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Enhancement of *O*-linked N-acetylglucosamine modification promotes metastasis in patients with colorectal cancer and concurrent type 2 diabetes mellitus

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Abstract. Reversible post-translational modification of serine and threonine residues by *O*-linked N-acetylglucosamine (*O*-GlcNAc), termed *O*-GlcNAcylation has been indicated to regulate the activities of a number of different proteins. Augmented *O*-GlcNAcylation contributes to the etiologies of type 2 diabetes mellitus (T_2DM) and cancer. Moreover, diabetic conditions increase the risk of colorectal cancer. However, the effect of *O*-GlcNAcylation in patients with colorectal cancer and concurrent T_2DM has not been elucidated. The current study evaluated the level of *O*-GlcNAcylation in patients with colorectal cancer with or without T_2DM. Notably, *O*-GlcNAcylation levels were significantly higher in tissues from patients with T_2DM compared with those in patients without T_2DM, and higher in cancer tissues compared with corresponding adjacent tissues. *O*-GlcNAcylation and cancer stage were more strongly correlated in cancer tissues from patients with T_2DM compared with those from patients without T_2DM. Additionally, distant metastasis was significantly correlated with *O*-GlcNAcylation in cancer tissues from patients with T_2DM. β-catenin levels in colorectal cancer tissues were the highest in patients with advanced-stage cancer and concurrent T_2DM. In SW480 human colon cancer cells, thiamet G (TMG) treatment and *OGA* silencing, which increased *O*-GlcNAcylation, significantly increased β-catenin and SNAIL in high-glucose, but not during normal-glucose conditions. These data suggest that *O*-GlcNAcylation is closely associated with distant metastasis, most likely through upregulation of the β-catenin/SNAIL signaling pathway in colorectal cancer patients with T_2DM.

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Introduction

O-linked β-N-acetylglucosaminylation (*O*-GlcNAcylation) is a post-translational modification that occurs at the serine and threonine residues of proteins present in the cytoplasm or nucleus (1). *O*-GlcNAc is attached to target proteins by *O*-GlcNAc transferase (OGT) and is removed by *O*-GlcNAcase (OGA) (2). Similar to phosphorylation, *O*-GlcNAcylation occurs quickly in response to environmental stimuli, such as stress, hormones, and nutrition, and plays various roles in the regulation of phosphorylation, protein-protein interactions, intracellular signaling, nuclear transport, proteolysis, and epigenetics, according to the histone code (3-5). Dysregulation of *O*-GlcNAcylation is closely associated with various diseases, including type 2 diabetes mellitus (T_2DM) (6,7), Alzheimer's disease (8), heart failure (9), and cancer (10,11). It is thought that control of *O*-GlcNAcylation may be an effective treatment approach for these diseases.

Under normal conditions, 2-3% of glucose taken up by the cells is shunted into the hexosamine biosynthesis pathway, which is a nutrient-sensing pathway that produces uridine diphospho (UDP)-GlcNAc, an energy substrate of OGT, as an end product (12). Under hyperglycemic conditions, more glucose flows toward the hexosamine biosynthesis pathway, the amount of UDP-GlcNAc increases, and *O*-GlcNAcylation of proteins is enhanced (13). In OGT transgenic mice, insulin resistance and leptin production are enhanced by increased levels of *O*-GlcNAc in muscles and adipose tissues (7). It

has been shown that the *O*-GlcNAcylation of glucose transporter 4 (GLUT-4), is enhanced in GLUT1-overexpressing muscles (14). Most proteins involved in the malignant transformation of cells, including β-catenin, p53, the pRb family, and c-Myc, are known to undergo *O*-GlcNAcylation (4). In colorectal and lung cancer tissues, *O*-GlcNAcylation and OGT expression are significantly enhanced, and *O*-GlcNAcylation promotes the growth of colorectal and lung cancer cells (15). Noticeably, *O*-GlcNAcylation is involved in both T_2DM and colorectal cancer.

Recent meta-analyses in epidemiological studies have demonstrated that T_2DM is a risk factor for colorectal cancer (16) and that the underlying mechanism may involve hyperglycemia, hyperinsulinemia, and insulin resistance (17). Thus, we hypothesized that *O*-GlcNAcylation may play an important role in colorectal cancer occurring concurrently with T_2DM. To prove this hypothesis, we aimed to elucidate the relationship between *O*-GlcNAcylation levels and colorectal cancer in patients with T_2DM. We report here our results, which provide important insights into the involvement of *O*-GlcNAcylation in cancer progression in patients with T_2DM.

