Silencing β-linked N-acetylglucosamine transferase induces apoptosis in human gastric cancer cells through PUMA and caspase-3 pathways

TI WEN[1], KEZUO HOU[1], ZHI LI[1], LU LI[2], HANJIE YU[3], YUNPENG LIU[1], YANGGUANG LI[2,4] and ZHINAN YIN[4]

[1]Department of Medical Oncology, The First Hospital of China Medical University, Heping, Shenyang, Liaoning 110001, P.R. China; [2]Wisconsin Institutes for Medical Research, University of Wisconsin, Madison, WI 53705, USA; [3]Laboratory for Functional Glycomics, College of Life Sciences, Northwest University, Beilin, Xi'an, Shaanxi 710069; [4]State Key Laboratory of Medicinal Chemical Biology, College of Life Sciences, Nankai University, Tianjin 300071, P.R. China

Received May 11, 2015; Accepted July 27, 2015

DOI: 10.3892/or.2015.4276

Abstract. β-linked N-acetylglucosamine (GlcNAc) is a monosaccharide that is catalyzed by O-GlcNAcylation transferase (OGT) to bind serine or threonine hydroxyl moieties of numerous nuclear and cytoplasmic proteins. Recent studies have shown that O-GlcNAcylation is elevated in various cancer types, which is associated with oncogenesis and tumor progression. However, whether OGT is expressed and/or plays a role in gastric cancer is unknown. In the present study, we used qPCR to determine that OGT mRNA levels are significantly elevated in gastric cancer tissues compared with that in corresponding adjacent tissues. In addition, *in vivo* silencing of OGT in nude mice suppressed tumor proliferation and decreased tumor burden. Furthermore, *in vitro* OGT knockdown induced more cell apoptosis through increasing PUMA and caspase-3 expression. We used a glycan-binding protein gene microarray to identify potential downstream target genes of OGT, and found that apoptosis-related genes such as *galectin* and *HBEGF* were decreased after OGT suppression, suggesting that OGT silencing induces apoptosis in gastric cancer tissues. We concluded that OGT plays a key role in gastric cancer proliferation and survival, and could be a potential target for therapy.

Correspondence to: Dr Ti Wen, Department of Medical Oncology, The First Hospital of China Medical University, 155 Nanjing North Street, Heping, Shenyang, Liaoning 110001, P.R. China
E-mail: tiwencmu@gmail.com

Dr Yangguang Li, Wisconsin Institutes for Medical Research, University of Wisconsin, Madison, 1111 Highland Ave., WI 53705, USA
E-mail: yli@medicine.wisc.edu

Key words: O-GlcNAc transferase, gastric cancer, apoptosis, PUMA, caspase-3

Introduction

O-GlcNAcylation (O-GlcNAc) is the covalent attachment of β-D-N-acetylglucosamine (GlcNAc) sugars to serine or threonine residues of nuclear and cytoplasmic proteins. The post-translational O-GlcNAc modification is reversible (1) and plays a critical role in regulating a wide panel of cellular processes, such as apoptosis, cell stress responses and signal transduction. O-GlcNAc transferase (OGT), an enzyme that transfers GlcNAc from uridine diphosphate (UDP) to serine/threonine residues of target proteins (2,3), induced elevated expression of O-GlcNAc in tissues.

Since OGT and O-GlcNAcylation plays an important role in normal biological process, aberrant regulation contributes to the development of wide range of diseases, including cancer. OGT and O-GlcNAcylation is elevated in breast cancer cell lines and tissues, particularly in metastatic lymph nodes. Also it has been demonstrated that O-GlcNAcylation could promote breast cancer tumorigenesis and metastasis (4,5). In colon and lung cancer, O-GlcNAcylation and OGT are also upregulated, compared with that in the corresponding adjacent tissues. Additionally, it demonstrated that O-GlcNAcylation enhanced cell growth and invasion, and may play important roles in lung and colon cancer formation and progression (6). In laryngeal cancer, OGT and O-GlcNAcase (OGA) mRNA level was related to larger tumor size, nodal metastases, higher grader and tumor size, their protein level showed a trend of more advanced tumors to be more frequently OGT and OGA positive, suggesting that O-GlcNAcylation may have an effect on tumor aggressiveness (7).

Gastric cancer is the fourth most common cancer worldwide and the second most frequent cause of cancer-related death (8,9), with ~27% 5-year survival rate (10). Although elevated OGT levels were reported in various epithelial cancers, it remains unclear whether OGT is upregulated and how OGT exerts its function in gastric cancer. In the present study, we show that OGT mRNA levels are upregulated in human gastric cancer compared with that in adjacent tissues. Additionally, OGT function is analyzed. Silencing OGT inhibits BCG-823

cell proliferation *in vitro*, and reduces tumorigenicity *in vivo*. We demonstrated that OGT silencing induces more cell apoptosis by increasing PUMA and caspase-3 protein levels. Also, we screened the potential targets of OGT using GBP array, our data suggest that silencing OGT promotes apoptosis, which may also be through *galectin* and *hbgef*.

