Cisplatin enhances protein *O*-GlcNAcylation by altering the activity of OGT, OGA and AMPK in human non-small cell lung cancer cells

DI WANG*, JIAAN WU*, DANDAN WANG, XIAOYAN HUANG, NAINING ZHANG and YIKANG SHI

National Glycoengineering Research Center, Shandong Provincial Key Laboratory of Carbohydrate Chemistry and Glycobiology, Shandong University, Qingdao, Shandong 266237, P.R. China

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Abstract. *O*-GlcNAcylation is a dynamic and reversible post-translational modification of proteins that is modulated by *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA). Alterations in the protein expression of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) can be induced by multiple factors. However, little is known of the effects of chemotherapeutic agents on *O*-GlcNAcylation and the relevant molecular mechanisms in cancer cells. In the present study, to investigate whether cisplatin alters protein *O*-GlcNAcylation and to explore whether protein *O*-GlcNAc modification affects the antitumor activity of cisplatin, experiments were performed *in vitro* and *in vivo*. The results indicated that cisplatin treatment resulted in an enhancement of global protein *O*-GlcNAc levels in the H1299, Hep G2 and MCF-7 cells *in vitro* and *in vivo*. Cisplatin upregulated the protein and mRNA expression levels of OGT and OGA in H1299 cells. Moreover, cisplatin induced the significant enhancement of the enzymatic activity of OGT in H1299 cells. On the contrary, the activation of OGA decreased in response to cisplatin exposure in H1299 cells. Cisplatin inhibited the activity of AMP-activated protein kinase (AMPK) by decreasing the AMP/ATP ratio. The present study also revealed that the decreased AMPK activation inhibited glutamine-fructose-6-phosphate aminotransferase (isomerizing) 1 (GFAT1) phosphorylation and subsequently promoted the activity of GFAT1. Cisplatin-induced GFAT1 activation elevated the production of the donor substrate, uridine 5-diphospho-N-acetylglucosamine (UDP-GlcNAc). However, alterations in the *O*-GlcNAc levels by the inhibition of OGT and OGA did not affect the sensitivity of lung cancer cells to cisplatin. On the whole, the present study demonstrates that cisplatin enhances protein *O*-GlcNAcylation by altering the activity of OGT, OGA and AMPK *in vitro* and *in vivo*.

Correspondence to: Dr Yikang Shi, National Glycoengineering Research Center, Shandong Provincial Key Laboratory of Carbohydrate Chemistry and Glycobiology, Shandong University, 72 Binhai Road, Qingdao, Shandong 266237, P.R. China
E-mail: shiyikang@sdu.edu.cn

*Contributed equally

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Introduction

Extracellular glucose is transferred into cells by glucose transporters and the majority of the transferred glucose is used for ATP synthesis through the glycolytic pathway and the tricarboxylic acid cycle. Only 2-5% of the transferred glucose enters the hexosamine biosynthetic pathway (HBP). Glutamine-fructose-6-phosphate aminotransferase (GFAT) is the rate-limiting enzyme of the HBP that catalyzes the conversion of fructose 6-phosphate to glucosamine 6-phosphate (GlcN6P). This reaction is the first step in the HBP. Subsequent steps metabolize GlcN6P to the major end product uridine 5-diphospho-*N*-acetylglucosamine (UDP-GlcNAc), which is the essential precursor of glycoproteins and glycolipids in the endoplasmic reticulum and Golgi apparatus. Moreover, UDP-GlcNAc is the direct donor for *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modification on numerous proteins (1).

O-GlcNAc is a post-translational modification of nuclear, cytoplasmic and mitochondrial proteins. Protein *O*-GlcNAcylation is a dynamic and reversible process carried out by two single enzymes: *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA). OGT transfers *O*-GlcNAc from UDP-GlcNAc to the hydroxyl groups of serine or threonine residues of target protein substrates, while OGA catalyzes its removal. *O*-GlcNAcylation is emerging as a key regulator of diverse cellular processes, such as signal transduction, transcriptional regulation and proteasomal degradation (2). Aberrant *O*-GlcNAcylation in cells is closely associated with a number of human diseases, including cancer, metabolic disorders and cardiovascular disease (3). Increased OGT and *O*-GlcNAc levels have been observed in various types of cancer and have been found to promote cancer growth and progression (4).

Protein *O*-GlcNAcylation and the donor substrate UDP-GlcNAc levels within the cell are modulated by the

availability of glucose, fatty acids, amino acids and nucleotides. Therefore, *O*-GlcNAc is proposed as a nutrient sensor and metabolic regulator (5). Moreover, there is increasing evidence to suggest that *O*-GlcNAc is a novel regulator of the cellular stress response. In response to numerous forms of cellular stress or injury, global *O*-GlcNAc levels are dynamically elevated in both *in vitro* and *in vivo* models of heat stress, oxidative stress, endoplasmic reticulum stress, hypoxia, ischemia-reperfusion injury and trauma hemorrhage (6). However, it has been demonstrated that *O*-GlcNAc levels of subproteome can decline (7). Alterations in the expression, activity, localization and targeting of OGT and OGA, as well as tje increased flux through the HBP, have been associated with stress-induced changes in *O*-GlcNAcylation (8). However, it remains unclear whether cells and tissues coordinate all of these mechanisms to affect stress-induced changes in *O*-GlcNAcylation, or whether different cells and tissues induce specific pathways depending on the type of stress (6,9).

Cisplatin is the most widely agent for the treatment of various types of solid malignancies. It generates intra- and inter-strand purine crosslinks that interfere with DNA replication, which leads to irreparable DNA damage, followed by apoptosis. Cisplatin has also been shown to bind to mitochondrial DNA, phospholipids and other molecules. However, resistance to cisplatin can develop, which limits its effectiveness in clinical practice (10). Recently, it was demonstrated that decreased *O*-GlcNAcylation through the inhibition of the HBP potentiates cisplatin cytotoxicity in non-small cell lung cancer cells (11). Conversely, another study demonstrated that suppressed *O*-GlcNAcylation via the downregulation of OGT decreased the sensitivity of ovarian cancer to cisplatin (12).

Global protein *O*-GlcNAcylation has been reported to increase in response to various types of stress; however, little is known with regards to the underlying mechanisms through which chemotherapeutic agents affect the levels of *O*-GlcNAc in cancer cells. In the present study, it was found that cisplatin elevated *O*-GlcNAc levels in lung cancer cells by altering the activity of OGT, OGA and AMP-activated protein kinase (AMPK). In addition, the sensitivity of cancer cells to cisplatin was not affected by the changes in *O*-GlcNAcylation induced by the inhibition of OGT and OGA *in vitro* and *in vivo*.