Materials and methods

Patients and samples. Among successive cases histologically diagnosed as colorectal cancer and subjected to surgical resection at our facility between 2008 and 2015, those patients whose condition could be followed up on the basis of clinical information in their medical records were retrospectively analyzed in this study. Patients with a history of treatment for T_2DM were classified into the DM group, and patients with no history of treatment for T_2DM were classified into the non-DM (NDM) group. Tumor and adjacent non-tumor tissue samples were then examined to determine correlations between the degree of *O*-GlcNAcylation and cancer stage, tumor volume, depth of invasion, lymph node metastasis, and distant metastasis. This study was approved by the ethics review committee of Osaka Medical College.

Cell culture. SW480 human colon cancer cells were obtained from the American Type Culture Collection and maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS containing 100 μg/ml streptomycin and 100 U/ml penicillin. Another human colon cancer cell line, LoVo cells, were obtained from Riken Bioresource Center (Riken BRC). Cells were maintained in DMEM/10% FBS containing 100 μg/ml streptomycin and 100 U/ml penicillin. Both cells were incubated at 37˚C in 5% CO_2 and 95% air.

Immunohistochemistry. Paraffin-fixed tumor and adjacent non-tumor tissue sections cut from surgical blocks were used for immunohistochemistry. The sections were deparaffinized using xylene, dehydrated using ethanol, and activated by microwaving three times in citrate buffer. Next, the sections were blocked using 3% H_2O_2 solution and M.O.M blocking reagent (Vector Laboratories). Next, the sections were stained using anti-*O*-GlcNAc antibody (cat. no. MA1-072; Thermo Fisher Scientific, Inc.) and anti-β-catenin antibody (cat. no. ab32572; Abcam) as primary antibodies (1:200), followed by staining with horseradish peroxidase-labeled donkey anti-mouse IgG (Universal LSAB2 kit, cat. no. K0675; 1:1000; Dako; Agilent Technologies, Inc.) as the secondary antibody. Color development was carried out using DAB substrate (Wako Pure Chemical Industries, Ltd.). Nuclei were counterstained using Mayer's hematoxylin Solution (Wako Pure Chemical Industries, Ltd.).

Histological analysis. The immunostained tissue sections were observed under a microscope, and three randomly chosen fields of view were photographed under the same conditions. All imaging analyses were performed using ImageJ software [National Institutes of Health (NIH)]. For evaluation of *O*-GlcNAcylation, to avoid effects of variations in glandular duct size, positive cells around all glandular ducts in each view were counted and divided by the circumference of the ducts; the resulting value was defined as the histological score. For evaluation of β-catenin expression, β-catenin-positive brown areas of the glandular ducts were extracted, and the integrated density in each view was measured.

Western blot analysis. Cells were lysed in 1% NP40, 0.25% deoxycholic acid, 0.1 M NaCl, and 25 mM Tris-HCl (pH 7.4). The lysates were subjected to SDS-PAGE and transferred to a PVDF membrane (EMD Millipore) using a Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad Laboratories, Inc.). The membrane was probed with antibodies of interest and exposed with Fusion FX (Vilber-Lourmat). The results were densitometrically analyzed with ImageJ software.

Co-immunoprecipitation. Cell lysates were incubated with SureBeads protein G (Bio-Rad Laboratories, Inc.) pre-incubated with anti-β-catenin antibody (#8480, Cell Signaling Technologies, Inc.). The precipitates were eluted according to manufacturer's instructions and subjected to western blot analysis using anti-*O*-GlcNAc antibody (RL-2, Thermo Fisher Scientific, Inc.).

Thiamet G (TMG) treatment. SW480 or LoVo cells were seeded in 6-well plates (Thermo Fisher Scientific, Inc.) at $1x10^5$ cells/well in high-glucose (25 mM) growth medium and cultured for 12 h. Next, the cells were cultured in the presence of 1 μM TMG (Cayman Chemical Chemical Company), an OGA inhibitor, for another 36 h. Next, cell lysates were prepared and subjected to western blot analysis.

siRNA-mediated OGA silencing. Three siRNAs against OGA (si*OGA* 1, 5'-actcatcccacggttaaaa-3', si*OGA* 2, 5'-gtgggtttacccttttaaa-3'), and the control siRNA (scrambled) were purchased from Nippon Gene. siRNA was transfected into SW480 cells using TransIT-X2 reagent (Takara Bio, Inc.) per the manufacturer's instructions. At 24 h after transfection, the cells were cultured in high-glucose (25 mM) or normal-glucose (5 mM) medium for 36 h. Next, cell lysates were prepared and subjected to western blot analysis.

