, MA, USA). The membranes were then blocked with 5% dried skimmed milk powder at 25°C for 1 hour and probed with primary antibodies at 4°C overnight. The following primary antibodies were used: anti-HMGA2 (1: 500) (Santa Cruz Biotechnology Inc., Dallas, TX, USA), anti-N-cadherin (1: 1000) (Thermofisher Scientific, Waltham, MA, USA), anti-Snail (1: 1000) (Abcam, Cambridge, MA, USA),

Table 1. Primer sequences for qRT-PCR assay.

| Gene | Forward primer | Reverse primer |
|-------------------|----------------------------|-------------------------------|
| Ms HMGA2 | 5'-TGAGTTTGGAGAACGCACCA-3' | 5'-TGAGAGTGGGAAGCGATGAGC-3' |
| Ms E-Cad | 5'-AGGCCAGAGTTTACCCAGGA-3' | 5'-TGACGATGGTGTAGGCGATG-3' |
| Ms N-Cad | 5'-GGCCTGTTCTTGCAGCAC-3' | 5'-ACACAACACAGACGCAAAGC-3' |
| Ms snail | 5'-TACCTCCCCATCCTCGCTGG-3' | 5'-TACAAAGGCACTCCATCAGCC-3' |
| Ms slug | 5'-TCCTGGTGCCTGTCCTCA-3' | 5'-TCAGTTCATCACTACACAAGTCA-3' |
| Ms β -actin | 5'-AGCTGAGAGGGAATCGTGC-3' | 5'-GAACCGCTCGTTGCCAATAG-3' |
| Ms GAPDH | 5'-TACTGGAACATGGTCTGGCG-3' | 5'-AGTGACCATAGGTGTGTGGGT-3' |

Ms – mouse.

anti-E-cadherin (1: 500) (BD, USA), anti- β -catenin (1: 500) (Merck Millipore, Burlington, MA, USA), anti-c-myc (1: 800) (Santa Cruz Biotechnology Inc., Dallas, TX, USA), anti-cyclin D1 (1: 1000) (Cell Signaling Technology, USA), anti-slug (1: 500) (Cell Signaling Technology, USA), and anti-GAPDH (1: 500) (ABclonal, Shanghai, China). After washing thoroughly, the PVDF membranes were incubated with the corresponding fluorescent secondary antibodies (LI-COR Biosciences, Lincoln, NE, USA) at room temperature for 1 hour. Following several washes with TBST, the membranes were scanned, and images were obtained using the Odyssey Imaging System (LI-COR Biosciences, Lincoln, NE, USA). ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used for quantitative analysis, and GAPDH was used as the internal reference. Each experiment was performed in triplicate.

Migration and invasion assays

The cell migration assay of A20 cells was performed using transwell chambers (Merck Millipore, Burlington, MA, USA). For the transwell migration assay, cells at a density of 5×10^5 per well in serum-free RPMI-1640 medium were seeded into the upper chambers of the 24-well plates with a non-coated membrane. RPMI-1640 medium with 10% FBS and different concentrations of glucose were added to the lower chambers as the chemoattractant. After incubation for 24 hours at 37°C, the cells in the lower chambers were fixed with methanol and then stained with 0.2% crystal violet (Beyotime, Shanghai, China) and counted. The invasion assay was conducted in a similar way but the membrane inserts applied were coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis

Data were analyzed using SPSS version 20.0 software (IBM, Chicago, IL, USA) and GraphPad Prism version 6.0 software (GraphPad Software, La Jolla, CA, USA). Data were presented as the mean \pm standard deviation (SD). The statistical significance

was assessed using Student's t-test for two independent groups and one-way analysis of variance (ANOVA) for three or more independent groups. A P-value <0.05 was considered to be with statistically significant.