Materials and methods

Sample. Human cancerous and adjacent normal parts of 7 gastric specimens were obtained from the First Hospital of China Medical University as frozen tissues. All tissue specimens were obtained with informed consent, and all investigations were approved by the local Ethics Committee. Each sample was divided into 2 parts, one for histopathological examination and the other was stored at -80˚C for protein extraction.

Cell culture. Gastric cancer cell line BCG-823 was maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 100 IU/ml penicillin and 100 μg/ml streptomycin, 2 mM L-glutamine and 10% fetal bovine serum (FBS) in a 5% CO_2 atmosphere at 37˚C.

Knockdown (KD) of OGT by shRNA. shRNAs targeting OGT were synthesized and inserted into pG-PU6/GFP/Neo vector by GenPharm Co. (Shanghai, China). A scrambled sequence provided by GenPharm was used as a negative control (shNC). The shRNA target sequence of OGT is GGATGCTTATATCA ATTTAGG (4). The shRNA was transfected into BGC-823 cells in 6-well culture plates using Lipofectamine™ 2000 (Invitrogen). At 48 h after transfection, G418 was added and screened for 2 weeks. Then cells were lysed, qPCR and western blotting were used to analyze the KD efficiency. The stable cells were used for function analysis.

Reverse transcription (RT)-PCR and quantitative real-time PCR (qRT-PCR). Total RNA of tissues or cells was extracted using the RNeasy Mini kit (Qiagen), according to the manufacturer's protocol. For each RNA sample, 1 μg was reverse-transcribed using a First Strand cDNA Synthesis kit (Invitrogen). Then, the first-strand cDNA was used to amplify genes of interest with gene specific primers. The number of PCR cycles was optimized for each gene to ensure linear amplification. Gene-specific primers are as follows: OGT forward, 5'-TTGCCTTCTGTGCATCCTCAT-3' and OGT reverse, 5'-TATCCTACACGCAGCCGACC-3'; galectin-2 forward, 5'-TCTGTTCGGACAACTTCCTTCA-3' and reverse, 5'-TTATTCTTTTAACTTGAAAGAGGA; HBEGF forward, 5'-CTCAGCCTTTTGCTTTGCTAAT-3' and reverse, 5'-GGAACTCACTTTCCCTTGTGTC-3'; GAPDH forward, 5'-ATGGGGAAGGTGAAGGTCG-3' and GAPDH reverse, 5'-GGGGTCATTGATGGCAACAATA-3'. RNA levels were normalized using GAPDH.

The amplification program consisted of one cycle of 95˚C for 10 min followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. Relative quantity (RQ) of gene expression was normalized to GAPDH and performed using the $2^{-\Delta\Delta Ct}$ method.

Western blotting. Western blotting was performed as previously described (11). Briefly, total protein extracts of different cell lines were prepared using RIPA buffer (Beyotime). Then, 20 μg of total proteins for each sample was separated by SDS/PAGE (10% gels) and transferred to PVDF membrane (Millipore). The membranes were blocked in 5% skimmed milk in PBST for 2 h at room temperature. After blocking, the membrane was probed with primary antibodies against the proteins of interest. Finally, the proteins were further detected using the horseradish peroxidase (HRP)-conjugated secondary antibody and chemiluminescence HRP substrate kit (Millipore). The primary rabbit anti-OGT and anti-O-GlcNAcylation were from Abcam. Caspase-3 and PUMA were from Cell Signaling. Mouse anti-β-actin (clone 6G3) was purchased from Tianjin Sungene.

Cell counting. Cells were treated with DMSO/OGT inhibitor (Benzyl-2-acetamido-2-deoxy-α-D-galactopyranoside; Santa Cruz). Cell number was measured with an automatic cell counter according to the manufacturer's instructions. Briefly, cells were harvested and suspended, and mixed with equal volume of 0.4% trypan blue. Cell suspension (10 μl) was loaded onto TC20 system (Bio-Rad) counting slides, and the number of viable cells was quantified on a TC20 automated cell counter (Bio-Rad).

MTT assay. Cells ($5x10^3$) were seeded into a 96-well culture plate and subsequently incubated with MTT reagent (0.5 mg/ml; Sigma) at 37˚C for 2 h and MTT assay was then performed.

Xenograft. BALB/c nude mice (4 weeks old) were purchased from the laboratory animal center of the Academy of Military Medical Sciences. The maintenances and experimental animal procedures were approved by the Animal Ethics Committee of China Medical University. BALB/c nude mice (4 weeks old) were randomly assigned to two groups (7 mice/group). BGC-823 cells (shOGT and shNC) were trypsinized and resuspended at a final concentration of $1x10^7$ cells/ml in phosphate-buffered saline (PBS). Then, 100 μl of cells were injected subcutaneously into the right flank of the mice. Tumor growth was monitored twice every week and recorded by measuring tumor length and width daily for 4 weeks, and tumor volume was calculated using the formula: 1/2 (length x $width^2$). After the experiments, mice were sacrificed and tumors weighed.

