

O-linked β -N-acetylglucosamine transferase is involved in pro-opiomelanocortin gene expression in mouse pituitary corticotroph AtT-20 cells

Koshi Makita^{a,1}, Shinobu Takayasu^{a,1,*}, Mari Usutani^a, Yuki Nakada-Nakayama^a, Kazunori Kageyama^a, Akira Sugawara^b, Makoto Daimon^a

^a Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine and Hospital, Hirosaki, Aomori, Japan

^b Department of Molecular Endocrinology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan

ARTICLE INFO

Keywords:

O-linked β -N-acetylglucosamine transferase
Pro-opiomelanocortin
Glucocorticoid
Cushing's disease

ABSTRACT

Glucocorticoids and glucocorticoid receptors (GRs) suppress pituitary pro-opiomelanocortin (*Pomc*) gene expression. O-linked β -N-acetylglucosamine (O-GlcNAc) modification, mediated by O-GlcNAc transferase (OGT), plays an important role during gene transcription. However, whether OGT is involved in the GR-mediated transrepression that occurs in pituitary corticotroph cells is currently unknown. Here, we report that OGT regulates *Pomc* expression in the mouse corticotroph cell line AtT-20. The overexpression of OGT has an additive effect on the GR-mediated negative transcription pathway. Both the knockdown of OGT by RNA interference and the use of a chemical OGT inhibitor abolished the repressive effects of *Pomc* expression induced by GRs. OGT inhibition leads to both the decreased recruitment of GRs and the increased recruitment of RNA polymerase II to the *Pomc* locus. O-GlcNAc modification is involved in the negative regulation of *Pomc* transcription in corticotroph cells. OGT may be a promising therapeutic target for the treatment of Cushing's disease.

1. Introduction

The pituitary pro-opiomelanocortin (*Pomc*) gene encodes the precursor protein necessary for adrenocorticotrophic hormone (ACTH) synthesis, which is the center of the hypothalamo-pituitary-adrenal axis. The hypothalamic corticotropin-releasing factor activates *Pomc* transcription and ACTH secretion. ACTH stimulates the synthesis and release of glucocorticoids (GCs) from the adrenal gland. GCs exert negative feedback on both pituitary corticotroph cells and hypothalamic corticotropin-releasing factor expressing neurons to maintain physiological levels of GCs. ACTH-producing pituitary tumors are resistant to GC-mediated negative feedback [1]. These tumors, which are defined as Cushing's disease (CD), result from chronic exposure to excessive cortisol production and secretion from the adrenal cortex. Patients with CD have increased risks of immune dysfunction, venous thromboembolism, and arterial thrombi. Hypertension, glucose intolerance, dyslipidemia, and osteoporosis are common complications observed in patients with

CD. Complications associated with CD can greatly affect quality of life and mortality. The primary treatment for CD is the surgical excision of the tumor; however, further therapeutic options are desirable in cases of unsuccessful excision. Cabergoline and somatostatin analogs can be used to suppress the production of ACTH, but their effects are limited. Clarifying the molecular defects involved in *Pomc* transcription is necessary to develop novel therapeutic options for CD.

O-linked β -N-acetylglucosamine (O-GlcNAc) modifies a variety of nuclear and cytoplasmic proteins at serine and threonine residues [2]. O-GlcNAc transferase (OGT) attaches O-GlcNAc to proteins, while O-GlcNAcase removes O-GlcNAc from proteins. This modification plays important roles in many regulatory pathways, such as transcription and translation, nutrient sensing, neuronal function, cell cycle, and stress [3]. The OGT-mediated O-GlcNAc modification of the C-terminal domain (CTD) of RNA polymerase II (Pol II) is involved in the gene transrepression mechanism induced by GCs [4,5]. The nuclear factor- κ B (NF- κ B) family member NF- κ B subunit RELA/p65 has been shown to be

Abbreviations: CD, Cushing's disease; CTD, C-terminal domain; GC, glucocorticoid; GR, glucocorticoid receptor; O-GlcNAc, O-linked β -N-acetylglucosamine; OGT, O-GlcNAc transferase; Pol II, RNA polymerase II

* Corresponding author at: Department of Endocrinology and Metabolism, Hirosaki University Graduate School of Medicine and Hospital, 5 Zaifu-cho, Hirosaki, Aomori 036-8562, Japan.