Statistical analysis. Statistical analyses were performed using GraphPad Prism 6 software (GraphPad Software, Inc.) as indicated in the figure legends (Figs. 1B and 3B, Tukey's test; Figs. 2E and S1, Student's t-test; Fig. 2A-D, Pearson's correlation analysis).

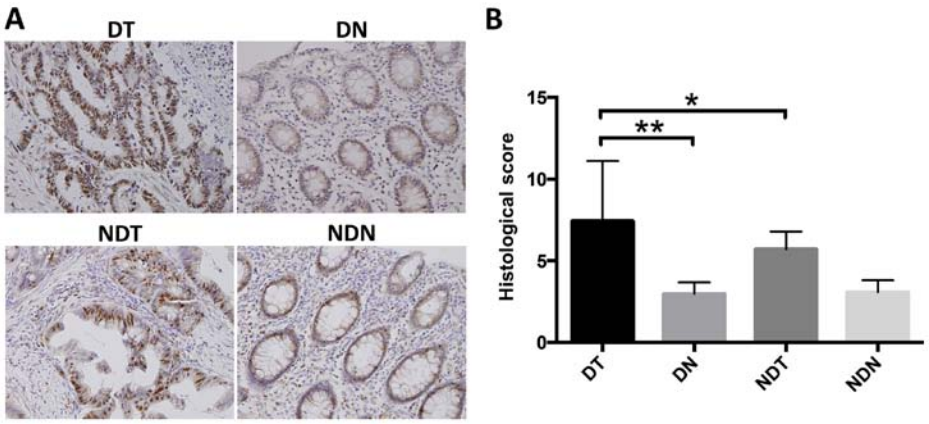

Figure 1. *O*-GlcNAcylation is enhanced in colorectal cancer tissues from patients with T_2DM. (A) Immunohistochemical staining for *O*-GlcNAc. Magnification, x400. DT, colorectal cancer tissues from patients with T_2DM; NDT, colorectal cancer tissues from patients without T_2DM; DN, non-cancerous tissues (adjacent normal tissues) from patients with T_2DM; NDN, non-cancerous tissues from patients without T_2DM. (B) Histological scores for each group. *P=0.016; **P<0.0001, Tukey's test. T_2DM, type 2 diabetes mellitus.

For western blot data, statistical analyses were performed using MEPHAS (http://www.gen-info.osaka-u.ac.jp/test-docs/tomocom/) as indicated in the figure legends (Figs. 4A-C, 5, and S2, Tukey's test; Fig. 4D, Dunnett's test). P<0.05 were considered to indicate a statistically significant difference.

Results

Patient characteristics. Thirty-one and 30 patients were enrolled in the DM and NDM group, respectively. No significant differences were found between the two groups in terms of mean age, tumor volume, tumor localization, macroscopic type, cancer stage, and presence or absence of metastasis to other organs. Body mass index was significantly higher in the DM group, and although there were no significant differences in sex between the two groups, there tended to be more men in the DM group (Table I). It was very difficult to distinguish DM patients with normal glucose from those with high glucose levels and thus, the DM group may contain patients with normal glucose. Therefore, blood glucose levels were compared between the DM and NDM groups; blood glucose levels were significantly higher in the DM group (Fig. S1).

O-GlcNAcylation is enhanced in colorectal cancer tissues, especially in patients with T_2DM. Next, we examined O-GlcNAc expression in colorectal cancer and adjacent non-cancerous tissues in patients of the DM and NDM groups. *O*-GlcNAc-positivity was detected mainly in the nucleus and cytoplasm of glandular ductal epithelial cells (Fig. 1A), suggesting that glycosylation may occur at these sites. Histological staining scores were significantly higher in cancer tissues than in non-cancerous tissues, and histological scores in cancerous tissues were significantly higher in the DM group than in the NDM group (Fig. 1B). These results suggested that *O*-GlcNAcylation is enhanced in colorectal cancer tissues, and especially, in patients with T_2DM.

In patients with T_2DM and colorectal cancer, O-GlcNAcylation is correlated with cancer stage. Next, we examined histological

Table I. Patient characteristics.