Apoptosis analysis. Cell apoptosis was performed using the allophycocyanin (APC)-Annexin V and 7-amino-actinomycin D (7-AAD) staining kit (Tianjin Sungene) following the manufacturer's instructions. Briefly, stable cell line (shOGT and shNC) was plated into a 6-well plate for 72 h with or without 5-fluorouracil (5-FU), cells were then trypsinized and washed twice with ice-cold PBS and then resuspended in binding buffer from the kit. APC-Annexin V and 7-AAD were added into the flow tube. Finally, flow cytometric analysis was performed within 1 h using FACS Calibur (BD Biosciences).

Glycan-binding protein (GBP) gene microarray. Total RNA was extracted as described above. RNA integrity was assessed by agarose gel electrophoresis and spectrophotometric analysis. tcRNA was obtained by linear amplification and labeled with Cy3/Cy5. Samples were hybridized to the array.

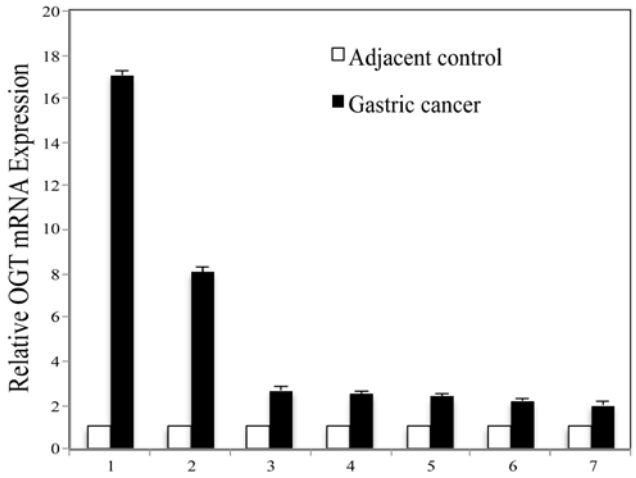

Figure 1. mRNA levels of OGT in gastric cancer and the corresponding non-cancer tissues were determined by qPCR. The OGT mRNA expression measured by qPCR increased significantly from non-cancer to cancer tissues.

Data were analyzed by GenePix Pro 3.0. Differential expression analysis was cut-off at fold-change of ±1.3. Heatmap was produced using the R program.

Statistical analysis. Data are expressed as means ± SEM. Student's t-test was used to evaluate the significance of differences between sample means obtained from three independent experiments. Statistical significance was defined as $P<0.05$.

Results

OGT is upregulated in gastric cancer. Highly expressed OGT in several types of cancer has been reported, except in gastric cancer. In order to determine OGT expression in gastric cancer, initial analysis was performed comparing OGT expression levels between cancerous and paired adjacent non-cancerous tissue mRNA derived from the same patient with gastric cancer. Seven gastric cancerous and adjacent tissues were used. Quantitative PCR results revealed differential expression, although OGT mRNA was detectable in the normal tissues, OGT is overexpressed in all cancerous tissues in comparison to the normal groups (Fig. 1).

OGT KD in BGC-823 cells decreases cell proliferation. To investigate the function of OGT in gastric cancer, we used RNA interference strategy to knock down endogenous OGT gene. Stable cells of control and OGT silencing group were constructed with that in G418 selection. The efficiency of inhibition of OGT was confirmed by qPCR and western