E-mail address: stakayas@hirosaki-u.ac.jp (S. Takayasu).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.neulet.2019.134407>

Received 23 April 2019; Received in revised form 12 July 2019; Accepted 29 July 2019

Available online 29 July 2019

0304-3940/ © 2019 Elsevier B.V. All rights reserved.

modified by OGT, and OGT is required for the E1A binding protein p300-mediated acetylation of chromatin-associated RELA during NF- κ B transcriptional activation [6].

We previously reported the involvement of NF- κ B and nuclear receptor 4A2 during the cytokine-induced transcription of *Pomc* in a pituitary corticotroph model cell line, AtT-20 [7]. GC receptors (GRs) mediate anti-inflammatory effects by directly binding to NF- κ B and AP-1, inhibiting their transcriptional activities [8]. Therefore, we examined whether O-GlcNAc modification suppresses *Pomc* expression and whether the GC/GR complex is involved in *Pomc* suppression. Here, we report that OGT represses the expression of *Pomc* in AtT-20 cells. *Ogt* knockdown or the use of an OGT inhibitor, STO45849, attenuated the GR-mediated reduction of *Pomc* expression. The suppression of *Pomc* expression is mediated by reduced GR recruitment at the *Pomc* locus, due to O-GlcNAc modification.

2. Materials and methods

2.1. Materials

STO45849 (STO) was purchased from Tim Tec (DE, USA).

2.2. Reverse transcription polymerase chain reaction

Reverse transcription-polymerase chain reaction (RT-PCR) was performed as described previously [7]. Total RNA was extracted using the RNeasy Mini Kit (Qiagen Sciences, Hilden, Germany), following the manufacturer's instructions. cDNAs were synthesized from total RNA (500 ng), with random hexamer as the primer, using the SuperScript First Strand Synthesis System for RT-PCR Kit (Thermo Fisher Scientific Waltham, MA, USA), according to the manufacturer's instructions. The obtained first-strand cDNAs were then used as templates for subsequent PCR reactions using Taq DNA polymerase. The primers used were as follows: *Ogt*: forward 5'-GCTGAGCAGCTAGAGAAGAATAG-3', and reverse 5'-TGCCTGGAATAGACTGCATAAG-3'.

2.3. Quantitative RT-PCR

Real-time RT-PCR was performed as described previously [7], with some modifications. The expression levels of *Pomc* and *Ogt* mRNA were evaluated using real-time quantitative PCR (qPCR), with specific sets of primers and probes (Thermo Fisher Scientific). Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) was used as a reference gene to standardize expression levels. Each reaction consisted of 1 \times TaqMan Universal PCR Master Mix (Thermo Fisher Scientific), 1 \times Assays-on-Demand Gene Expression Products (Mm00435874_m1 for *Pomc*, Mm00507317_m1 for *Ogt*, and Mm99999915_g1 for *Gapdh*), and 2 μ l of cDNA, in a total volume of 25 μ l, using the following parameters on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific): 95 $^{\circ}$ C for 10 min, and then 40 cycles of 95 $^{\circ}$ C for 15 s and 60 $^{\circ}$ C for 1 min. All expression data were determined as a function of the threshold cycle (CT) for quantitative analyses, using the StepOnePlus Real-Time PCR System.

2.4. RNA interference experiments

Control and *Ogt* Smartpool siRNAs were obtained from Thermo Fisher Scientific (for control and *Ogt*), and 20 μ M siRNA were introduced into 50,000 AtT-20 cells, which were cultured in 12-well plate for 72 h, using Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher Scientific), following the manufacturer's instructions, as described previously [9].

2.5. Plasmid construction

The plasmid POMC-Luc was described previously [7]. The OGT

expression vector was constructed by inserting the mouse *Ogt* cDNA into the pRc/RSV plasmid. HindIII and KpnI sites were engineered, and *Ogt* cDNA was obtained by PCR from a cDNA library of the mouse corticotroph cell line AtT-20 using the following primer set: forward 5'-ATGGCGTCTCCGTGGGCAA-3', and reverse 5'-TCAGGCTGACTCG GTGACTTCAA-3'.

2.6. Transfection and cell culture

Cell culture and transfections were performed as described previously [7], with some modifications. AtT-20 cells (D16v) were cultured in 10-cm-diameter culture dishes with low-glucose Dulbecco Modified Eagle Medium (DMEM, Wako, Osaka, Japan), supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin and 100 U/mL penicillin, in a 5% CO₂-95% air atmosphere, at 37 $^{\circ}$ C. The cells were plated in 24-well plates at approximately 70% confluence and transfected transiently with the test plasmid DNA, using X-tremeGENE 9 DNA Transfection Reagent (Roche, Basel, Switzerland), for 24 h.