Characteristic	DM	NDM	P-value
Number of cases	31	30	
Age, years, mean ± SD	68.2±10.7	70.0±8.2	0.460[a]
Sex			0.600[b]
Female	7 (22.6%)	14 (46.7%)	
Male	24 (77.4%)	16 (53.3%)	
BMI, mean ± SD	23.9±3.4	21.4±3.5	0.007[a]
Volume, mm^2, mean ± SD	1,497±1080	1,740±1279	0.43[a]
Location			0.88[c]
Rectum	15 (48.4%)	17 (56.7%)	
Sigmoid	10 (32.3%)	5 (16.6%)	
Descending	0 (0%)	2 (6.7%)	
Transverse	3 (9.7%)	4 (13.3%)	
Ascending	2 (6.5%)	2 (6.7%)	
Cecum	1 (3.1%)	0 (0%)	
Type			0.82[c]
0	3 (9.7%)	3 (10%)	
1	0 (0%)	0 (0%)	
2	22 (71%)	24 (80%)	
3	5 (16.1%)	2 (6.7%)	
5	1 (3.2%)	1 (3.3%)	
Stage (UICC)			0.94[c]
0	0 (0%)	1 (3.3%)	
I	5 (16.1%)	4 (13.3%)	
II	15 (48.4%)	7 (23.4%)	
III	5 (16.1%)	13 (43.3%)	
IV	6 (19.4%)	5 (16.7%)	
Metastasis			1.0[b]
-	25 (80.6%)	25 (83.3%)	
+	6 (19.4%)	5 (16.7%)	

[a]Student's t test; [b]Fisher's exact test; [c]two-way ANOVA. SD, standard deviation; BMI, body mass index; UICC, Union for International Cancer Control.