Results

High glucose upregulated the expression of HMGA2 and induced epithelial-mesenchymal transition (EMT) in A20 murine diffuse large B-cell lymphoma (DLBCL) cells

To investigate the effects of glucose on the expression of HMGA2 and the process of epithelial-mesenchymal transition (EMT), the A20 cells were treated with different concentrations of glucose, including normal glucose (NG) at 5 mM, high glucose-1 (HG-1) at 25 mM, and high glucose-2 (HG-2) at 30 mM for 48 hours. The glucose concentration at 5 mM in the culture media was equivalent to the normal physical level in human blood, whereas 25 mM or 30 mM of glucose corresponded with the levels in patients with diabetes mellitus [14]. The results showed that the expression of HMGA2 mRNA was significantly increased in A20 cells treated with 25 mM and 30 mM of glucose compared with those cultured with 5 mM of glucose (Figure 1A). Treatment with high glucose (30 mM) upregulated the expression of HMGA2 at the protein level in A20 cells (Figure 1B).

We next investigated the expression of EMT markers in A20 cells by using quantitative real-time polymerase chain reaction (qRT-PCR) and Western blot. The results of qRT-PCR indicated that high glucose (30 mM) significantly increased the expression of the mesenchymal phenotype marker N-cadherin and key transcription factors of EMT including snail and slug. However, the expression of the epithelial marker E-cadherin was reduced when compared with the NG group (Figure 1C). Western blot confirmed that stimulation of high glucose (30 mM) in A20 cells resulted in the upregulation of N-cadherin and snail proteins,