2.7. Luciferase assay

The luciferase assay was performed as previously described [7,10].

2.8. Western blotting

Western blotting was performed as previously described [10,11], with some modifications. AtT-20 cells were cultured in 12-well plates. The anti-OGT antibody (sc-32921; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and anti-O-GlcNAc antibody (ab2739; abcam, Cambridge, UK) were used.

2.9. Cell proliferation assay

The cells were incubated at 37 $^{\circ}$ C for 72 h with the indicated concentrations of si*Ogt* or 48 h with STO. Viable cells were measured using a Cell Counting Kit-8 (Dojin, Kumamoto, Japan). All samples from each experiment were measured in the same assay.

2.10. Chromatin Immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation (ChIP) assay was performed and analyzed as described previously, with some modifications [9]. The sonication buffer was composed of 20 mM Tris-HCl, pH 8, 150 mM NaCl, 2 mM EDTA, 0.1% SDS, and 1% Triton X-100. We used a sonicator (TOMY ultrasonic disruptor UR-20 P) and sonicated the samples at approximately 50 W using 20 \times 2 s pulses for 5 cycles (60 s pause between cycles). Samples were kept on ice at all times. The extract was incubated overnight at 4 $^{\circ}$ C with mixture of 20 μ l protein A and 20 μ l protein G Dynabeads (Invitrogen), which were pre-incubated with 6 μ g of the appropriate antibodies. Beads were washed once with the sonication buffer, once with a buffer composed of 20 mM Tris-HCl, pH 8.0, 500 mM NaCl, 2 mM EDTA, 0.1% SDS, and 1% Triton X-100, once with a buffer composed of 10 mM Tris-HCl, pH 8.0, 250 mM LiCl, 2 mM EDTA, and 1% NP40, and once with Tris-EDTA buffer (TE) containing 50 mM NaCl. qPCR was performed using a StepOnePlus Real-Time PCR System, and the results were analyzed using the accompanying software.

2.11. Data analyses

Each value is expressed as the mean \pm standard error of the mean (SEM). Statistical analyses were performed using analysis of variance (ANOVA), followed by *post hoc* test. The level of statistical significance was set at $P < 0.05$.

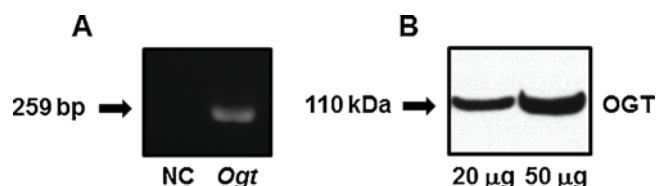


Fig. 1. (A) Expression of *Ogt* mRNA (259 bp, NC; negative control), amplified by RT-PCR analysis using AtT-20 cells. (B) Expression of OGT protein. Loading amounts of protein for western blotting are indicated.

3. Results

3.1. OGT expression in AtT-20 cells and the effects of OGT and GCs on *Pomc* promoter activity

We first examined whether OGT is expressed in AtT-20 cells. RT-PCR analysis showed *Ogt* mRNA expression that corresponded with OGT protein expression (Figs. 1A and B). To assess the roles played by OGT and GCs during *Pomc* transcription in AtT-20 cells, we performed a luciferase assay using POMC-Luc. POMC-Luc was significantly suppressed by both OGT and the synthetic GC dexamethasone (DEX), and the response to these combined treatments showed additive effects (0.78 ± 0.04 , 0.77 ± 0.03 , and 0.61 ± 0.04 , respectively) (Fig. 2A). However, the overexpression of OGT did not affect the activity of GR-dependent transcription via glucocorticoid response elements (Fig. 2B).

3.2. Effect of *Ogt* knockdown on *Pomc* expression

To determine whether OGT is necessary for the repression of *Pomc* expression, we examined the effects of *Ogt* siRNA. Increased amount of *Ogt* siRNA resulted in gradual decrease of cell proliferation (Fig. 3A). Treatment with 20 μ M siOgt did not show any effects on O-GlcNAc protein levels in AtT-20 cells (Fig. 3B), while that treatment resulted in reductions of approximately 55% and 25% for *Ogt* mRNA and OGT protein expression levels, respectively (Figs. 3C and D). DEX treatment did not affect *Ogt* mRNA expression levels (Fig. 3C left panel). *Ogt* knockdown did not affect *Pomc* expression under basal conditions (1.00 ± 0.04 (control) vs 0.90 ± 0.04 , ANOVA; $P = 0.12$) (Fig. 3C, right panel). Surprisingly, siOgt significantly attenuated the DEX-mediated reduction of *Pomc* expression (0.49 ± 0.04 vs 0.61 ± 0.06 , ANOVA; $P < 0.05$) (Fig. 3C, right panel).