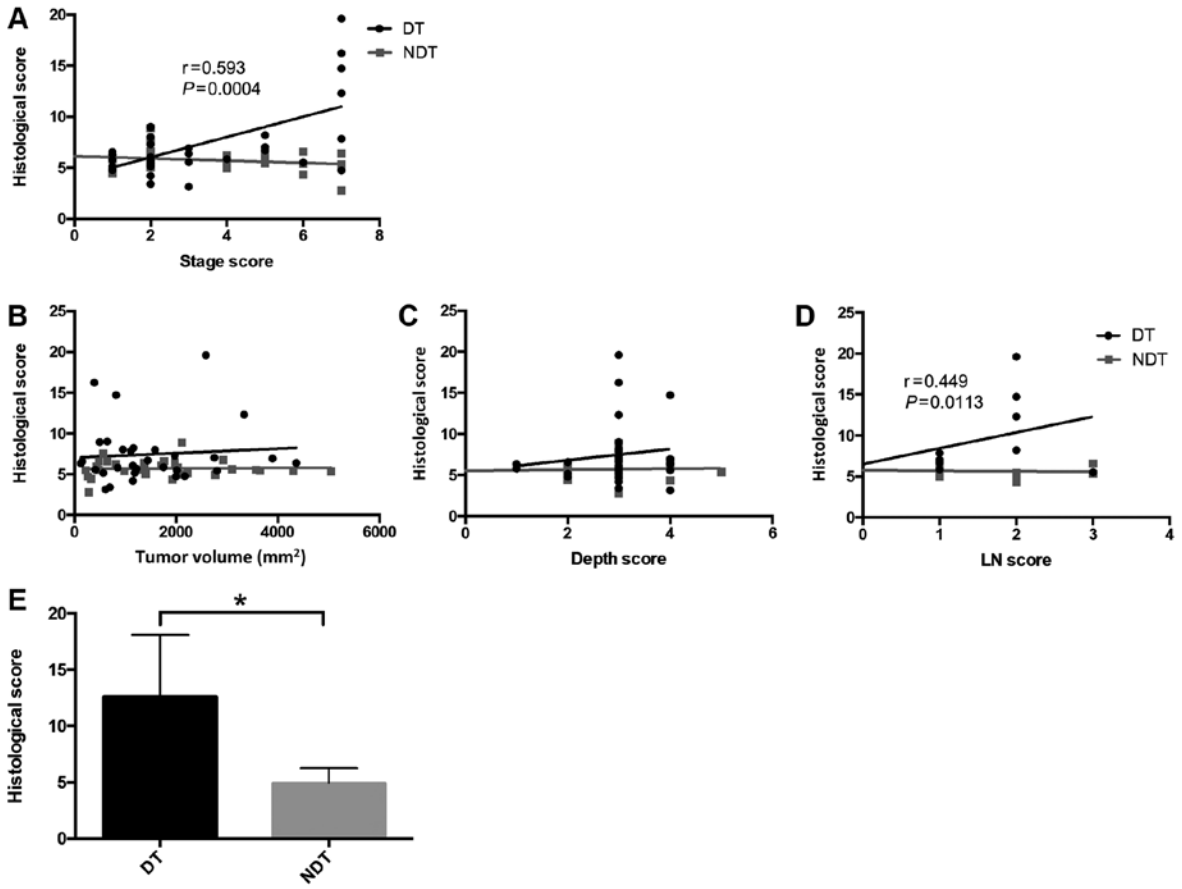

Figure 2. *O*-GlcNAcylation is correlated with cancer stage, particularly, with distant metastasis. (A) Correlation between histological score of *O*-GlcNAcylation and stage score. Stage score was defined as UICC stage I=0, IIA=1, IIB=2, IIC=3, IIIA=4, IIIB=5, IIIC=6, IVA=7 and IVB=8. (B) Correlation between the histological score and tumor volume. Tumor volume was calculated by multiplying the major tumor axis by the minor tumor axis. (C) Correlation between the histological score and depth score. Depth score was defined by the depth of tumor invasion as M=0, SM=1, MP=2, SS/A=3, SE=4, SI/AI=5 points. (D) Correlation between the histological score and LN score. LN score was defined by lymph node metastasis as UICC N0=0, N1=1, N2a=2, N2b=3 points. (E) Comparison between T_2DM (n=6) and non-T_2DM groups (n=5) of the histological score in cases with metastasis. *P=0.0147. Statistical analyses were performed using Pearson's correlation analysis for (A-D) and Student t-tests for (E). T_2DM, type 2 diabetes mellitus; UICC, the Union for International Cancer Control; LN, lymph node.

scores of O-GlcNAcylation in the DM and NDM groups according to colorectal cancer stage. In the DM group, the histological score increased with cancer progression, revealing a correlation between cancer stage and histological score. In contrast, such a tendency was not found in the NDM group (Fig. 2A). These findings suggest that in colorectal cancer and comorbid T_2DM, *O*-GlcNAcylation may play a more important role in cancer progression than in colorectal cancer alone.

In patients with T_2DM and colorectal cancer, O-GlcNAcylation is correlated with distant metastasis. In the TNM classification established by the Union for International Cancer Control (UICC), cancer is staged on the basis of tumor size and depth of invasion (T factor), lymph node status (N factor), and presence or absence of distant metastasis (M factor). To examine which of these factors promoted *O*-GlcNAcylation, we examined the correlations among histological scores, tumor volume, depth of invasion, lymph node metastasis, and distant metastasis. No correlations were found between histological score and tumor volume or depth of invasion; however, there was a weak correlation with lymph node metastasis (Fig. 2B-D). Moreover, in patients with distant metastasis, the histological score was significantly higher in the DM group than in the NDM group (Fig. 2E). These data suggested that in patients with DM, *O*-GlcNAcylation may be involved in the distant metastasis of colorectal cancer.

In patients with T_2DM, the progression of colorectal cancer is accompanied by increased intracellular accumulation of β-catenin in epithelial cells. Using immunohistochemical staining, we examined the expression of β-catenin, which is involved in cancer progression, in patients with colorectal cancer with or without T_2DM. In patients with less advanced cancer stages (e.g., stage IIA), no differences were found between the two groups in terms of immunoreactivity; however, in those with advanced cancer stages (e.g., stage IV), staining for β-catenin was stronger in cases of colorectal cancer with comorbid T_2DM than in those without T_2DM. In addition, β-catenin expression was higher in patients with advanced cancer than in patients with earlier cancer (Fig. 3A and B). β-catenin was localized in the cytoplasm (Fig. 3A). These results suggested that β-catenin accumulation in the cytoplasm might be involved in the progression of colorectal cancer, particularly in patients with T_2DM.