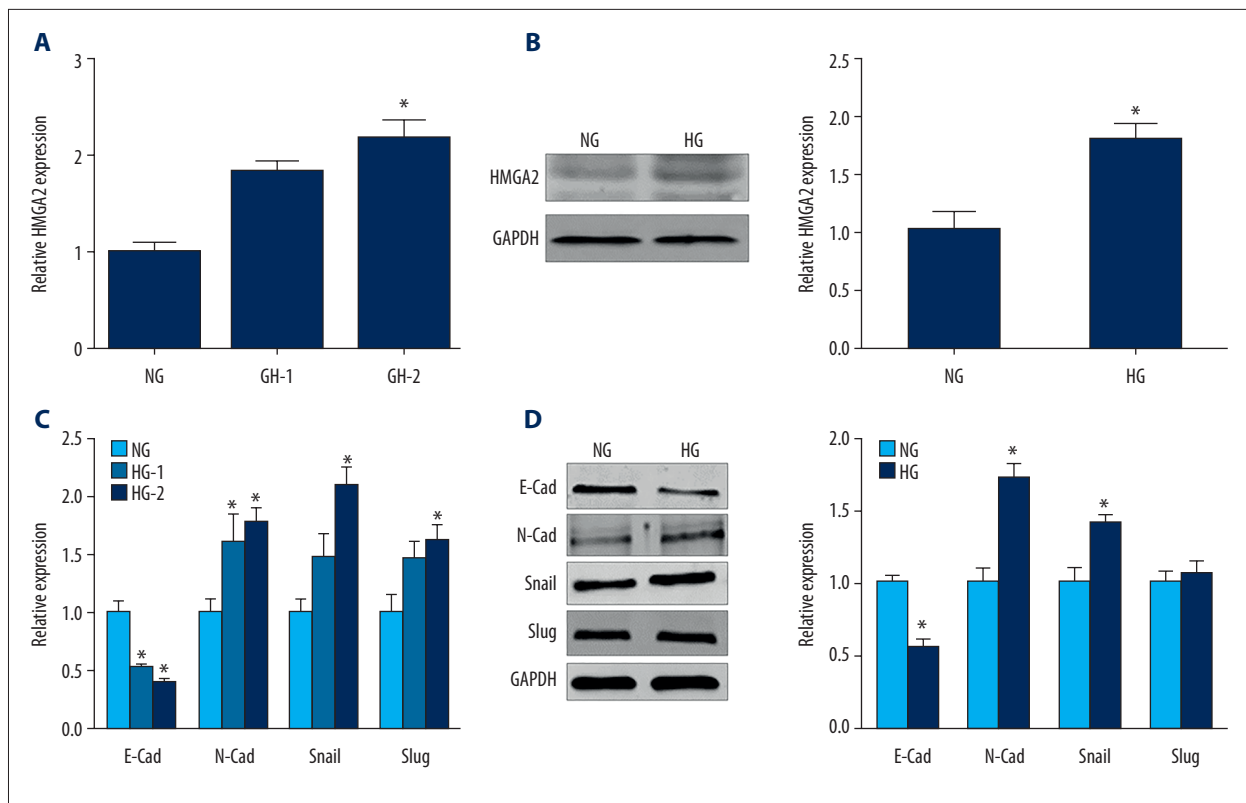


Figure 1. Effects of glucose on the expression of HMGA2 and markers of epithelial-mesenchymal transition (EMT) in A20 murine diffuse large B-cell lymphoma (DLBCL) cells. A20 cells in RPMI-1640 medium and 10% fetal bovine serum (FBS) were treated with different concentrations of glucose, including normal glucose (NG), 5mM, high glucose-1 (HG-1) 25mM, and high glucose-2 (HG-2), 30 mM. Gene expression was analyzed 48 hours later by quantitative real-time polymerase chain reaction (qRT-PCR) for HMGA2 (A) and EMT markers including E-cadherin, N-cadherin, snail, and slug (C). The protein levels of HMGA2 (B) and EMT markers (D) in A20 cells treated with high glucose (30 mM) and normal glucose (5 mM) were assessed by Western blot. Quantification analysis by ImageJ software was performed, as shown as a graph on the right. GAPDH was used as an internal reference. Each experiment was performed in triplicate, and the representative images are shown. Data are presented as the mean \pm standard deviation (SD). * $P < 0.05$, compared with the Control (normal glucose) group.

and down-regulation of the key adherence protein E-cadherin, which was consistent with the results of qRT-PCR (Figure 1D). These data indicated that A20 cells treated with high glucose had undergone EMT marked by phenotypic transition.

High glucose induced cell migration and invasion of A20 cells

The transwell assay analyzed the effects of high glucose on the migration of A20 cells. The findings showed that A20 cells treated with high glucose concentrations (30 mM) showed an enhanced migratory capacity compared with the normal glucose group (Figure 2A). Cells cultured with high glucose (30 mM) also had significantly increased invasion when compared with those grown in normal concentrations of glucose (Figure 2B).

Knockdown of HMGA2 inhibited high glucose-induced EMT in A20 cells

Previous studies have reported that HMGA2 was involved in the process of EMT as a transcription regulator [15]. Short-interfering RNA (siRNA) was used to knockdown HMGA2 to further validate its functional role in high glucose-induced EMT in A20 cells. Knockdown of HMGA2 resulted in a significant decrease in HMGA2 expression compared with the normal control (NC) group, and also reduced high glucose-induced upregulation of HMGA2 both at gene and protein level compared with the NC+HG group (Figures 3A, 4A).

Knockdown of HMGA2 significantly reduced the expression of mesenchymal phenotype markers including N-cadherin and slug, and enhanced the expression of the epithelial marker E-cadherin. Knockdown of HMGA2 also reduced high glucose-induced upregulation of N-cadherin and slug as well as down-regulation of E-cadherin, as shown by the mRNA level (Figure 3B).