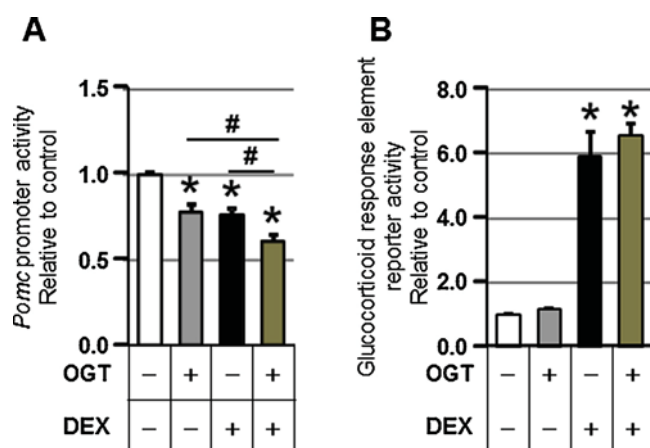


Fig. 2. Effects of OGT overexpression and/or 100 nM DEX treatment on *Pomc* promoter activity (A) and transcriptional activity of glucocorticoid response elements reporter plasmids (B) in AtT-20 cells. Statistical analyses were performed using a ANOVA, followed by a *post hoc* test. * $P < 0.05$ (compared with control), # $P < 0.05$.