Materials and methods

Cell lines and cell culture. The lung cancer cell line NCI-H1299 (CRL-5803), breast cancer cell line, MCF-7 (HTB-22) and the hepatoblastoma cell line, Hep G2 (HB8065) were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. All three cell lines were initially derived from ATCC. The taxol-resistant cell line, H1299/Taxol, was a gift from Dr Hongying Zhen (Department of Cell Biology, School of Basic Medical Sciences, Peking University Health Science Center). All cell lines were maintained in a humidified atmosphere containing 5% CO_2 at 37˚C in RPMI-1640 medium or DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin.

Reagents. Cisplatin, PUGNAc, alloxan, 6-diazo-5-oxo-L-nor-Leucine (DON), oligomycin and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) were obtained from Sigma-Aldrich; Merck KGaA. Adriamycin was obtained from Shanghai Biochempartner Co., Ltd. Vincristine was obtained from Guangrun Biotechnology.

Sulforhodamine B (SRB) assay. Cancer cells were incubated in 96-well plates for 24 h. Following the addition of 0, 2, 8 and 16 μg/ml of CDDP or in combination with 10 μM of alloxan or 100 μM of PUGNAc, the plates were incubated at 37˚C for an additional 48 h in a 5% CO_2 incubator. The culture medium was then discarded and the cells were fixed *in situ* by the gentle addition of 100 μl of cold 10% (w/v) trichloroacetic acid followed by incubation for 60 min at 4˚C. The supernatant was discarded and the plates were washed 5 times with tap water and air-dried. SRB solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added and the plates were incubated for 20 min at room temperature. After staining, the unbound dye was removed by washing 5 times with 1% acetic acid and the plates were air-dried. The bound stain was subsequently solubilized with 10 mM Tris (pH 10.5) and the absorbance was read at 515 nm on a Bio-Rad 550 ELISA microplate reader (Bio-Rad Laboratories, Inc.). The optical density (OD) was then analyzed with SPSS software 17.0 (IBM Corp.).

Western blot analysis. The cells were trypsinized, washed with PBS and then lysed with buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 2 mM EGTA, 1 mM dithiothreitol, 1% Nonidet P-40, 0.1% SDS, protease inhibitors (1 mM PMSF, 5 mg/ml aprotinin, 5 mg/ml leupeptin and 5 mg/ml pepstatin) and phosphatase inhibitors (20 mM β-glycerophosphate, 50 mM NaF, and 1 mM Na_3VO4). The tissues of nude mice (described below) were washed with PBS and were then ground with the same lysis buffer. The lysates were incubated at 4˚C for 20 min and centrifuged at 12,000 x g for 15 min at 4˚C. Equal amounts of the lysate (20 or 30 μg) were resolved by a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (EMD Millipore). The membranes were blocked in 5% non-fat skim milk/TBST [20 mM Tris-HCl (pH7.4), 150 mM NaCl and 0.1% Tween-20] at room temperature for 2 h and detected with primary antibodies at room temperature for 2 h. The membranes were then blotted for 1 h at room temperature with an appropriate horseradish peroxidase-linked secondary antibody (dilution 1:5,000, ZB-2301, ZB-2305; ZSGB-Bio), followed by enhanced chemiluminescence western blot detection reagents (Amersham Pharmacia Biotech). Proteins were analyzed using Image Lab software (Bio-Rad Laboratories, Inc.).

The primary antibodies, OGT (SAB2702273, dilution 1:1,000), OGA (HPA036141, dilution 1:1,000), *O*-GlcNAc (MABS157, dilution 1:1,000), anti-AMPK α1 antibody (cat. no. 07-350, dilution 1:1,000), p-AMPK α (pThr172) (SAB4503754, dilution 1:1,000), p-serine (PSR-45) (P5747, dilution 1:1,000) were purchased from Sigma-Aldrich; Merck KGaA. GFAT1 (D-9) (sc-377479, dilution 1:1,000), glucose transporter member 1 (Glut1; sc-7903, dilution 1:1,000), pyruvate kinase isozyme M2 (PKM2; sc-365684, dilution 1:1,000), phosphofructokinase 1 (PFK1; sc-67028, dilution 1:1,000), HK2 (sc-374091, dilution 1:1,000) and β-actin (sc-47778, dilution 1:1,000) were purchased from Santa Cruz Biotechnology, Inc.

Immunoprecipitation assays. Cells were harvested in lysis buffer (50 mM Tris, 150 mM NaCl, 1% NP-40 and 0.5% sodium deoxycholate) and supernatants incubated with rotation at 4˚C with either anti-GFAT1 overnight. Protein G-agarose (Invitrogen; Thermo Fisher Scientific, Inc.) was added the following day, and lysates were placed on the rotator at 4˚C for 4 h. Protein G-agarose beads were isolated by centrifugation at 200 x g for 1 min at 4˚C, washed 3 times with lysis buffer and heated for 5 min at 100˚C in loading buffer. Samples were run on 10% SDS-PAGE and then probed by western blot analysis for an antibody specific for the phosphorylation of serine, as described above.

Reverse transcription-quantitative PCR (RT-qPCR). Total mRNA was isolated from the H1299 cells using TRIzol® reagent, and complementary DNA (cDNA) was synthesized from 100 ng total RNA using the cDNA Synthesis kit (Invitrogen; Thermo Fisher Scientific, Inc.). qPCR was performed with SYBR-Green qPCR SuperMix (Invitrogen; Thermo Fisher Scientific, Inc.). The sequences of the primers for OGT, OGA and GAPDH amplification were as follows: OGT forward, 5'-TCCTGATTTGGTACTGTGTTCGC-3' and reverse, 5'-AAGCTACTGCAAAGTTCGGTT-3'; OGA forward, 5'-GAAGGAGAGTCAAGCGACGTT-3' and reverse, 5'-TCCATAACCCAAGGTCTTCCAT-3'; GAPDH forward, 5'-GGAGCGAGATCCCTCCAAAAT-3' and reverse, 5'-GGC TGTTGTCATACTTCTCATGG-3'. The expression of OGA and OGT was normalized to that of GAPDH and analyzed using the $2^{-\Delta\Delta Cq}$ method (13).

Enzymatic activity assay of OGT and OGA. OGT activity was measured in whole-cell lysates, which were incubated with reaction buffer containing recombinant glutathione S-transferase-tagged p62 and 100 μM UDP-GlcNAc for 2 h, as previously described (14). Recombinant His-tagged p62 fragment used as a substrate for OGT was extracted from *Escherichia coli* (Tiangen Biotech Co., Ltd.) and purified on Ni-NTA His Bind Resin (Merck KGaA). The reaction was terminated by the addition of 2 mM glutathione, and supernatants were mixed with SDS-PAGE sample buffer, subjected to 10% SDS-PAGE, and immunoblotted using anti-*O*-GlcNAc antibodies.