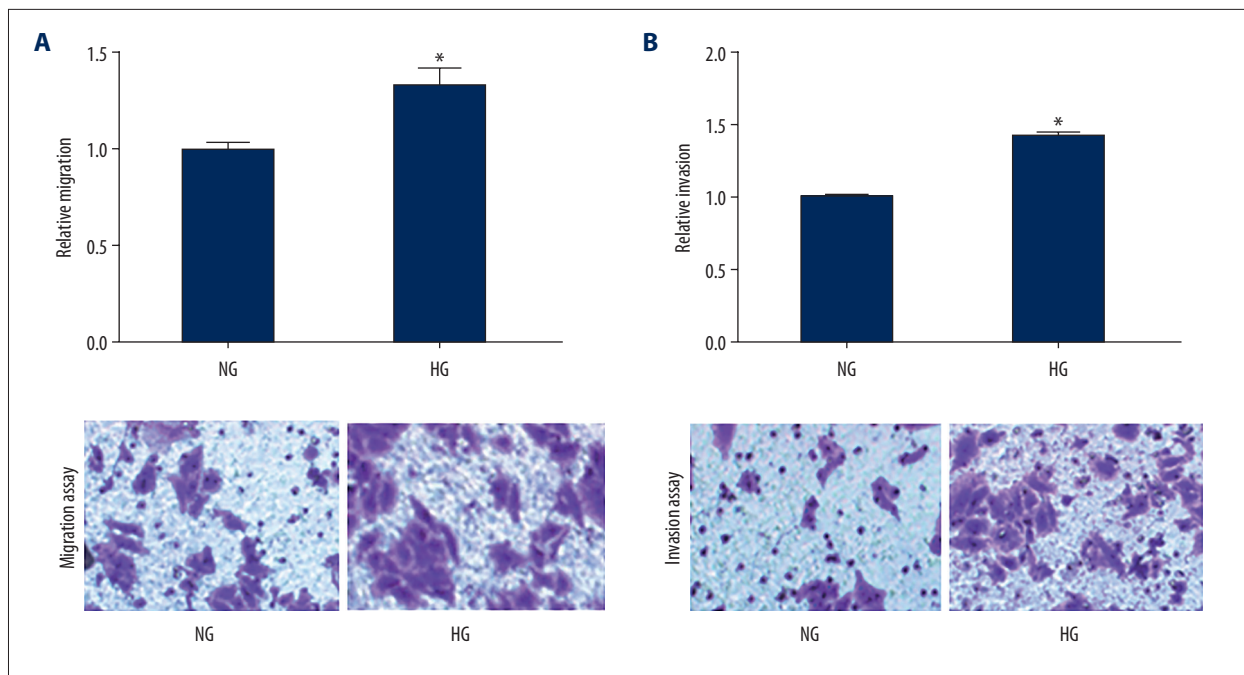


Figure 2. Effects of glucose on migration and invasion of A20 murine diffuse large B-cell lymphoma (DLBCL) cells. A20 cells were treated with different concentrations of glucose, including normal glucose (NG), 5 mM, high glucose (HG), 30 mM. Cell migration (A) and invasion (B) of A20 cells were evaluated by transwell assays. Quantification analysis showed that migration and invasion in A20 cells treated with high glucose were enhanced compared with the normal glucose group. Data are presented as the mean \pm standard deviation (SD). * $P < 0.05$, compared with the Control (normal glucose) group.

Also, transfection with HMGA2 siRNA alone without HG treatment did not significantly alter the EMT transcription factor, snail mRNA expression, when compared with the NC group. However, in the presence of high glucose, transfection with HMGA2 siRNA led to a significant decrease in snail mRNA expression compared with non-transfected cells (Figure 3B).

Consistent with the findings at mRNA level, Western blot showed that in high glucose conditions, transfection with HMGA2 siRNA significantly reduced the expression of N-cadherin and snail, and increased the expression of E-cadherin at the protein level, when compared with non-transfected cells (Figure 4B). Also, high glucose (30 mM) significantly increased the expression of slug, and knockdown of HMGA2 significantly reduced high glucose-induced upregulation of slug compared with non-transfected cells. However, there was no significant variation in the expression of slug before and after treatment with high glucose or knockdown of HMGA2 at the protein level. The most likely reason for these differences is that there may be complex post-transcriptional control and post-translational modification processes involved in the regulation of slug protein in the context of DLBCL. These findings support that HMGA2 is a valid transcription regulator that modulates the progression of high glucose-induced EMT in A20 cells.

HMGA2 has a role in high glucose-induced EMT of A20 cells by regulating the Wnt/ β -catenin signaling pathway

To further determine the underlying mechanisms of HMGA2 involved in high glucose-induced EMT in A20 cells, the role of HMGA2 in the Wnt/ β -catenin signaling pathway was investigated. This approach was taken due to the close link between Wnt/ β -catenin and EMT and cancer progression by modulating cell adhesion and migration. After transfection with HMGA2 siRNA and treatment with high glucose (30 mM), expression of β -catenin, cyclin D1 and c-myc were investigated, as key proteins in the Wnt/ β -catenin signaling pathway [16]. Western blot showed that in conditions of high glucose, transfection with HMGA2 siRNA led to a significant decrease in the expression of β -catenin and c-myc proteins compared with non-transfected cells (Figure 5). Also, there was a trend in the reduction of expression of cyclin D1 protein after knockdown of HMGA2 in high glucose-treated A20 cells, but this effect did not reach statistical significance (Figure 5). These findings suggest that HMGA2 was involved in high glucose-induced EMT of A20 cells by directly regulating the Wnt/ β -catenin signaling pathway.