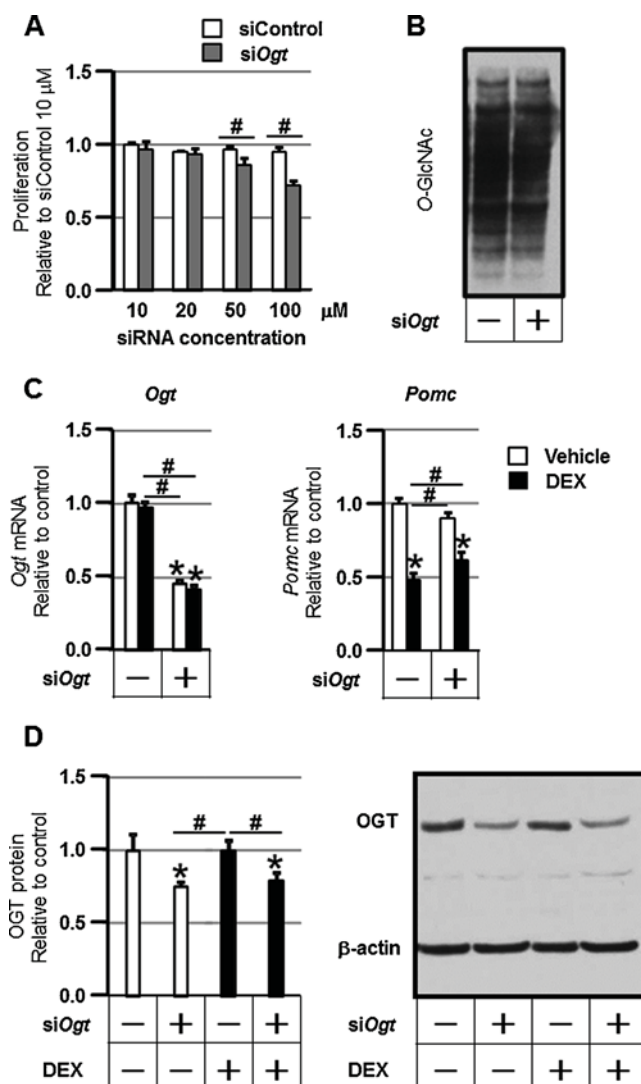


Fig. 3. (A) Effects of *Ogt* knockdown on cell proliferation in AtT-20 cells. Data are presented as the levels of cell proliferation relative to that for 10 μ M siControl condition. Cells were treated at least in triplicate, and the average of three independent experiments is shown. (B) Effects of 20 μ M siOgt on O-GlcNAc protein levels in AtT-20 cells, as analyzed by western blotting. (C) Effects of *Ogt* knockdown on *Ogt* (left panel) and *Pomc* (right panel) mRNA expression levels in AtT-20 cells were analyzed by qPCR. Cells were incubated for 24 h with either vehicle or 100 nM DEX. Data are presented as amounts relative to the levels in cells treated with siControl and vehicle (control). Cells were treated at least in duplicate, and the averages of at least three independent experiments are shown. (D) Effects of *Ogt* knockdown on OGT protein expression in AtT-20 cells, as analyzed by western blotting. Cells were incubated for 24 h with either vehicle or 100 nM DEX. β -actin was used as a housekeeping protein. Three independent experiments were averaged for analysis (left panel), and a representative blot is shown (right panel). Statistical analyses were performed using a ANOVA, followed by a *post hoc* test. * $P < 0.05$ (compared with control), # $P < 0.05$.

3.3. Effect of the OGT inhibitor STO on *Pomc* transcription

We further examined the necessity of OGT during *Pomc* expression using an OGT inhibitor. First, AtT-20 cells were incubated with STO to determine its dose-dependent effects on cell proliferation. This study showed that 100 μ M STO significantly decreased cell proliferation, while 10 μ M STO had no effects on proliferation, regardless the presence or absence of DEX (Fig. 4A). Treatment with 10 μ M STO did not affect O-GlcNAc protein modification in AtT-20 cells (Fig. 4B). At 10 μ M STO, the DEX-mediated reduction of *Pomc* expression was slightly, but