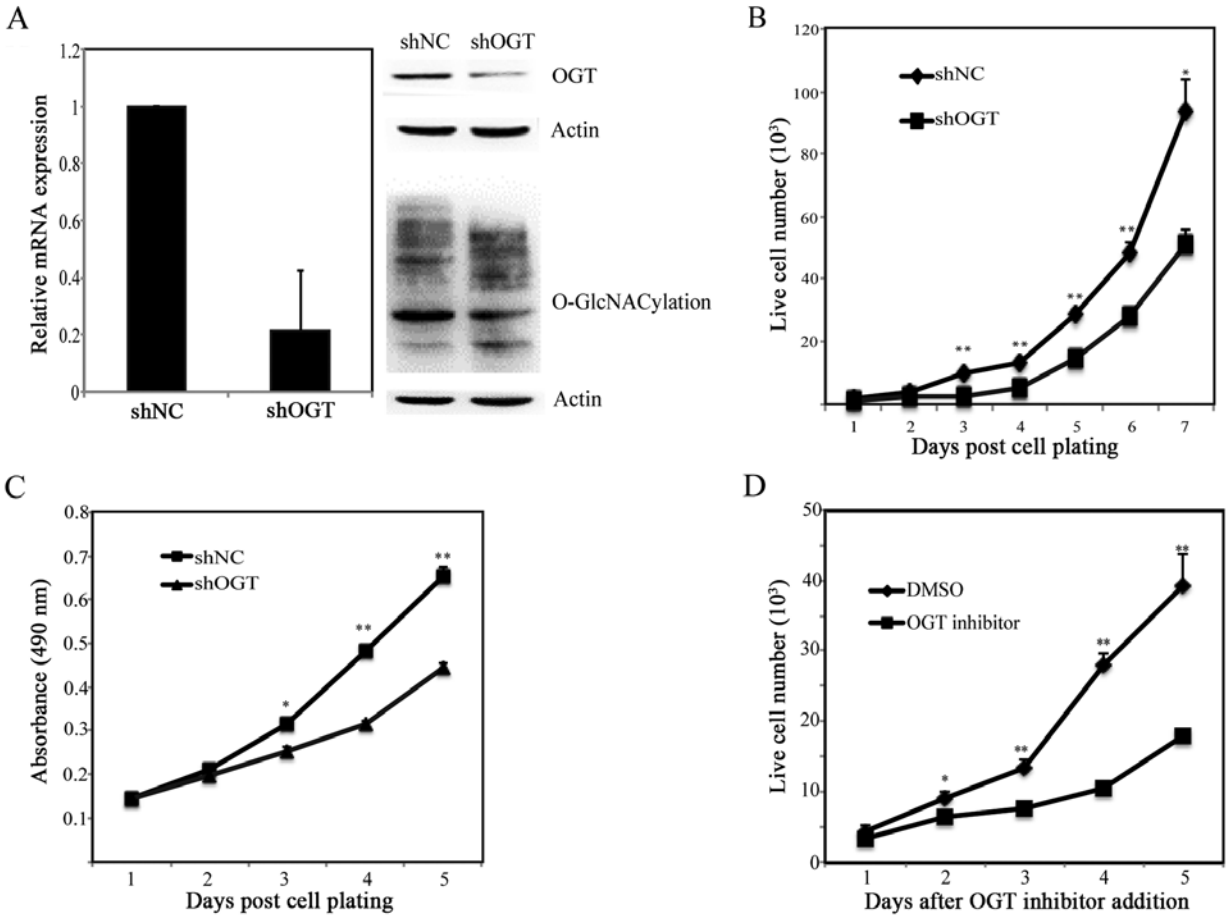

Figure 2. OGT silencing suppresses gastric cancer growth *in vitro*. (A) OGT silencing efficiency was measured by qPCR and western blotting. mRNA and protein levels were significantly reduced after OGT knowdown. (B and C) Cell growth curves of OGT expressed, and the unexpressed cells by cell counting and MTT assay. (D) Growth curve after treatment with DMSO/OGT inhibitor.

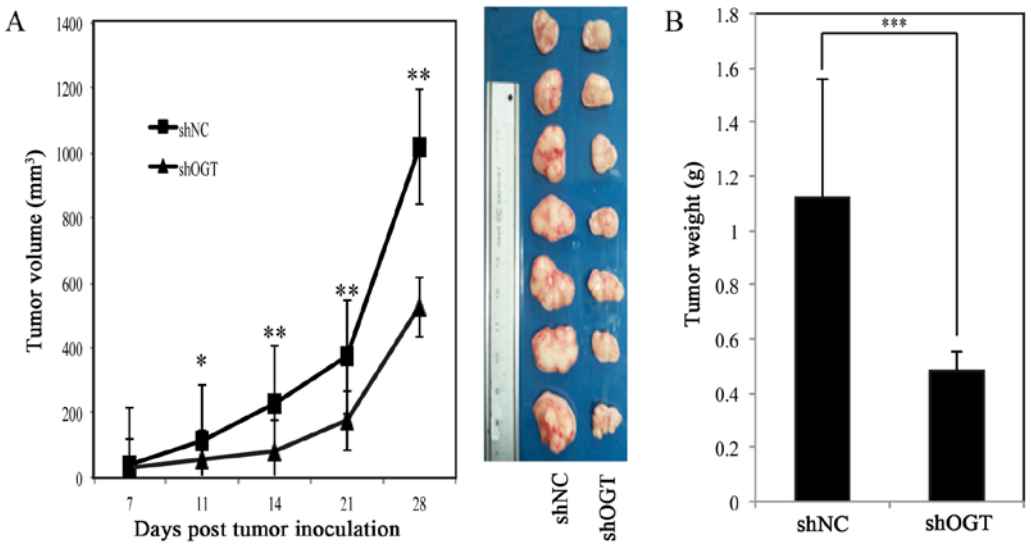

Figure 3. OGT silencing inhibits tumor growth *in vivo*. (A) Left panel, growth curves represent tumor size in OGT expressed and unexpressed BGC-823 cells in xenograft mice at different time points (n=7). Right panel, image of tumor size. (B) Representative results of tumor weight (n=7).