OGA activity assays were performed as described in a previous study (14). The whole-cell lysates were incubated with assay buffer (50 mM sodium cacodylate, pH6.5, 50 mM N-acetylgalactosamine, 2 mM p-nitrophenyl-*N*-acetyl-D-glucosaminide) for 1 h at 37˚C. The reaction was terminated by the addition of 0.5 M sodium carbonate. Hydrolyzed p-nitrophenol was measured at 400 nm using a spectrophotometer (Agilent Technologies, Inc.).

Measurement of UDP-GlcNAc levels. The levels of UDP-GlcNAc were measured in cell extracts as previously described with minor modifications (15). Cells were counted using a hemocytometer (Sigma-Aldrich; Merck KGaA) and the cell extracts were homogenized at room temperature for 10 min in 4 volumes of perchloric acid (300 mM). The solution was adjusted to pH 7.0 with 1 M NaOH and then boiled for 10 min. The precipitates were centrifuged at 13,800 x g for 10 min at room temperature. The lipid was extracted from the supernatants with 2 volumes of tri-n-octylamine:1,1,2-trichlorofluoroethane (1:4). The aqueous phase was filtered through a 0.22-μM filter and then stored at -80˚C until analysis by HPLC. HPLC was performed on a YMC-Pack Polyamine II HPLC Columns (250x4.6 mm, 5 μm), eluted with 10 mM potassium dihydrogen phosphate for 60 min at a flow rate of 0.5 ml/min. UDP-GlcNAc levels were quantified using a UV spectrophotometer (Agilent Technologies, Inc.) at 254 nm, compared with the standard curve.

Enzymatic activity assay of GFAT1. The enzymatic activity of GFAT was examined the established glutamate dehydrogenase method (16). The generation of glutamate, one of the products in the GFAT1 reaction, was assayed as the reduction of acetylpyridine adenine dinucleotide (APAD) to APADH by the glutamate dehydrogenase reaction with glutamate, which is determined directly using a UV spectrophotometer (Agilent Technologies, Inc.) at 370 nm. Briefly, the H1299 cells were collected using GFAT buffer (50 mM Tris, 5 mM EDTA, 5 mM glutathione, 5 mM D-Glucose 6-phosphate disodium salt hydrate and 50 mM KCl, pH 8.5). Cells were disrupted with an ultrasonic cytometer and centrifuged at 13,800 x g for 10 min at 4˚C. The supernatants were collected for protein quantification and activity assay. The aliquots of the cell lysate were incubated in 100 μl of the reaction mixture (10 mM fructose-6-phosphate, 6 mM glutamine, 0.3 mM APAD, 50 mM KCl, 100 mM KH_2PO_4 and 6 U of glutamate dehydrogenase) at 30˚C for 2 h in a 96-well plate. The change in absorbance by the reduction of APAD was monitored at 370 nm using a microplate spectrometer (Model 680; Bio-Rad Laboratories, Inc.). The absorbance values of the reaction mixture containing GFAT buffer instead of the cell lysate were employed as a reference.

Intracellular glucose contents assay. The glucose oxidase method was used to measure the glucose contents in the cells following the manufacturer's instructions (Applygen Technologies, Inc.). Briefly, the H1299 cells were counted and the cell extracts were disrupted by ultrasonic in buffer. The cell lysate was kept at 95˚C for 10 min and then centrifuged at 8,000 x g at 4˚C. The supernatants were used to determine the glucose contents.

Measurement of ATP and AMP/ATP ratios. H1299 cells were treated in the presence or absence of 8 μg/ml cisplatin or 2 μg/ml oligomycin for 24 h. Endogenous levels of ATP and AMP in lysates of treated cells were detected using an Enhanced ATP Assay kit following the manufacturer's instructions (Shanghai Biyuntian Biological Co., Ltd.). The luciferase enzyme included in the assay was used to catalyze the generation of light from ATP and luciferin. ADP was measured by its conversion to ATP, which was detected using the same reaction. The intracellular ATP and AMP contents were calculated according to the standard curve made by ATP standards and normalized to the cell number in each sample.

Inhibition of tumor growth in vivo. The research protocol was in accordance with the institutional guidelines of the Animal Care and Use Committee of Shandong University (no. 2016020, Jinan, China). The mice were housed in

pathogen-free ventilated cages and fed with standard commercial diets and water in a temperature-controlled environment (25±2˚C) with a 12-h day/night cycle. The nude mouse experiment was divided into the pre-experiment and formal experiment stage. The purpose of the preliminary experiment was to determine the optimal dosage of PUGNAc which can alter protein *O*-GlcNAcylation. In the preliminary experiment, 40 female BALB/c (nu/nu) mice (weighing, 20±2 g; 4-6 weeks old) were purchased from the Animal Center of the China Academy of Medical Sciences (Beijing, China). The H1299 lung cancer cells ($5.0x10^6$) suspended in 100 μl PBS, were subcutaneously inoculated into the lower right flanks of the nude mice. When the tumors reached a volume of 100-150 mm^3, 27 mice were used to determine the appropriate dosage of PUGNAc. The mice were divided randomly into 9 groups (n=3 in each group) as follows: i) The control group (PBS); ii) PUGNAc (1 mg/kg, 24 h) group; iii) PUGNAc (1 mg/kg, 48 h) group; iv) PUGNAc (1 mg/kg, 72 h) group; v) PUGNAc (1 mg/kg, 96 h) group; vi) PUGNAc (2 mg/kg, 24 h) group; vii) PUGNAc (2 mg/kg, 48 h) group; viii) PUGNAc (2 mg/kg, 72 h) group; and ix) PUGNAc (2 mg/kg, 96 h) group. PUGNAc (1 mg/kg or 2 mg/kg) was then injected intravenously for each group, and the mice were sacrificed after 24, 48, 72 or 96 h, respectively. The tumor tissues were obtained by dissection from mice that were subjected to cervical dislocation following deep anesthesia by an intraperitoneal injection with pentobarbital sodium injection (100 mg/kg). Protein *O*-GlcNAc levels in tumors were measured by western blot analysis. In the formal experiment, a total of 34 female BALB/c (nu/nu) mice (weighing, 20±2 g; 4-6 weeks old) were purchased from the Animal Center of the China Academy of Medical Sciences (Beijing, China). The H1299 lung cancer cells ($5.0x10^6$) suspended in 100 μl PBS, were subcutaneously inoculated into the lower right flanks of the nude mice. When the tumors reached a volume of 100-150 mm^3, 24 mice were divided randomly into 4 groups (n=6 in each group): Control group (PBS), CDDP group, PUGNAc group and CDDP + PUGNAc group. The mice were injected intravenously 4 times with 100 μl of PBS, CDDP (6 mg/kg), PUGNAc (1 mg/kg), or a combination of CDDP (6 mg/kg) and PUGNAc (1 mg/kg) respectively during the 16 days of treatment. The doses of PUGNAc were determined based on the preliminary experiment and a previous study (17). The diameter of the tumor was measured twice a week using a caliper. Tumor volume was calculated with the following formula: $v=ab^2/2$, where 'a' and 'b' are the long diameter and the perpendicular short diameter of the tumor, respectively. The body weights were measured twice a week. The mice with tumor volumes >2,000 mm^3 or a weight loss of >20% were sacrificed in advance. On the 16th day, all mice were injected with pentobarbital sodium injection (100 mg/kg) intraperitoneally, and were then subjected to cervical dislocation following deep anesthesia. Tumor, liver and lung tissues from mice were obtained by dissection.