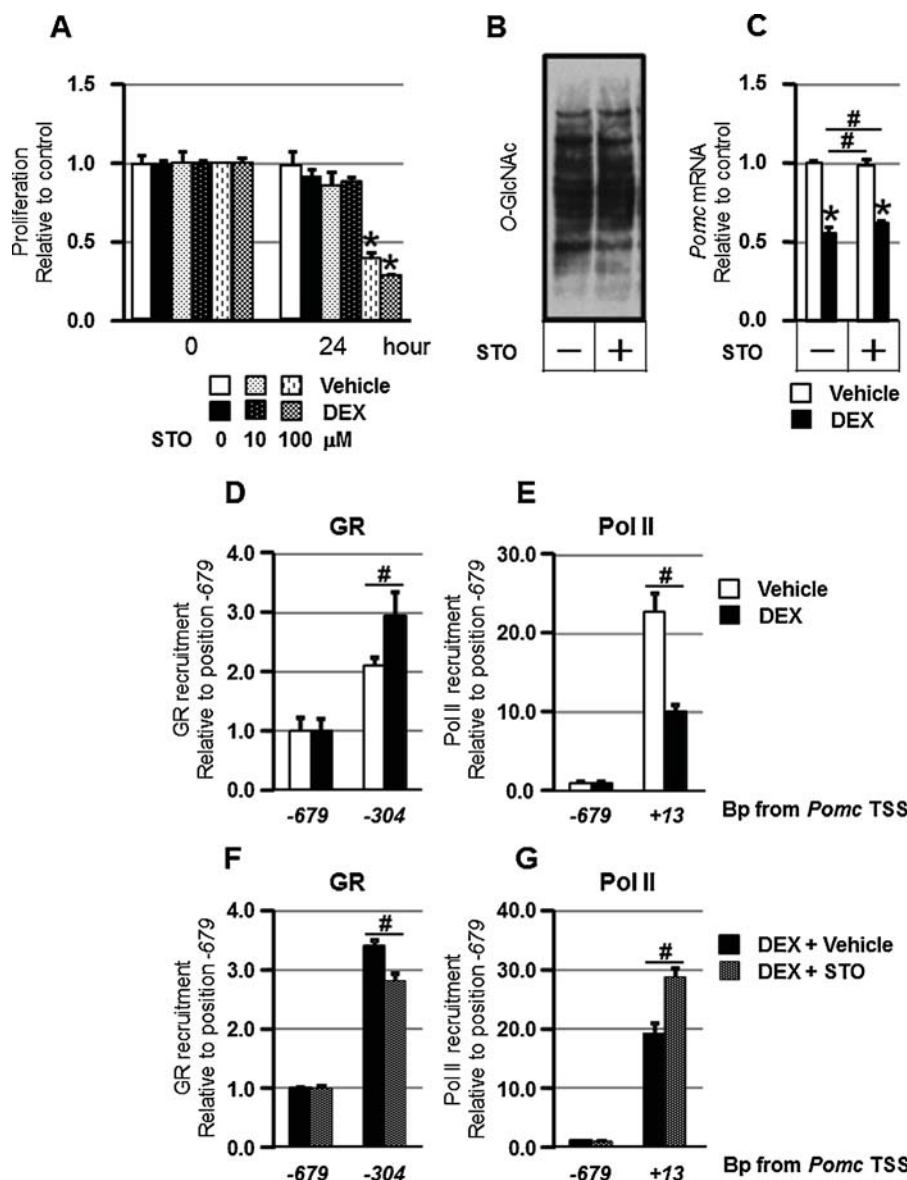


Fig. 4. (A) Dose-response effects of STO on cell proliferation in AtT-20 cells. Cells were pre-incubated for 1 h with STO, and then incubated for 24 h with either vehicle or 100 nM DEX. Data are presented as the levels of cell proliferation relative to that for each basal condition. Cells were treated in triplicate, and the average of two independent experiments is shown. (B) Effects of 10 μ M STO on O-GlcNAc protein modification in AtT-20 cells, as analyzed by western blotting. (C) Effects of 10 μ M STO on *Pomc* mRNA expression by qPCR. Cells were pre-incubated for 1 h with STO, and then incubated for 24 h with either vehicle or 100 nM DEX. Data are presented as the levels relative to those for the condition without STO or DEX treatment (control). Cells were treated in duplicate, and the average of four independent experiments is shown. (D–G) ChIP analysis of AtT-20 cells. GR recruitment to the *Pomc* -300 bp enhancer and Pol II recruitment to the *Pomc* transcription start site were assessed. Cells were incubated for 20 min with either vehicle or 100 nM DEX (D, E). Cells were pre-incubated for 24 h with STO, and then incubated for 20 min with 100 nM DEX (F, G). DNA levels were analyzed by qPCR, and the average of at least three independent experiments is shown. Statistical analyses were performed using a ANOVA, followed by a *post hoc* test. * $P < 0.05$ (compared with control), # $P < 0.05$.

significantly, attenuated (0.56 ± 0.04 vs 0.62 ± 0.01 , ANOVA; $P < 0.05$) (Fig. 4C).

We then assessed the recruitment of GR and Pol II to the *Pomc* locus. As we observed during the ChIP-seq analysis [9], GR was recruited to the *Pomc* -300 bp enhancer, while Pol II was recruited to the *Pomc* transcription start site. The levels of GR and Pol II recruitment were increased and decreased by DEX, respectively (Fig. 4D and E). STO reduced the levels of DEX-mediated GR recruitment and attenuated the DEX-induced reduction of Pol II recruitment (Fig. 4F and G).

4. Discussion

In the present study, we showed that OGT potently reduced *Pomc* transcriptional activity. GCs and their receptors, GRs, exert negative feedback on pituitary corticotroph cells, and the transcription of *Pomc* is subject to GC/GR-induced repression. In addition, the overexpression of OGT has been reported to potentiate GR-mediated transrepression, whereas OGT depletion by siRNA has been reported to abolish this repression [5]. However, in this study, the reducing effect of OGT on *Pomc* transcription levels was independent of the GR-mediated effect. These results indicate that OGT is essential for the transcriptional repression of *Pomc* transcriptional activity. To react strongly to an

immune suppressor, OGT and GR may exert additive effects on the repression of *Pomc* transcriptional activity.

O-GlcNAcylation has been reported to modulate cell proliferation, survival, and apoptosis [5,12–14]. We examined whether OGT contributes to cell proliferation and *Pomc* transcription. We confirmed that both large amounts of *Ogt* siRNA and a high concentration of STO cause decreased cell proliferation in AtT-20 cells. These results suggest that O-GlcNAcylation can maintain cell growth and proliferation in pituitary corticotroph cells. GC/GR has potent inhibitory effects on pituitary corticotroph cell proliferation, and the growth of AtT-20 cells is inhibited by GC/GR [9]. Because the inhibitory effects of GC/GR appeared within 2 days after culture [9], the effects of siOgt and OGT inhibition were examined within 24 h after treatment with DEX to avoid cell growth effects. In this condition, neither siOgt nor OGT inhibition showed any effects on *Pomc* transcription in basal conditions. However, both siOgt and OGT inhibitor reduced *Pomc* transrepression by 24 h after DEX treatment.