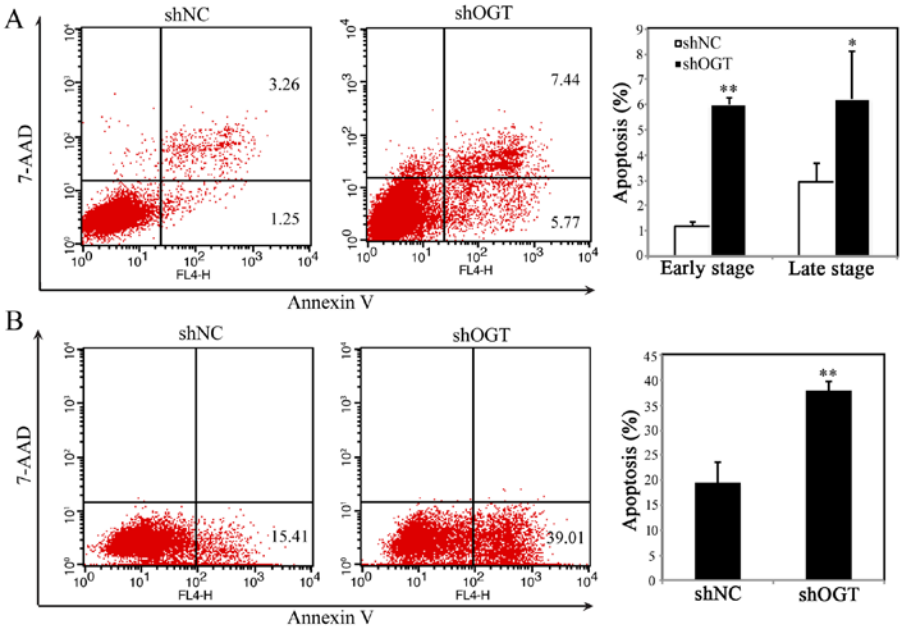

Figure 4. OGT silencing induces cell apoptosis. (A) BGC-823 cells expressing shNC/shOGT were cultured for 72 h, and then apoptosis was determined by APC-Annexin V and 7-AAD staining with flow cytometric analysis. The subpopulations of APC-Annexin V^-/7-AAD^- were viable cells. The APC-Annexin V^+/7-AAD^- and APC-Annexin V^+/7-AAD^+ subpopulations indicated cells under early and late apoptosis, respectively. Cells that stained positive with APC-Annexin V were counted as total apoptotic cells. APC-Annexin V^-/7-AAD^+ represents necrotic cells. (B) shNC/shOGT cells were treated with 5-FU for 24 h, apoptosis was then analyzed.

blotting. OGT mRNA and protein level were significantly reduced compared with that in control cells (Fig. 2A). We further analyzed whether the O-GlcNAcylation expression was changed after OGT silencing. As expected, silencing OGT led to significant reduction in global O-GlcNAcylation compared with control cells. Therefore these stable cells were used for following functional experiments. Proliferation of OGT KD cells was first assessed by cell counting and MTT assay. Both results showed that OGT silencing inhibited cell growth (Fig. 2B and C). In addition, to confirm this result, inhibitor specifically targeting OGT was used. Cell counting assay showed similar results (Fig. 2D). Taken together, these results indicated that OGT is required for gastric cancer cell proliferation.

Silencing OGT inhibits tumor progression in vivo. To examine the effects of OGT on tumorigenicity of BGC-823 cells *in vivo* and explored the therapeutic potential of OGT gene silencing in BGC-823, we compared the tumor growth in immuno-compromised nude mice after shNC/shOGT cell inoculation. Tumor size was monitored twice weekly by cationic palpation. At the end of the experiments, animals were sacrificed and the tumors weighed. As shown in Fig. 3A, silencing OGT could significantly suppress tumor growth. The tumors harvested