Statistical analysis. SPSS software version 17.0 (IBM Corp.) and GraphPad Prism 6 (GraphPad Software, Inc.) were used for statistical analysis. All data represent the means ± SD of 3 independent experiments. Statistical tests included independent a samples t-test and one-way ANOVA with post hoc Tukey's test. P-values <0.05 were considered to indicate statistically significant differences.

Results

Chemotherapeutic agents increase global protein O-GlcNAcylation. To investigate whether chemotherapeutic agents lead to increases in total protein *O*-GlcNAcylation in various types of cancer cells, the H1299, HepG2 and MCF-7 cells were treated with cisplatin, adriamycin and vincristine for 24 h, respectively. The results of western blot analysis revealed that all cancer cells exhibited an elevation in *O*-GlcNAc levels upon treatment with the different cytotoxic drugs; vincristine treatment led to a slight increase in *O*-GlcNAc levels, with no significant difference (Fig. 1A). In addition, the effect of cisplatin on the *O*-GlcNAc levels was examined in the multi-drug resistant cells, H1299/Taxol. *O*-GlcNAcylation was enhanced upon treatment of the H1299/Taxol cells with cisplatin at high concentrations (Fig. 1B). Furthermore, cisplatin increased the *O*-GlcNAc levels in both a time- and concentration-dependent manner in the H1299 cells (Fig. 1C and D). In the present study, cisplatin at concentrations of 2, 8 and 16 μg/ml inhibited cell growth by ~10, 45 and 67% after the H1299 cells were treated for 48 h (Fig. S1). The *O*-GlcNAc levels increased gradually and reached maximal levels at 24 h and then declined to baseline levels at 72 h when the H1299 cells were exposed to 8 μg/ml cisplatin for various periods of time (Fig. 1C). These results suggested that the the global protein *O*-GlcNAc levels were upregulated in cancer cells in response to certain types of chemotherapeutic agents.

Cisplatin increases the enzymatic activity of OGT and OGA. As shown in Fig. 1D, the results of western blot analysis revealed that cisplatin increased both the OGT and OGA protein levels in the H1299 cells. Moreover, the OGT and OGA mRNA levels were significantly enhanced following treatment of the H1299 cells with cisplatin (Fig. 2A). OGT and OGA activity assays were then performed with total lysates from the H1299 cells. OGT activity toward the p62 peptide substrate was increased; however, OGA activity toward the PNP-GlcNAc substrate gradually decreased and was 83.9, 78.1 and 71.5% of the control following treatment of the H1299 cells with cisplatin at the concentrations of 2, 8 and 16 μg/ml for 24 h (Fig. 2B and C).

Alloxan, an inhibitor of OGT, markedly downregulated the global *O*-GlcNAc levels and reversed the cisplatin-induced increase in *O*-GlcNAc levels in the H1299 cells (Fig. 3A). The results of SRB assay revealed that the decrease in *O*-GlcNAc levels induced by alloxan did not result in any changes in cisplatin cytotoxicity (Figs. 3B and S2A). In addition, the OGA inhibitor, PUGNAc, was used to increase the global protein *O*-GlcNAc levels. Although treatment with 100 μM PUGNAc led to a 2.2-fold increase in the *O*-GlcNAc levels in the H1299 cells compared with the control (Fig. 3C), PUGNAc alone did not affectd cell survival (Fig. S2B). PUGNAc enhanced the cisplatin-induced elevation in *O*-GlcNAc levels; however, combined treatment with PUGNAc and cisplatin induced the same cell growth rate as with cisplatin treatment alone (Fig. 3C and D). The aforementioned results suggested that alteration of global O-GlcNAc levels via the inhibition of