Some studies [5,15] have reported that the O-GlcNAc modification is involved in the subcellular localization of transcription factors. Li et al. suggested that the recruitment of OGT by ligand-bound GRs resulted in increased levels of O-GlcNAcylated Pol II-CTD and decreased levels of phosphorylated Pol-II-CTD at the proximal promoter region of

the target genes [5]. ChIP experiments showed that the OGT inhibitor reduced the DEX-mediated recruitment of GRs and attenuated the DEX-induced reduction of Pol II recruitment. These data support the hypothesis that the inhibition of OGT mediates the GC-induced repression of *Pomc* transcription by regulating GR recruitment, leading to changes in Pol II recruitment or activity. These results may represent a protective mechanism for cell survival. When the levels of most OGTs fall below certain thresholds, decreased O-GlcNAc-modified proteins presumably affect cell proliferation and survival. Treatment with 20 μ M siOgt or 10 μ M STO did not show any significant effects on O-GlcNAc protein levels in AtT-20 cells. The concentration of each treatment may be equal to or smaller than the thresholds. In the condition, the remaining OGTs may be consumed to maintain cell proliferation and cellular activities in survival cells. As a result, decreasing amounts of the OGT/GR complex may lead to the increased recruitment of Pol II. Whether OGT regulates the O-GlcNAcylation of GR itself or the translocation of GR from the cytosol to the nucleus were not clarified in this study. Whether O-GlcNAcylation regulates the transcriptional initiation and pausing, clearance, or elongation of Pol II is also unknown. These mechanisms must be elucidated. However, medicines that increased the delivery of OGT to the *Pomc* locus, facilitate OGT function, or stabilize the O-GlcNAcylation of Pol II may represent therapeutic candidates for the treatment of CD.

An additional question is whether other proteins are involved in this repression mechanism. Bilodeau et al. showed that SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4 (SMARCA4/BRG1, hereafter BRG1) is required to stabilize interactions between GR and histone deacetylase 2 (HDAC2). The recruitments of GR and HDAC2 are ligand-dependent and result in the histone H4 deacetylation of the *Pomc* locus [16]. They also reported that the nuclear expression levels of BRG1 and HDAC2 are deficient in less than 50% of human and dog pituitary corticotroph adenoma cases, and these factors may contribute to GC-resistance [16]. This observation raises the possibility that the recruitment of the OGT/GR/BRG1/HDAC2 complex may act in concert with the transcriptional repression of *Pomc*. The misexpression or dysfunction of OGT may cause GC-resistance in CD.

Immunoprecipitation-grade OGT antibodies are currently not available for our experiments. Further study is required to determine the protein-protein interactions between OGT and GR and the recruitments of glycosylated and phosphorylated Pol II to the *Pomc* locus. Furthermore, whether the OGT inhibitor can decrease plasma ACTH levels and pituitary *Pomc* mRNA levels must also be elucidated in vivo.

In conclusion, we demonstrated important roles played by OGT during *Pomc* transcription in AtT-20 cells. OGT may be a promising therapeutic target for the treatment of CD.

Funding

This research was not funded through specific grants from any funding agencies in the public, commercial, or not-for-profit sectors.

Compliance with ethical standards

Human participants were not involved in this research.

Authors' contributions

All authors participated in writing the manuscript and approved the final manuscript.

Declaration of Competing Interest

None of the authors have any potential conflicts of interest associated with this research.

Acknowledgments

We thank Ms. Kanako Niioka for her excellent technical assistance. We thank Lisa Giles, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