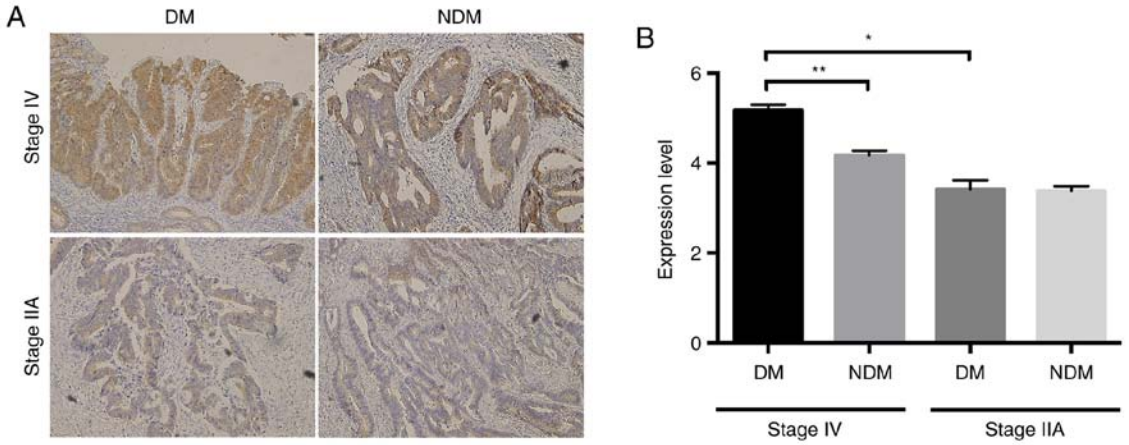

Figure 3. β-catenin expression is enhanced in advanced-stage cancer in patients with T_2DM. (A) Immunohistochemical staining for β-catenin. Magnification, x200. DM, colorectal cancer tissues from patients with T_2DM; NDM, colorectal cancer tissues from patients without T_2DM. (B) Quantitative analysis of β-catenin expression based on immunohistochemical staining. Four cases from each of the DM and NDM groups for patients with stage IV or stage IIA cancer were analyzed. β-catenin expression was defined as the integrated density of brown color from immunohistochemical staining measured using ImageJ software. *P<0.0001, **P<0.0001, Tukey's test. T_2DM, type 2 diabetes mellitus.

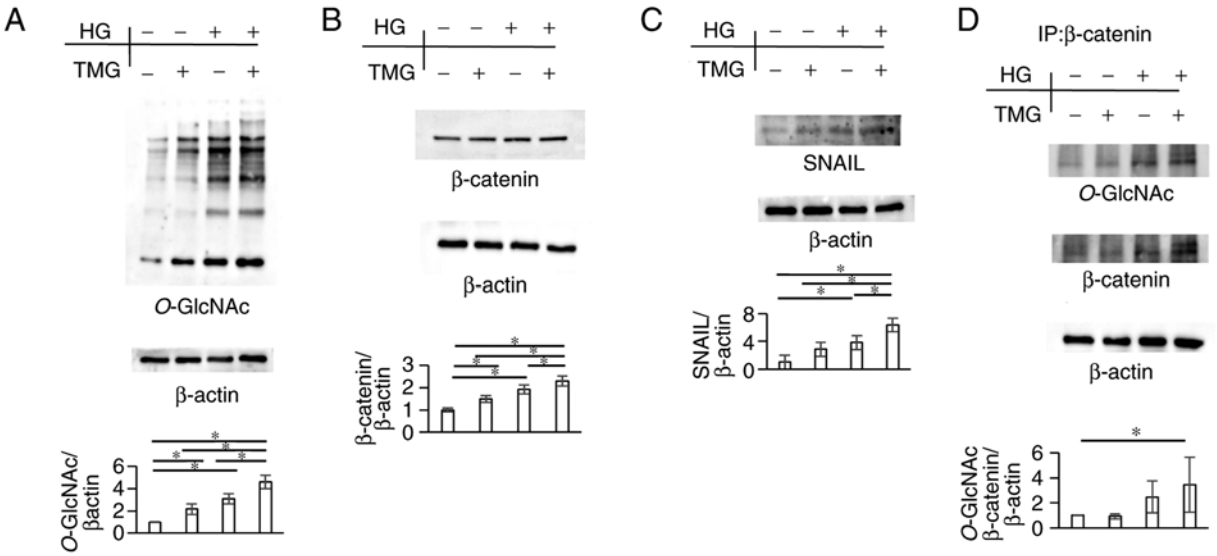

Figure 4. TMG treatment in high-glucose condition induces the β-catenin/SNAIL signaling pathway. SW480 human colon cancer cells were cultured in the presence or absence of 1 μM TMG for 36 h in normal (5 mM) or high (25 mM) glucose. (A-C) The cells were lysed and subjected to western blotting with (A) anti-*O*-GlcNAc, (B) β-catenin and (C) SNAIL antibodies. Densitometry was performed using ImageJ. *P<0.05, Tukey's test. (D) The lysates were also co-immunoprecipitated with anti-β-catenin, followed by probing with anti-*O*-GlcNAc and anti-β-catenin antibodies to determine *O*-GlcNAcylated β-catenin. β-actin was used as a loading control. *P<0.05, Dunnett's test. TMG, thiamet G.