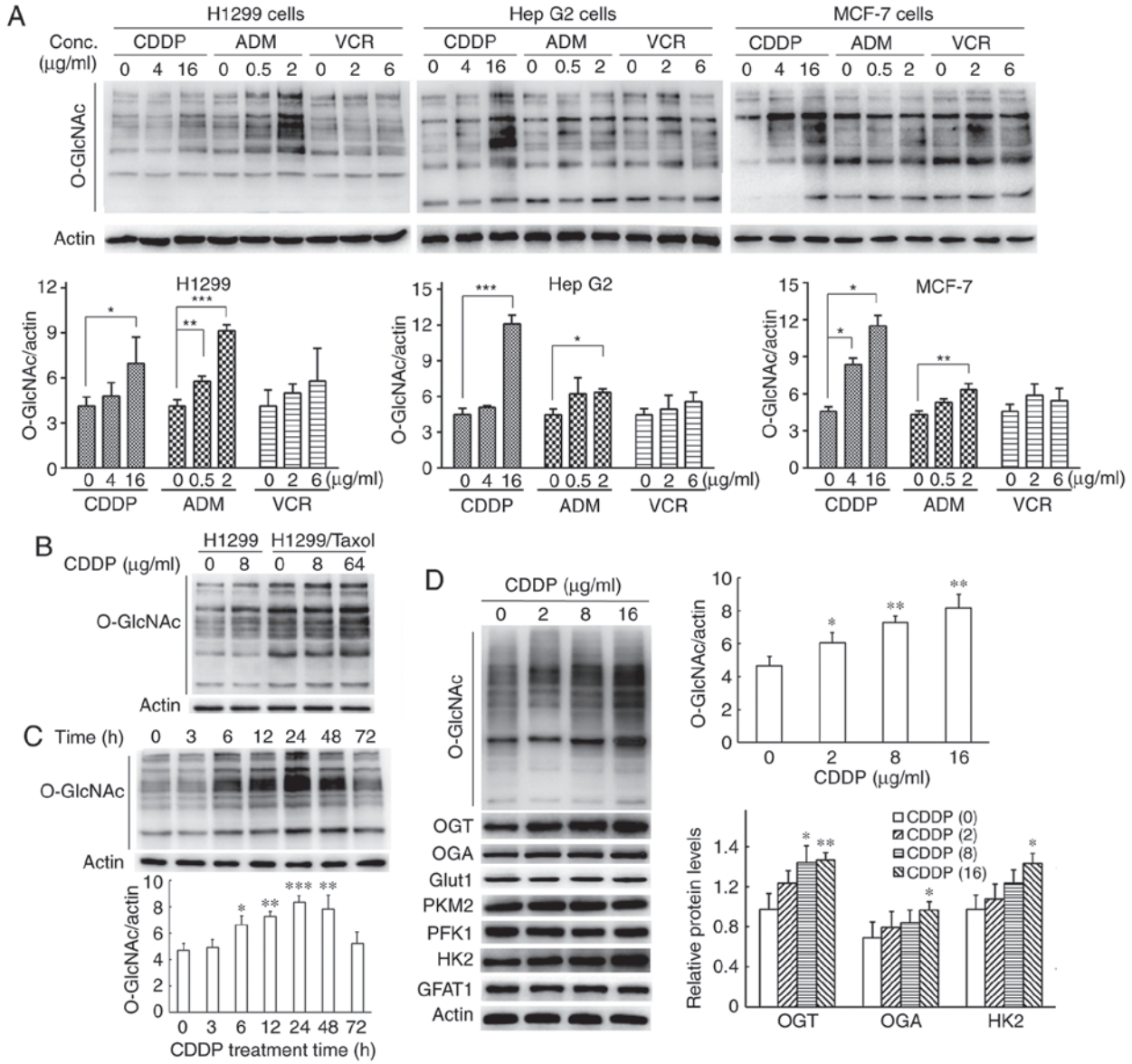

Figure 1. Chemotherapeutic agents increase global protein *O*-GlcNAc levels in cancer cells. (A) The lung cancer cell line, H1299, hepatoblastoma cell line, Hep G2, and the breast cancer cell line, MCF-7, were treated with cisplatin, adriamycin and vincristine at various concentrations for 24 h. Total cell extracts were harvested for western blot analysis. Protein *O*-GlcNAc levels were semi-quantified by densitometry and normalized against those of β-actin. (B) The multi-drug resistant cancer cells, H1299/Taxol, and parental cells, H1299, were treated with cisplatin for 24 h. Total cell extracts were harvested for western blot analysis. (C) H1299 cells were exposed to 8 μg/ml cisplatin for the indicated times and then lysed for western blot analysis. Protein O-GlcNAc levels were semi-quantified by densitometry and normalized against that of β-actin. (D) H1299 cells were exposed to cisplatin for 24 h at the indicated concentrations and then lysed for western blot analysis. Protein *O*-GlcNAcylation and other protein expression levels were semi-quantified by densitometry and normalized against that of β-actin. All data are shown as the means ± SD of 3 independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. control. CDDP, cisplatin; AMD, Adriamycin; VCR, vincristine; OGT, *O*-GlcNAc transferase; OGA, *O*-GlcNAcase.

OGT or OGA did not influence the sensitivity of H1299 cells to cisplatin.

To further explore the mechanisms underlying the upregulation of the global *O*-GlcNAc levels induced by cisplatin, the protein expression levels of enzymes related to glycolysis and the HBP were examined. The results of western blot analysis revealed that only HK2 expression was increased upon cisplatin treatment and no changes were observed in the protein levels of enzymes, such as Glut1, PFK1, PKM2 and GFAT1 (Fig. 1D).

Cisplatin increases UDP-GlcNAc via the activation of GFAT1 and the inhibition of AMPK activity. It is commonly known that the activity of OGT is sensitive to the intracellular concentration of UDP-GlcNAc. Therefore, intracellular UDP-GlcNAc was measured using HPLC in H1299 cells following cisplatin treatment. Compared with the control cells, intracellular UDP-GlcNAc levels were enhanced 1.77-, 2.29- and 2.85-fold when the H1299 cells were exposed to cisplatin at 2, 8 and 16 μg/ml for 24 h (Fig. 4A). The UDP-GlcNAc levels increased as the duration of treatment increased and were 5.10-fold greater at 72 h than at 0 h (Fig. 4B).

DON is a glutamine analogue that selectively inactivates glutamine-utilizing pathways to irreversibly inhibit GFAT1 activity (18,19). As shown in Fig. 4C, DON significantly down-regulated the UDP-GlcNAc levels in a concentration-dependent

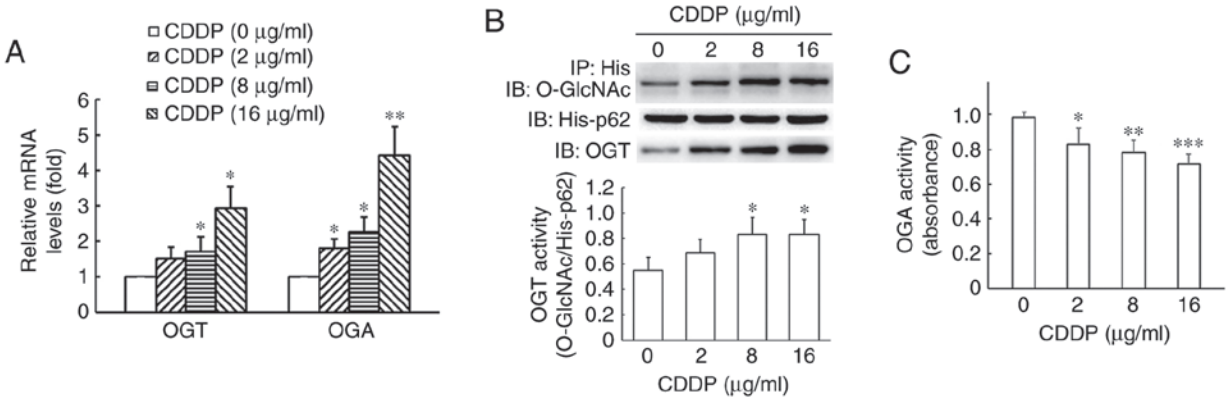

Figure 2. Cisplatin treatment alters the mRNA levels and enzymatic activity of OGT and OGA in H1299 cells. (A) Reverse transcription-quantitative PCR assay was performed in H1299 cells treated with cisplatin for 24 h at the indicated concentrations. (B) H1299 cells were treated with cisplatin for 24 h at the indicated concentrations, and then whole cell lysates were assayed for OGT activity through immunoprecipitation and western blotting analysis. The *O*-GlcNAc levels of His-p62 were taken as a measurement of OGT activity and normalized to the amount of His-p62. (C) Whole cell lysates from H1299 cells treated with cisplatin for 24 h were assayed for OGA activity as described in the Materials and methods section. Hydrolyzed p-nitrophenol was measured spectrophotometrically at 400 nm. All data are shown as the means ± SD of three independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. control. CDDP, cisplatin; OGT, *O*-GlcNAc transferase; OGA, *O*-GlcNAcase.