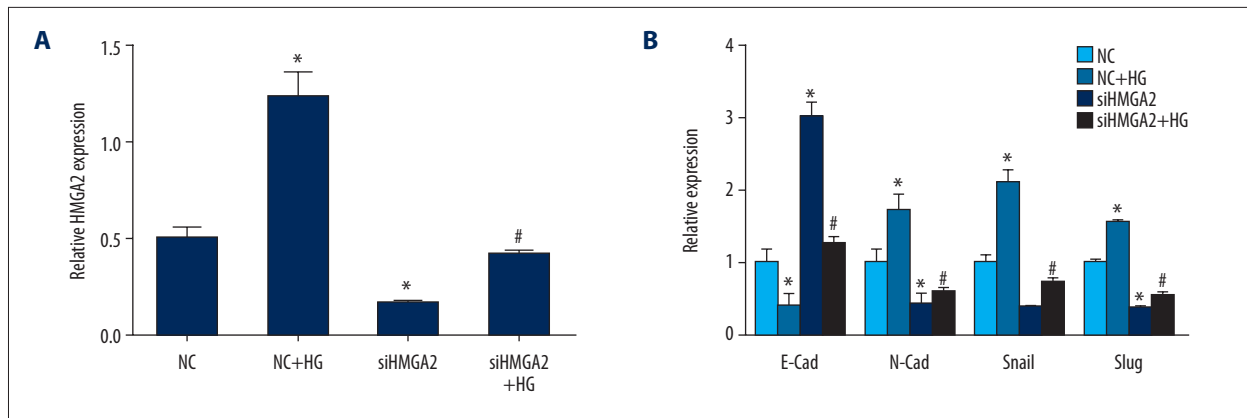


Figure 3. Effects of knockdown of the HMGA2 gene on the expression of markers of epithelial-mesenchymal transition (EMT) in A20 murine diffuse large B-cell lymphoma (DLBCL) cells. A20 cells were transfected with a murine HMGA2-targeting siRNA (siHMGA2) at a final concentration of 50 nM, and then treated with or without high glucose (30 mM). Gene expression was assayed 48 hours later by quantitative real-time polymerase chain reaction (qRT-PCR) for HMGA2 (A) and EMT markers including E-cadherin, N-cadherin, snail, and slug (B). Each experiment was performed in triplicate. Data are presented as mean \pm SD. * $P < 0.05$, compared with NC group; # $P < 0.05$, compared with NC+HG group.