References

- [1] J. Drouin, 60 YEARS OF POMC: transcriptional and epigenetic regulation of POMC gene expression, *J. Mol. Endocrinol.* 56 (2016) T99–T112, <https://doi.org/10.1530/JME-15-0289>.
- [2] N.E. Zachara, G.W. Hart, Cell signaling, the essential role of O-GlcNAc! *Biochim. Biophys. Acta.* 1761 (2006) 599–617, <https://doi.org/10.1016/j.bbali.2006.04.007>.
- [3] G.W. Hart, C. Slawson, G. Ramirez-Correa, O. Lagerlof, Cross talk between O-GlcNAcylation and phosphorylation: roles in signaling, transcription, and chronic disease, *Annu. Rev. Biochem.* 80 (2011) 825–858, <https://doi.org/10.1146/annurev-biochem-060608-102511>.
- [4] S.M. Ranuncolo, S. Ghosh, J.A. Hanover, G.W. Hart, B.A. Lewis, Evidence of the involvement of O-GlcNAc-modified human RNA polymerase II CTD in transcription in vitro and in vivo, *J. Biol. Chem.* 287 (2012) 23549–23561, <https://doi.org/10.1074/jbc.M111.330910>.
- [5] M.D. Li, H.B. Ruan, J.P. Singh, L. Zhao, T. Zhao, S. Azarhoush, J. Wu, R.M. Evans, X. Yang, O-GlcNAc transferase is involved in glucocorticoid receptor-mediated transrepression, *J. Biol. Chem.* 287 (2012) 12904–12912, <https://doi.org/10.1074/jbc.M111.303792>.
- [6] D.F. Allison, J.J. Wamsley, M. Kumar, D. Li, L.G. Gray, G.W. Hart, D.R. Jones, M.W. Mayo, Modification of RelA by O-linked N-acetylglucosamine links glucose metabolism to NF- κ B acetylation and transcription, *Proc. Natl. Acad. Sci. U.S.A.* 109 (2012) 16888–16893, <https://doi.org/10.1073/pnas.1208468109>.
- [7] S. Takayasu, Y. Iwasaki, T. Nigawara, M. Asai, M. Yoshida, K. Kageyama, T. Suda, Involvement of nuclear factor- κ B and Nurr-1 in cytokine-induced transcription of proopiomelanocortin gene in AtT20 corticotroph cells, *Neuroimmunomodulation* 17 (2010) 88–96, <https://doi.org/10.1159/000258691>.
- [8] C.K. Glass, K. Saijo, Nuclear receptor transrepression pathways that regulate inflammation in macrophages and T cells, *Nat. Rev. Immunol.* 10 (2010) 365–376, <https://doi.org/10.1038/nri2748>.
- [9] A. Roussel-Gervais, C. Couture, D. Langlais, S. Takayasu, A. Balsalobre, B.R. Rueda, Lr. Zukerberg, D. Figarella-Branger, T. Brue, J. Drouin, The Cables1 gene in glucocorticoid regulation of pituitary corticotrope growth and cushing disease, *J. Clin. Endocrinol. Metab.* 101 (2016) 513–522, <https://doi.org/10.1210/jc.2015-3324>.
- [10] Y. Watanuki, S. Takayasu, K. Kageyama, Y. Iwasaki, S. Sakihara, K. Terui, T. Nigawara, T. Suda, Involvement of Nurr-1/Nur77 in corticotropin-releasing factor/urocortin1-induced tyrosinase-related protein 1 gene transcription in human melanoma HMV-II cells, *Mol. Cell. Endocrinol.* 370 (2013) 42–51, <https://doi.org/10.1016/j.mce.2013.01.022>.
- [11] Y. Asari, K. Kageyama, Y. Nakada, M. Tasso, S. Takayasu, K. Niioka, N. Ishigame, M. Daimon, Inhibitory effects of a selective Jak2 inhibitor on adrenocorticotrophic hormone production and proliferation of corticotroph tumor AtT20 cells, *Onco. Ther.* 10 (2017) 4329–4338, <https://doi.org/10.2147/OTT.S141345>.
- [12] N. O'Donnell, N.E. Zachara, G.W. Hart, J.D. Marth, Ogt-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability, *Mol. Cell. Biol.* 24 (2004) 1680–1690, <https://doi.org/10.1128/MCB.24.4.1680-1690.2004>.
- [13] S.A. Caldwell, S.R. Jackson, K.S. Shahriari, T.P. Lynch, G. Sethi, S. Walker, K. Vosseller, M.J. Reginato, Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1, *Oncogene* 29 (2010) 2831–2842, <https://doi.org/10.1038/ncr.2010.41>.
- [14] L. Wang, S. Chen, Z. Zhang, J. Zhang, S. Mao, J. Zheng, Y. Xuan, M. Liu, K. Cai, W. Zhang, Y. Guo, W. Zhai, X. Yao, Suppressed OGT expression inhibits cell proliferation while inducing cell apoptosis in bladder cancer, *BMC Cancer* 18 (2018) 1141, <https://doi.org/10.1186/s12885-018-5033-y>.
- [15] S.S. Andrali, Q. Qian, S. Ozcan, Glucose mediates the translocation of NeuroD1 by O-linked glycosylation, *J. Biol. Chem.* 282 (2007) 15589–15596, <https://doi.org/10.1074/jbc.M701762200>.
- [16] S. Bilodeau, S. Vallette-Kasic, Y. Gauthier, D. Figarella-Branger, T. Brue, F. Berthelet, A. Lacroix, D. Batista, C. Stratakis, J. Hanson, B. Meij, J. Drouin, Role of Brg1 and HDAC2 in GR trans-repression of the pituitary POMC gene and mis-expression in Cushing disease, *Genes Dev.* 20 (2006) 2871–2886, <https://doi.org/10.1101/gad.1444606>.