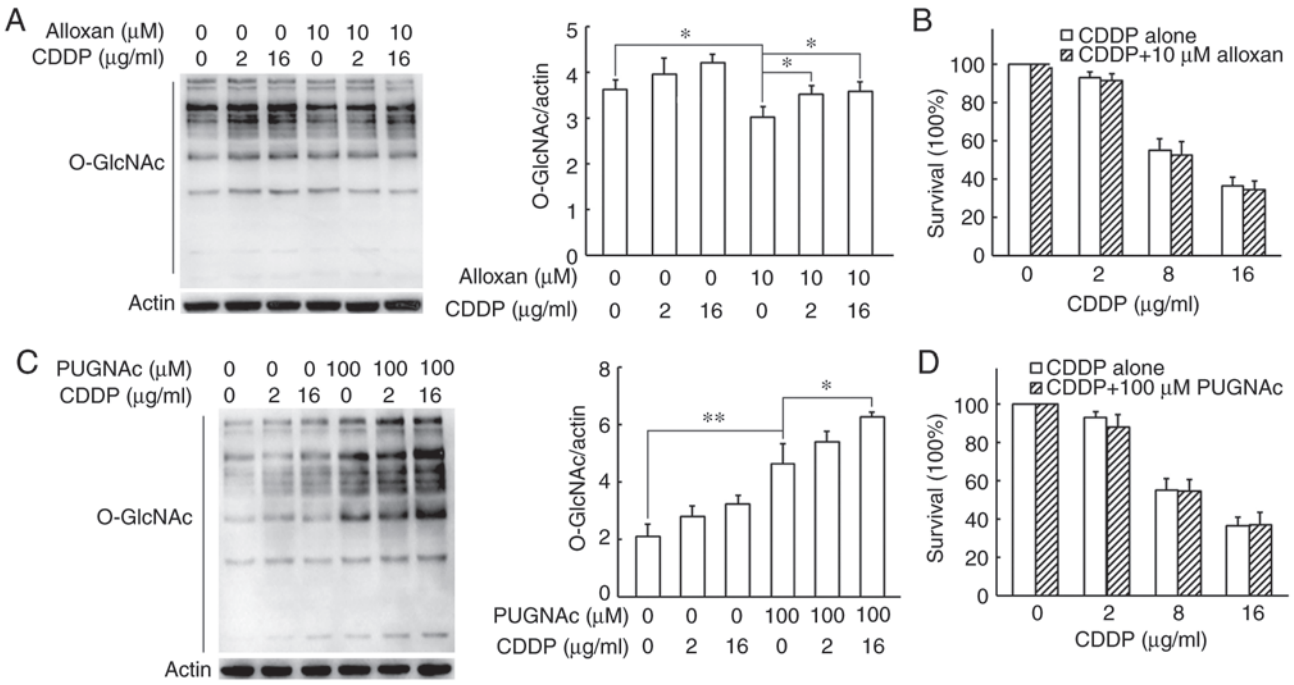

Figure 3. Effects of inhibition of OGT and OGA on cisplatin-induced *O*-GlcNAcylation and cisplatin cytotoxicity in H1299 cells. (A) H1299 cells were treated with cisplatin alone or in combination with the OGT inhibitor, alloxan, for 24 h. Cells were collected and lysed for western blot analysis. Protein *O*-GlcNAc levels were semi-quantified by densitometry and normalized against those of β-actin. (B) In SRB assay, H1299 cells were exposed to 0, 2, 8 or 16 μg/ml cisplatin or the combination with 10 μM of alloxan for 48 h respectively. (C) H1299 cells were treated with cisplatin alone or in combination with the OGA inhibitor, PUGNAc for 24 h. Cells were collected and lysed for western blot analysis. Protein *O*-GlcNAc levels were semi-quantified by densitometry and normalized against those of β-actin. (D) SRB assay was performed in H1299 cells. The cells were exposed to 0, 2, 8 or 16 μg/ml cisplatin or the combination with 100 μM of PUGNAc for 48 h respectively. All data are shown as the means ± SD of 3 independent experiments. *P<0.05, **P<0.01. CDDP, cisplatin; OGT, *O*-GlcNAc transferase; OGA, *O*-GlcNAcase.

manner in the H1299 cells. Moreover, DON significantly reversed the cisplatin-induced elevation in the UDP-GlcNAc levels, thereby resulting in significant decreases in global *O*-GlcNAc levels in the H1299 cells treated with DON and cisplatin (Fig. 4D and E). Taken together, these findings indicated that cisplatin resulted in the elevation of intracellular UDP-GlcNAc, which was involved in the regulation of global protein *O*-GlcNAcylation.

GFAT1 is the first step of the HBP and the rate-limiting enzyme for the synthesis of UDP-GlcNAc, the end product of the HBP. The GFAT1 enzymatic activity assay using cell lysates indicated that cisplatin treatment enhanced GFAT1 activity in a concentration-dependent manner (Fig. 4F). AICAR, an activator of AMPK, can reduce GFAT1 activity by enhancing GFAT1 phosphorylation (20). In the present study, GFAT1 was enriched in advance by immunoprecipitation, and the