Blockage of OGA in high-glucose condition upregulates β-catenin/SNAIL signaling in human colon cancer cells. Next, we examined whether the β-catenin/SNAIL signaling pathway is upregulated by augmented O-GlcNAcylation and whether the upregulation is amplified in high-glucose condition, using SW480 cells. After the cells were cultured in the presence or absence of 1 μM TMG in normal- or high-glucose condition for 36 h, the expression levels of β-catenin and SNAIL were determined by western blot analyses. While both TMG treatment and high glucose significantly increased *O*-GlcNAcylation, a synergistic increase was observed under TMG treatment in high-glucose condition (Fig. 4A). TMG treatment in normal-glucose condition slightly, albeit insignificantly, increased the expression of β-catenin and SNAIL (Fig. 4B and C, left 2 lanes). In contrast, TMG treatment in high-glucose condition significantly increased the expression of both proteins (Fig. 4B and C, right 2 lanes). A co-immunoprecipitation assay showed that TMG treatment did not increase *O*-GlcNAcylated β-catenin in normal glucose, whereas *O*-GlcNAcylation was significantly increased in high glucose (Fig. 4D). To confirm that the elevation of protein *O*-GlcNAcylation affected the metastatic phenotype of colon cancer cells as indicated by the high β-catenin and SNAIL expression levels, we used siOGA to increase protein *O*-GlcNAcylation genetically. SW480 cells were treated with siOGA1 or 2 and then subjected to western blot analysis. OGA expression in SW480 cells was effectively decreased by siOGA transfection (Fig. 5A), and, subsequently, protein *O*-GlcNAcylation was increased (Fig. 5B). The

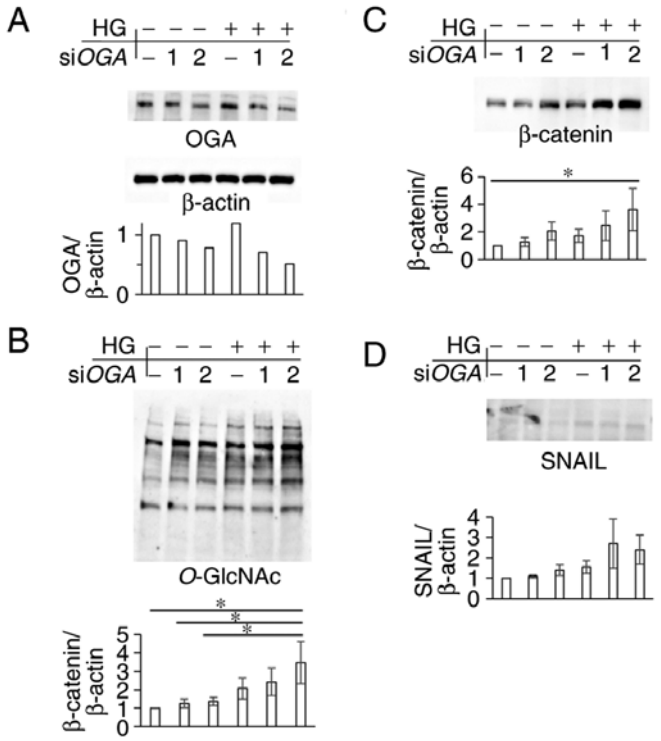

Figure 5. siRNA-mediated silencing of *OGA* in high-glucose condition induces β-catenin and SNAIL. si*OGA* 1, si*OGA* 2, or control siRNA (-) was transfected into SW480 cells. Twenty-four hours later, the cells were cultured in normal (5 mM) or high (25 mM) glucose for another 36 h. The cells were lysed and subjected to western blot analysis using anti-OGA, β-actin (A), *O*-GlcNAc (B), β-catenin (C), and SNAIL (D) antibodies. Densitometric was performed using ImageJ (B-D). β-actin (A) was used as a loading control and for normalization in densitometry. *P<0.05, Tukey's test. si, small interfering; OGA, *O*-GlcNAcase.

expression levels of β-catenin and SNAIL were increased in high-glucose condition after silencing of OGA (Fig. 5C and D), which was consistent with the findings in TMG-treated cells. To evaluate whether the effect of augmented *O*-GlcNAcylation observed in SW480 cells can be generalized to other colon cancer cells, we used another human colon cancer cell line, LoVo. The β-catenin/SNAIL signaling pathway was upregulated in high-glucose, but not in normal-glucose condition in LoVo cells after TMG treatment, consistent with the findings in SW480 cells (Fig. S2). These data suggested that high glucose has a synergistic effect on the upregulation of the β-catenin/SNAIL signaling pathway, probably through *O*-GlcNAcylation of β-catenin, in colorectal cancer tissues where *O*-GlcNAcylation is augmented.