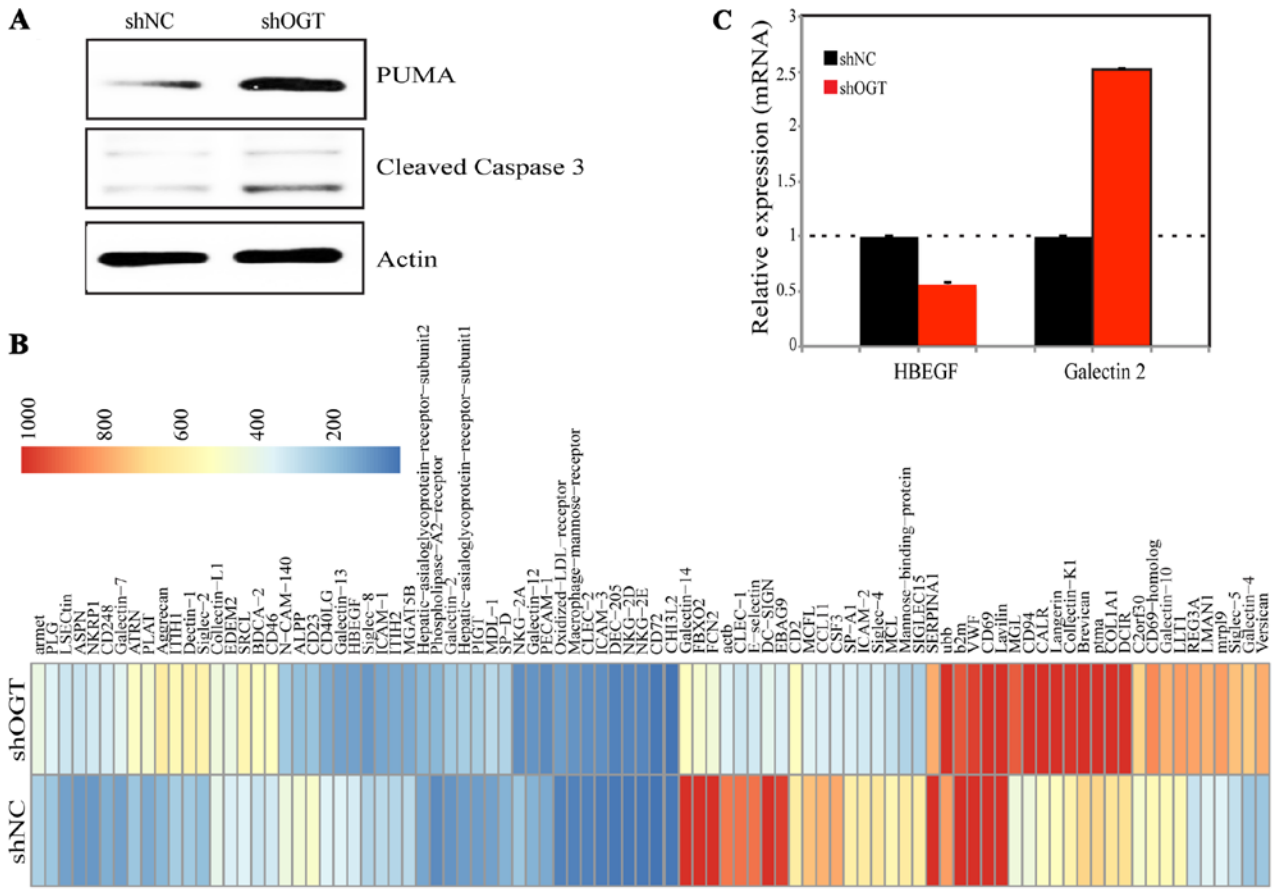

Figure 5. Silencing OGT activates apoptosis pathway. (A) PUMA and cleaved caspase-3 expression level were determined by w estern blotting. (B) Heat map of gene expression levels in shNC and shOGT BGC-823 cells by GBP. The color bar indicates expression levels from low in blue to high in red. (C) Validation of levels of HBEGF and galectin 2 by qPCR.

from the OGT KD group also weighed less (Fig. 3B), these suggesting that OGT could be a therapeutic target in gastric cancer.

Suppression of OGT induces cell apoptosis. To investigate whether the inhibition of proliferation in OGT KD cells was due to cell apoptosis, we used APC-Annexin V/7-AAD double staining kit followed by FACS analysis. As shown in Fig. 4A, percentage of APC-Annexin V^+/7-AAD^- (early apoptosis) and APC-Annexin V^+/7-AAD^+ (late apoptosis) cells markedly increased after OGT silencing, which indicated that OGT has an anti-apoptosis role in gastric cancer cells. In addition, OGT reversed anti-chemotherapeutic drug-mediated apoptosis. If shOGT/shNC cells were treated with 5-FU, silencing OGT could increase apoptosis, which suggested elevated level of OGT in gastric cancer may be one of the drug-resistant mechanisms.

Silencing OGT induces cell apoptosis through upregulation of PUMA and caspase-3. As silencing OGT promoted cell apoptosis, PUMA and caspase-3 were detected by western blotting. Data showed that PUMA and caspase-3 were significantly upregulated after OGT KD (Fig. 5A), which could be the reason of OGT KD inducing apoptosis. In order to examine the downstream genes of OGT, GBP gene microarray was performed. Ninety genes were assessed in this array, they were well known as glycan-binding protein genes based on the database of functional glycomics and uniprot. RNA from shNC/shOGT was investigated. As shown in Fig. 5B, many apoptosis-related genes were changed after OGT silencing. Of these, HBEGF were most significantly reduced, whereas *galectin* 2 and *galectin* 7 were increased after OGT suppression. qPCR was used for validation, as shown in Fig. 5C. These genes have apoptotic-related function indicating that OGT KD may target these proteins to modulate PUMA and caspase-3.

Discussion

O-GlcNAc is the covalent addition of a GlcNAc moiety to serine/threonine residues of cytosolic and nuclear proteins. OGT could transfer GlcNAc form UDP-GlcNAc to substrate proteins, whereas O-GlcNAcase (OGA) could remove GlcNAc. For O-GlcNAcylation and OGT, the expression level has been examined in various types of cancer, including breast (4,5,12), prostate (13), lung, colorectal (6), liver (14,15) and non-solid cancers such as chronic lymphocytic leukemia (15). However, the role of OGT in gastric cancer was not reported.