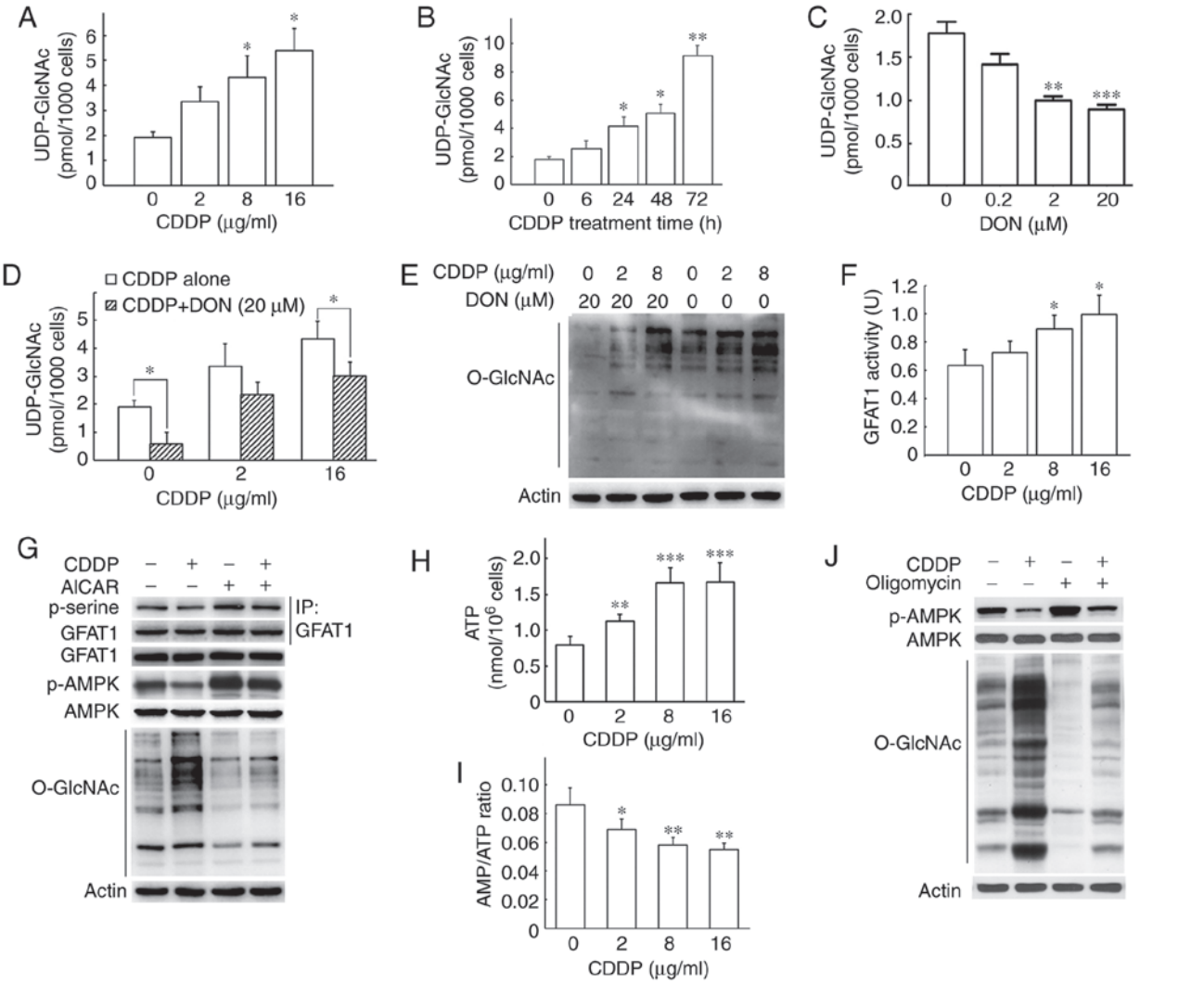

Figure 4. Cisplatin treatment enhances the intracellular UDP-GlcNAc content by altering the activity of AMPK and GFAT1 in H1299 cells. (A) Cisplatin increased intracellular UDP-GlcNAc levels. UDP-GlcNAc was measured using HPLC after the H1299 cells were treated with cisplatin for 24 h at the indicated concentrations. (B) Intracellular UDP-GlcNAc was measured using HPLC after the H1299 cells were treated with 8 μg/ml cisplatin for the indicated periods of time. (C) The GFAT1 inhibitor, DON, inhibited the production of UDP-GlcNAc. H1299 cells were treated with DON for 24 h and the cell lysate was used for HPLC analysis. (D) DON prevented the cisplatin-induced increase in UDP-GlcNAc levels. H1299 cells were treated with cisplatin in the presence or absence of DON for 24 h and the cell lysate was used for HPLC analysis. (E) DON prevented cisplatin-induced increase in protein *O*-GlcNAcylation. H1299 cells were treated with cisplatin in the presence or absence of DON for 24 h and the cell lysate was used for western blot analysis. (F) Cisplatin enhanced GFAT1 activity. GFAT1 activity was measured after the H1299 cells were treated with cisplatin at the indicated concentrations. The supernatants of cell lysate were mixed with reaction buffer and the absorbance was monitored at 370 nm using a microplate spectrometer. (G) Cisplatin counteracted the AMPK-induced phosphorylation of GFAT1. H1299 cells were treated with cisplatin in the presence or absence of the AMPK activator, AICAR, for 24 h. The cell lysate was used for immunoprecipitation and western blot analysis. GFAT1 was first enriched by immunoprecipitation, and then p-serine antibody, a broad-spectrum phosphorylated serine antibody, was used to detect the phosphorylation of GFAT1. (H and I) Cisplatin decreased the AMP/ATP ratio. Intracellular ATP and AMP were detected after H1299 cells were treated with cisplatin for 24 h. H1299 cells cultured in a 96-well plate were mixed with reaction buffer containing luciferin and luciferase and light was measured using a luminometer. (J) The AMP/ATP ratio affected cisplatin-induced AMPK activation and protein *O*-GlcNAc levels. H1299 cells were treated with cisplatin (8 μg/ml) in the presence or absence of ATP synthase oligomycin (2 μg/ml) for 24 h. The cell lysate was used for western blot analysis. All data are shown as the means ± SD of 3 independent experiments. *P<0.05, **P<0.01, ***P<0.001 vs. control. CDDP, cisplatin; AMPK, AMP-activated protein kinase; GFAT1, glutamine-fructose-6-phosphate aminotransferase (isomerizing) 1; DON, 6-diazo-5-oxo-L-nor-Leucine; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside.

phosphorylation of GFAT1 was then detected by a p-serine antibody to reflect its activation. Treatment with cisplatin not only decreased GFAT1 phosphorylation, but also counteracted the AICAR-induced increase in GFAT1 phosphorylation (Fig. 4G), suggesting that cisplatin upregulated GFAT1 activity by inhibiting GFAT1 phosphorylation. As shown in Fig. 4G, cisplatin decreased AMPK phosphorylation. The phosphorylation of Thr172 is the hallmark of AMPK activation. Furthermore, the AICAR-induced AMPK activation decreased the *O*-GlcNAc levels, an effect which was prevented by cisplatin treatment, indicating that the cisplatin-induced inactivation of AMPK was involved in the upregulation of *O*-GlcNAc levels in cells treated with cisplatin (Fig. 4G). AMPK is primarily involved in monitoring cellular energy status by sensing the AMP/ATP and/or ADP/ATP ratios (21). Thus, the present study measured the ATP and AMP levels in H1299 cells treated with cisplatin. Cisplatin led to an increase in ATP levels and to a significant decrease in the ratio of AMP/ATP (Fig. 4H and I). Intracellular