Discussion

It is well known that patients with T_2DM are at significantly higher risk for many types of cancer, but potential biological links between the two diseases are not fully elucidated. Here, we showed one of the mechanisms through which T_2DM influences the prognosis of colorectal cancer patients. There was a significant correlation between *O*-GlcNAcylation and cancer stage only in the DM group. However, a comparison based on factors regulating the stage of cancer progression revealed no association between *O*-GlcNAcylation and horizontal or vertical progression, namely infiltration (Fig. 2B and C), whereas a weak association was found with lymph node metastasis (Fig. 2D). Additionally, in patients with metastasis to other organs, *O*-GlcNAcylation was more strongly enhanced in the DM group (Fig. 2E), suggesting that *O*-GlcNAcylation may play a more important role in the distant metastasis of colorectal cancer with comorbid T_2DM than in that of colorectal cancer without T_2DM.

Epithelial-mesenchymal transition (EMT) is believed to be required for cancers to undergo metastasis (18,19). In healthy living organisms, EMT is involved in embryogenesis, organ formation, wound healing, and fibrosis, and contributes to the maintenance of homeostasis (20). However, in cancer cells, the transition of epithelial cells toward highly mobile mesenchymal cells causes a loosening of intercellular adhesion; as a result, cancer cells are more likely to infiltrate by passing through the basal membrane (21). Various molecules, including β-catenin, are involved in EMT.

Abnormal activation of the β-catenin/SNAIL signaling pathway has been found in various types of malignant tumors, including colorectal cancer (22,23). Studies in breast cancer and oral squamous cell carcinoma have shown that enhancement of the β-catenin/SNAIL signaling pathway is associated with EMT and cancer cell invasiveness (24,25). According to our immunohistochemical analysis, β-catenin expression was strongly enhanced in patients with stage IV colorectal cancers with comorbid T_2DM (Fig. 3A and B). Thus, EMT mediated by β-catenin/SNAIL signaling may be more strongly involved in the metastasis of colorectal cancer with comorbid T_2DM.

Our findings suggest that *O*-GlcNAcylation, which was enhanced in colorectal cancer tissues from patients with T_2DM, may be involved in the development of distant metastases. Given that this was a retrospective study and that there were no cases of early-stage cancer with markedly enhanced *O*-GlcNAcylation, the possibility of enhancement of *O*-GlcNAcylation as a result of the development of distant metastases cannot be ruled out. However, based on the findings from recent molecular biology studies of EMT (26,27), we hypothesize that enhanced *O*-GlcNAcylation of β-catenin in colorectal cancer tissues of patients with T_2DM may have promoted β-catenin/SNAIL-mediated EMT, which may have promoted the development of distant metastases. For example, Jiang *et al* reported that *O*-GlcNAcylation, which is negatively regulated by *miR-101*, likely promotes colorectal cancer metastasis by enhancing enhancer of zeste homolog 2 (EZH2) protein stability and function, which plays important roles in EMT (28). In turn, *O*-GlcNAcylation in cancer cells is promoted, stimulating EMT-mediated metastasis in colorectal cancer (28). In addition, according to Ling *et al*, EMT in podocytes is controlled by demethylation of the promoter region of *MMP-9*, which occurs under hyperglycemic conditions in diabetic nephropathy (29). These findings strongly support our hypothesis that EMT may be involved in the development of distant metastasis in the presence of colorectal cancer and hyperglycemia.

In conclusion, we found that *O*-GlcNAcylation was more strongly enhanced in colorectal cancer tissues from patients with T_2DM than in those from patients without T_2DM. Moreover, it was shown that this modification promoted

cancer progression, particularly the development of distant metastasis, through upregulation of the β-catenin/SNAIL pathway, probably through *O*-GlcNAcylation of β-catenin. *O*-GlcNAcylation has been shown to be enhanced in cancer tissues and in patients with diabetes, although no previous studies have examined *O*-GlcNAcylation in cancer tissues from patients with T_2DMtional studies with more participants are needed to elucidate the underlying mechanisms; however, our findings provided important insights into the development and etiopathology of colorectal cancers with comorbid T_2DM, and may facilitate the establishment of therapeutic strategies targeting *O*-GlcNAcylation for the treatment and prevention of colorectal cancer with comorbid T_2DM.

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Availability of data and materials

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

Authors' contributions

TO, MA and KH designed the current study. YN, TO, TN, EK, YK, YT, HT, YHira, KKaw, and KKak conducted the current study. TI, TT, SF, YHiro and KU analyzed the data. TO, TN, and MA wrote the manuscript. KH revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

This study was approved by the ethics review committee of Osaka Medical College. All patients provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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