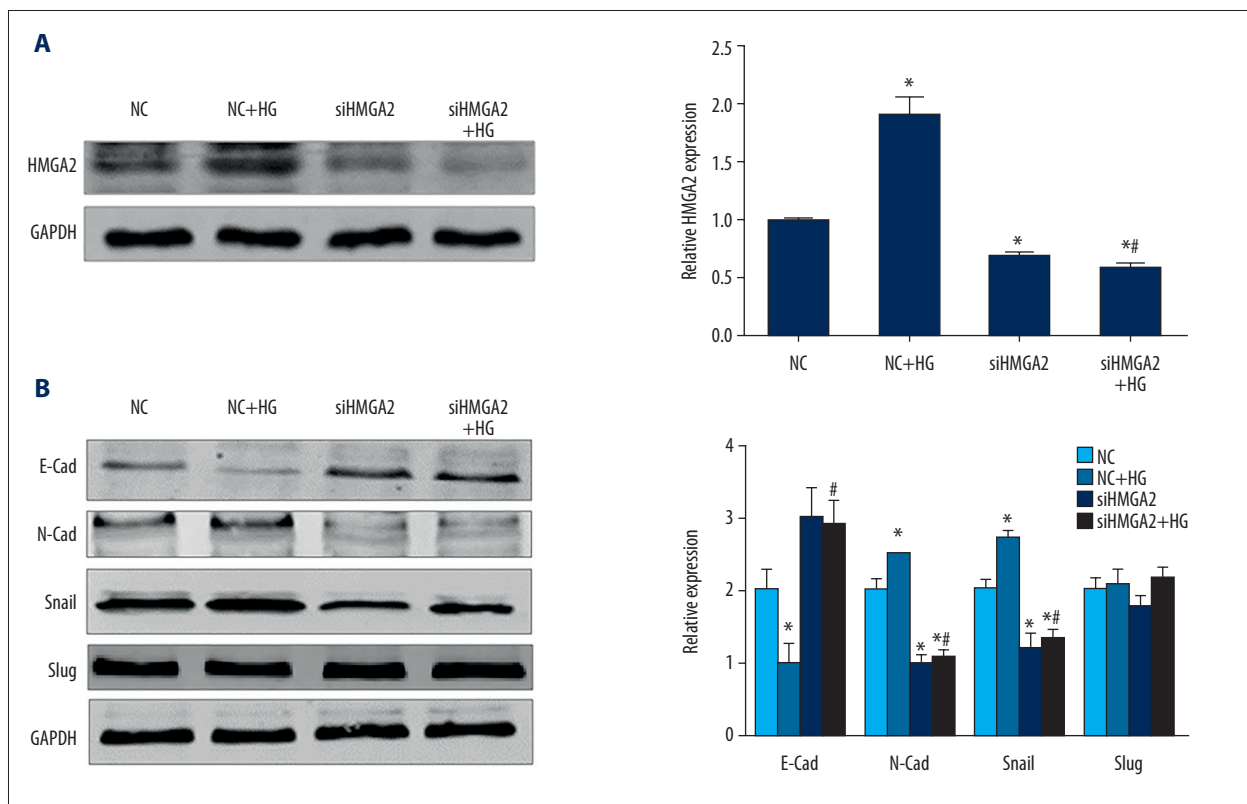


Figure 4. Effects of knockdown of the HMGA2 gene on the expression of protein markers of epithelial-mesenchymal transition (EMT) and HMGA2 in A20 murine diffuse large B-cell lymphoma (DLBCL) cells. A20 cells were transfected with siHMGA2 (50 nM), and then treated with or without high glucose (30 mM) for 48 hours. The protein levels of HMGA2 (A) and EMT markers (B) were assessed by Western blot separately, and quantification analysis was performed and shown as a graph right next to the images. GAPDH was used as an internal reference. Each experiment was performed in triplicate, and the representative images are shown. Data are presented as the mean \pm standard deviation (SD). * $P < 0.05$, compared with NC group; # $P < 0.05$, compared with NC+HG group.