The present study was designed to investigate the expression and function of OGT in gastric cancer. We observed the elevated expression of OGT at mRNA level in gastric cancerous tissues compared with that in adjacent tissues. Silencing OGT decreased cell proliferation both *in vitro* and *in vivo* due to apoptosis induction. The reduction of OGT results in pro-apoptosis effect in the presence or absence

of 5-FU. In addition, PUMA and caspase-3 were increased after OGT KD. Furthermore, the GBP array results showed expression change of various apoptosis-related genes after OGT KD, pointing to a tumor genesis function of OGT in gastric cancer.

In order to determine the level of OGT in gastric cancer, the mRNA level of 7 cases of gastric cancer and adjacent non-cancer tissues was measured by qPCR. The results showed that OGT is overexpressed in all cancer tissues when compare with that in non-cancerous tissues. Due to the limit number of samples, the protein level and whether OGT is associated with different stages and patient survival are unknown, which need to be further studied.

Several studies have shown that O-GlcNAcylation plays a key role in cell growth, division and invasion. In ES cells, OGT deletion is lethal (16), and OGT tissue-specific mutation results in the loss of O-GlcNAcylation in specific tissues and causes T-cell apoptosis (17). Reduction of O-GlcNAcylation through RNA interference in breast cancer cells leads to inhibition of tumor growth both *in vitro* and *in vivo*. Reduced O-GlcNAcylation also decreased lung and colon cancer invasion in a context-dependent manner. Also, O-GlcNAcylation regulates cancer cell metabolism, increasing glucose and glutamine uptake in cancer cells (18,19).

In order to investigate the function of OGT in gastric cancer, the stable cell line expressing shRNA that targets OGT was constructed. Cell counting and MTT assays showed OGT suppression decreased cell proliferation. To confirm this result, the inhibitor targeting OGT was used, similar results were shown. Additionally, OGT KD cells showed less tumor growth in nude mice. We demonstrated that OGT was necessary for the proliferation of gastric cancer.

To explore the mechanism of silencing OGT in inhibiting cancer cell proliferation, cell cycle was determined by PI staining, but there was no change in these two cell lines (data not shown). Apoptosis was then examined by Annexin V and 7-AAD staining. Our results showed that OGT inhibition induced more cell apoptosis, both early and late stage of apoptosis. Our data are consistent with a previous study (17) showing that loss of OGT could cause T cell apoptosis. Notably, OGT KD also increased the sensitivity of 5-FU on BCG-823 suggesting that cancer cell could use the anti-apoptosis effect of OGT in drug-resistance.

Since OGT KD showed pro-apoptosis effect, we examined apoptosis-related proteins by western blotting. Importantly, the levels of PUMA and caspase-3 were significantly upregulated in OGT KD cells, which are critical apoptosis proteins (20). These results indicated that the pro-apoptosis function of OGT KD may be through the mitochondrial pathway, which was consistent with a recent study (19). PUMA was regulated by p53, and p53 was activated by survival signals (21). To determine which protein was involved in OGT KD inducing apoptosis, we used a glycan-binding protein gene array downstream of OGT. The results showed many genes were altered after OGT silencing. Of these, *HBEGF* and *CCL11* are associated with survival or anti-apoptosis, which were reduced (22). Whereas, *galectin* 2, 4, 7 and 10 were increased, which have pro-apoptosis effects (23-25). However, whether they are the direct target of OGT or not needs to be further verified.

In summary, the present study has shed light on the expression and function of OGT in gastric cancer. Our data showed that OGT is overexpressed in gastric cancer tissues. Silencing OGT inhibited cell growth *in vitro* and *in vivo*, due to inducing cell apoptosis. Importantly, we showed that PUMA and caspase-3 were upregulated after OGT silencing, which is the mechanism of OGT suppression-induced apoptosis. In addition, we found various candidate genes downstream of OGT. Further studies need to be carried out, to investigate whether these genes were associated with apoptosis in OGT KD cancer cells. Collectively our results suggested that OGT is an oncogene in gastric cancer.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (no. 31300743), the Fund of the Education Department of Liaoning province (no. L2012278) and Science and Technology Plan Project of Liaoning Province (no. 2011404013-1, 2012225001, 2014225013).