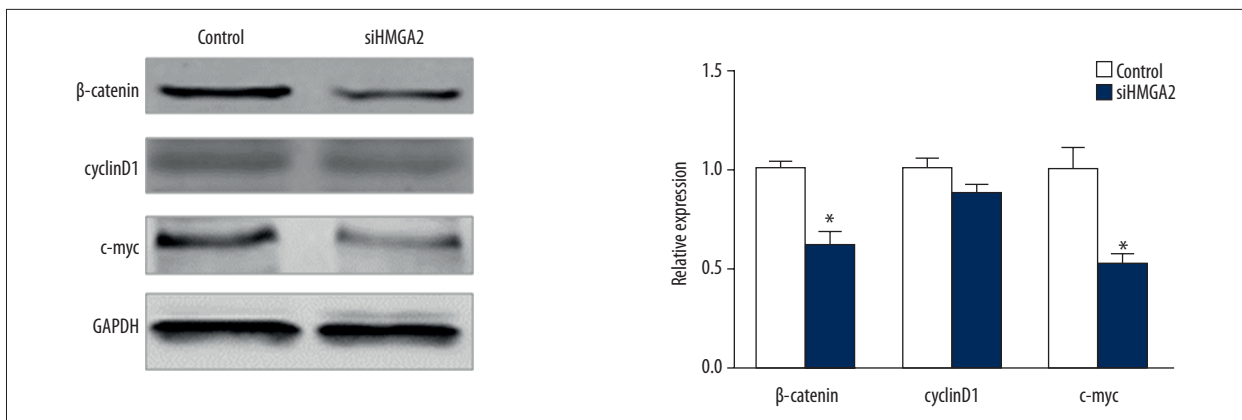


Figure 5. HMGA2 expression was involved in epithelial-mesenchymal transition (EMT) induced by high glucose in A20 murine diffuse large B-cell lymphoma (DLBCL) cells by regulating the Wnt/ β -catenin signaling pathway. A20 cells were transfected with siHMGA2 (50 nM), and then treated with high glucose (30 mM) for 48 hours. Western blot was performed to explore the effects of HMGA2 knockdown on the Wnt/ β -catenin signaling pathway. The expression of key proteins including β -catenin, cyclin D1 and c-myc in the signaling pathway was assessed, and quantification analysis was performed and shown as a graph right next to the images. GAPDH was used as an internal reference. Each experiment was performed in triplicate and representative images are shown. Data are presented as the mean \pm standard deviation (SD). * $P < 0.05$, compared with the Control (normal glucose) group.

Discussion

This study aimed to investigate the effects of high glucose on HMGA2 expression and epithelial-mesenchymal transition (EMT) in A20 murine diffuse large B-cell lymphoma (DLBCL) cells. The findings showed that high glucose upregulated the expression of HMGA2 and induced EMT, identified by phenotypic change in A20 cells, which supported the role of high glucose in the progression of EMT in murine DLBCL cells *in vitro*. High glucose could induce and accelerate migration and invasion of A20 cells, while knockdown of HMGA2 significantly inhibited high glucose-induced EMT. Also, because the Wnt/ β -catenin signaling pathway was previously proposed to have a close link with EMT and cancer progression by modulating cell adhesion and migration, the role of HMGA2 in this signaling pathway was investigated. HMGA2 directly regulated the expression of key proteins of the Wnt/ β -catenin signaling pathway. This study showed, for the first time, that high glucose in A20 cells had an effect on HMGA2 regulated by the Wnt/ β -catenin signaling pathway. Although this was an *in vitro* study in murine cells, a proposed hypothesis for the effects of high glucose and the induction of EMT in the promotion of invasion and metastasis in DLBCL is presented in Figure 6.

Impaired metabolism and chronic inflammation are two important hallmarks of cancer which facilitate the progression to an invasive and metastatic stage [17,18]. Type 2 diabetes mellitus (T2DM) is a factor involved in the progression of several malignant tumors [19,20]. Recent evidence suggests that patients with T2DM have an increased incidence of non-Hodgkin's lymphoma (NHL) when compared with non-diabetes, and

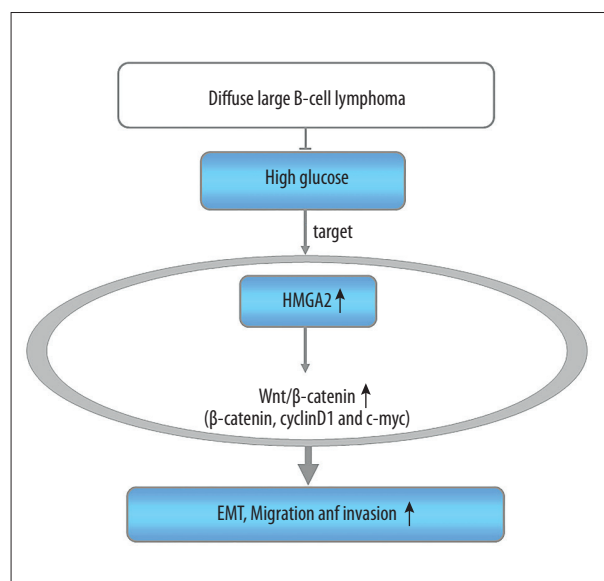


Figure 6. Graphic conclusion. High glucose upregulates HMGA2 to regulate the Wnt/ β -catenin signaling pathway and induces epithelial-mesenchymal transition (EMT), further promoting invasion and metastasis in diffuse large B-cell lymphoma (DLBCL).

hyperglycemia has been proposed to be a risk factor [4,21]. The effects of T2DM on lymphoma may be attributed to its association with chronic inflammation and autoimmune disorders, which are two driving forces for the development of lymphoma, including DLBCL [19,22]. Importantly, high glucose has been shown to induce EMT, thus enabling cells to become invasive and to metastasize, in colon cancer, pancreatic cancer,

and breast cancer cells [8–10]. These previous findings support those of the present study that identified a role for high glucose in the progression of EMT, and invasion in murine DLBCL cells *in vitro*.

HMGA2 is a key transcription regulator that is abundantly expressed in a variety of tumors, and the expression is considered to be closely related to tumor invasion and metastasis [12,13]. Also, HMGA2 is reported to be aberrantly expressed in some human hematopoietic malignancies as well as canine lymphoma [23,24], suggesting that HMGA2 may have a functional role in the progression of hematopoietic neoplasia. This study firstly demonstrated that high glucose upregulated HMGA2 expression, and specifically targeting HMGA2 effectively inhibited high glucose-induced EMT in murine DLBCL cells *in vitro*. The findings of the present study are supported by previous studies that showed that high glucose levels act via HMGA2 to induce EMT, including cell migration and invasion in colorectal cancer cells [8], and are supported by the previous finding that HMGA2 was a key regulator of EMT in bladder cancer and nasopharyngeal cancer [13,15].

Several signaling pathways are considered to be involved in HMGA2-mediated EMT, including TGF- β /Smad2, Notch, and TGF β /ERK signaling [25,26]. Also, the Wnt/ β -catenin pathway is reported to have a close link with EMT and cancer progression by modulating cell differentiation, cell adhesion, and migration. Also, β -catenin, cyclin D1 and c-myc have been identified as critical target genes of the Wnt/ β -catenin signaling pathway [16]. Therefore, we focused on the role of HMGA2 in the Wnt/ β -catenin signaling pathway. Western blot showed that in the conditions of high glucose, transfection with HMGA2 short-interfering RNA (siRNA) resulted in a significant decrease in the expression of β -catenin and c-myc proteins, suggesting that HMGA2 participates in high glucose-induced EMT of A20 cells by directly regulating the Wnt/ β -catenin signaling pathway. Also, this study showed a decreasing trend in the expression of cyclin D1 protein following knockdown of HMGA2 in high glucose-treated A20 cells, but this effect was with not statistically significant.

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This study was limited by the small study sample size, the use of a single cell line, and the culture time of the cells treated with glucose. Therefore, further studies on the role of cyclin D1, and the *in vivo* effects of the Wnt/ β -catenin signaling pathway deserve further study. However, the findings of this study are also supported by those from a previous study that showed HMGA2 had a fundamental role in EMT by activating the Wnt/ β -catenin signaling pathway in bladder cancer [15]. Therefore, specifically targeting HMGA2 and its downstream signaling pathways with molecular interventions might provide a novel approach for the prevention and treatment of DLBCL. Further studies are required to determine whether HMGA2 could directly activate the Wnt/ β -catenin pathway in conditions of high glucose and whether there are any other pathways involved in this process.

This study had several limitations. As hyperglycemia is the most characteristic feature of T2DM, we analyzed its effects in isolation, which is a simplistic representation of a complex disease process. T2DM is a complicated disease with a series of metabolic changes that include hyperinsulinemia and hyperlipidemia [27]. These other metabolic disorders should be included in future *in vivo* studies of hyperglycemia on DLBCL.

Conclusions

The aims of this study were to investigate the effects of high glucose on cell migration, invasion and epithelial-mesenchymal transition (EMT), and the expression of high mobility group A2 (HMGA2) in A20 murine diffuse large B-cell lymphoma (DLBCL) cells. The findings showed that high glucose upregulated the expression of HMGA2 to induce EMT and promote cell migration and invasion through the Wnt/ β -catenin signaling pathway. These findings require further investigation to determine the significance of glucose levels in the progression of DLBCL. The findings of this study support the possibility that managing hyperglycemia and molecular targeting of the involved signaling networks may provide novel therapeutic strategies in patients with DLBCL, and possibly in other types of non-Hodgkin's lymphoma (NHL).

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