References

1. Slawson C, Copeland RJ and Hart GW: O-GlcNAc signaling: A metabolic link between diabetes and cancer? Trends Biochem Sci 35: 547-555, 2010.
2. Haltiwanger RS, Holt GD and Hart GW: Enzymatic addition of *O*-GlcNAc to nuclear and cytoplasmic proteins. Identification of a uridine diphospho-N-acetylglucosamine:peptide beta-N-acetylglucosaminyltransferase. J Biol Chem 265: 2563-2568, 1990.
3. Dong DL and Hart GW: Purification and characterization of an *O*-GlcNAc selective *N*-acetyl-β-D-glucosaminidase from rat spleen cytosol. J Biol Chem 269: 19321-19330, 1994.
4. Caldwell SA, Jackson SR, Shahriari KS, Lynch TP, Sethi G, Walker S, Vosseller K and Reginato MJ: Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. Oncogene 29: 2831-2842, 2010.
5. Gu Y, Mi W, Ge Y, Liu H, Fan Q, Han C, Yang J, Han F, Lu X and Yu W: GlcNAcylation plays an essential role in breast cancer metastasis. Cancer Res 70: 6344-6351, 2010.
6. Mi W, Gu Y, Han C, Liu H, Fan Q, Zhang X, Cong Q and Yu W: O-GlcNAcylation is a novel regulator of lung and colon cancer malignancy. Biochim Biophys Acta 1812: 514-519, 2011.
7. Starska K, Forma E, Brzezińska-Błaszczyk E, Lewy-Trenda I, Bryś M, Jóźwiak P and Krześlak A: Gene and protein expression of O-GlcNAc-cycling enzymes in human laryngeal cancer. Clin Exp Med: Oct 15, 2014 (DOI 10.1007/s10238-014-0318-1).
8. Hartgrink HH, Jansen EP, van Grieken NC and van de Velde CJ: Gastric cancer. Lancet 374: 477-490, 2009.
9. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
10. Cunningham D, Jost LM, Purkalne G, Oliveira J and Force EGT: ESMO Minimum Clinical Recommendations for diagnosis, treatment and follow-up of gastric cancer. Ann Oncol 16 (Suppl 1): i22-i23, 2005.
11. Li Y, Wen T, Zhu M, Li L, Wei J, Wu X, Guo M, Liu S, Zhao H, Xia S, *et al*: Glycoproteomic analysis of tissues from patients with colon cancer using lectin microarrays and nanoLC-MS/MS. Mol Biosyst 9: 1877-1887, 2013.
12. Krześlak A, Forma E, Bernaciak M, Romanowicz H and Bryś M: Gene expression of *O*-GlcNAc cycling enzymes in human breast cancers. Clin Exp Med 12: 61-65, 2012.
13. Kamigaito T, Okaneya T, Kawakubo M, Shimojo H, Nishizawa O and Nakayama J: Overexpression of O-GlcNAc by prostate cancer cells is significantly associated with poor prognosis of patients. Prostate Cancer Prostatic Dis 17: 18-22, 2014.
14. Zhu Q, Zhou L, Yang Z, Lai M, Xie H, Wu L, Xing C, Zhang F and Zheng S: O-GlcNAcylation plays a role in tumor recurrence of hepatocellular carcinoma following liver transplantation. Med Oncol 29: 985-993, 2012.

15. Shi Y, Tomic J, Wen F, Shaha S, Bahlo A, Harrison R, Dennis JW, Williams R, Gross BJ, Walker S, *et al*: Aberrant O-GlcNAcylation characterizes chronic lymphocytic leukemia. Leukemia 24: 1588-1598, 2010.
16. O'Donnell N, Zachara NE, Hart GW and Marth JD: *Ogt*-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. Mol Cell Biol 24: 1680-1690, 2004.
17. Shafi R, Iyer SP, Ellies LG, O'Donnell N, Marek KW, Chui D, Hart GW and Marth JD: The O-GlcNAc transferase gene resides on the X chromosome and is essential for embryonic stem cell viability and mouse ontogeny. Proc Natl Acad Sci USA 97: 5735-5739, 2000.
18. Marshall S: Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: A nutritional perspective of diabetes, obesity, and cancer. Sci STKE 2006: re7, 2006.
19. Ferrer CM, Lynch TP, Sodi VL, Falcone JN, Schwab LP, Peacock DL, Vocadlo DJ, Seagroves TN and Reginato MJ: O-GlcNAcylation regulates cancer metabolism and survival stress signaling via regulation of the HIF-1 pathway. Mol Cell 54: 820-831, 2014.
20. Ding WX, Ni HM, Chen X, Yu J, Zhang L and Yin XM: A coordinated action of Bax, PUMA, and p53 promotes MG132-induced mitochondria activation and apoptosis in colon cancer cells. Mol Cancer Ther 6: 1062-1069, 2007.
21. Nakano K and Vousden KH: PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 7: 683-694, 2001.
22. Yotsumoto F, Yagi H, Suzuki SO, Oki E, Tsujioka H, Hachisuga T, Sonoda K, Kawarabayashi T, Mekada E and Miyamoto S: Validation of HB-EGF and amphiregulin as targets for human cancer therapy. Biochem Biophys Res Commun 365: 555-561, 2008.
23. Sturm A, Lensch M, André S, Kaltner H, Wiedenmann B, Rosewicz S, Dignass AU and Gabius HJ: Human galectin-2: Novel inducer of T cell apoptosis with distinct profile of caspase activation. J Immunol 173: 3825-3837, 2004.
24. Paclik D, Danese S, Berndt U, Wiedenmann B, Dignass A and Sturm A: Galectin-4 controls intestinal inflammation by selective regulation of peripheral and mucosal T cell apoptosis and cell cycle. PLoS One 3: e2629, 2008.
25. Kuwabara I, Kuwabara Y, Yang RY, Schuler M, Green DR, Zuraw BL, Hsu DK and Liu FT: Galectin-7 (PIG1) exhibits pro-apoptotic function through JNK activation and mitochondrial cytochrome *c* release. J Biol Chem 277: 3487-3497, 2002.