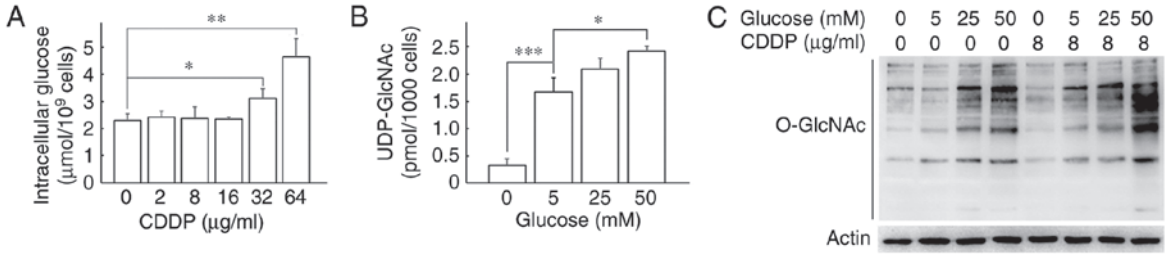

Figure 5. Effects of glucose on cisplatin-induced *O*-GlcNAcylation in H1299 cells. (A) Intracellular glucose contents were influenced by cisplatin treatment. H1299 cells were treated with cisplatin for 24 h and then were extracted by sonication. The supernatants were mixed with reaction buffer to produce red quinone compounds, which were measured at 505 nm. (B) Extracellular glucose contents enhanced intracellular UDP-GlcNAc. UDP-GlcNAc was measured using HPLC after the H1299 cells were treated with 8 μg/ml cisplatin for 24 h in medium containing various glucose concentrations. (C) Extracellular glucose increased cisplatin-induced *O*-GlcNAcylation. Western blot analysis was performed to determine *O*-GlcNAc levels in cisplatin-treated H1299 cells cultured in medium containing various glucose concentrations. All data are shown as the means ± SD of 3 independent experiments. *P<0.05, **P<0.01, ***P<0.001. CDDP, cisplatin.

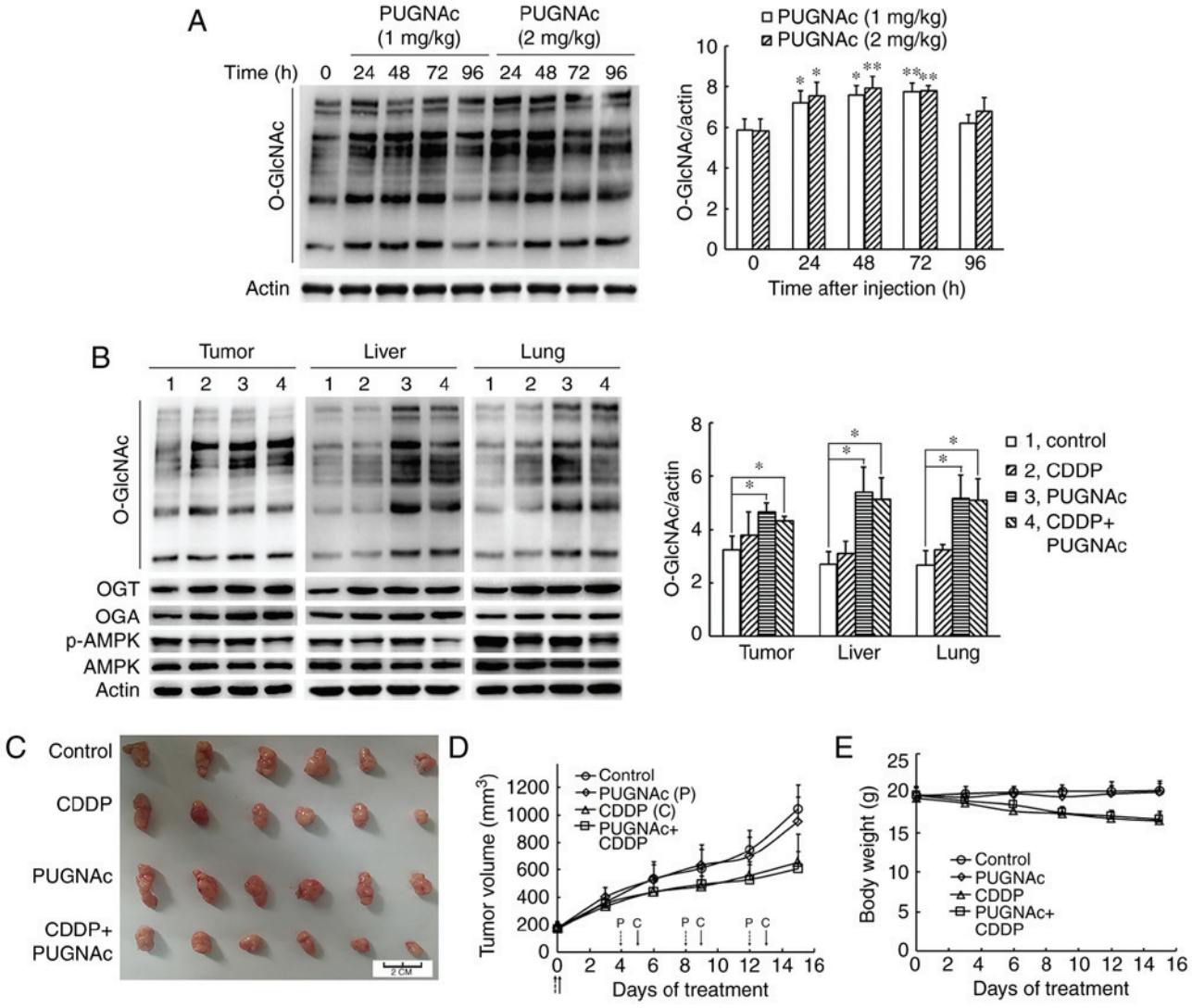

Figure 6. Cisplatin increases protein *O*-GlcNAcylation and *O*-GlcNAcylation does not influence the antitumor activity of cisplatin in a nude mouse xenograft tumor model. (A) Protein *O*-GlcNAc levels in tumors were measured by western blot analysis in order to determine the suitable dosage of PUGNAc. A total of 27 nude mice injected with H1299 cells were divided into 9 groups (n=3 per group). PUGNAc was injected intravenously for each group. Mice were sacrificed at the indicated days. Tumors were used for western blot analysis. Protein *O*-GlcNAc levels were semi-quantified by densitometry and normalized against those of β-actin. The data are shown as the means ± SD of 3 mice. *P<0.05, **P<0.01 vs. control. (B) A total of 26 nude mice injected with H1299 cells were divided into 4 groups (n= 6 per group). Western blot analysis was performed in tissues from nude mice following treatment with cisplatin or in combination and PUGNAc for 16 days. Protein *O*-GlcNAc levels were semi-quantified by densitometry and normalized against those of β-actin. (C) Imaging of the tumors dissected from the mice at the end of the treatment. Nude mice were treated as described in the Materials and methods section. (D)Tumor growth curves during the treatment period were calculated using the volume size of individual tumors. Dotted arrow represents the date of injection of PUGNAc. Solid arrow represents the date of injection of cisplatin. (E) Mean body weights for each group during the treatment period. The data in (B-E) are shown as the means ± SD of 6 mice. *P<0.05, **P<0.01 vs. control.

ATP is produced either through glycolysis or through oxidative phosphorylation in the mitochondria. However, ATP is mainly produced by ATP synthase in human cells (22). Oligomycin is a known inhibitor of the membrane motor of the mitochondrial ATP synthase for >50 years (23). In the present study, oligomycin treatment at a concentration of 2 μg/ml led to a 14% decrease in cell viability, decreased the intracellular ATP contents from 0.81 nmol/10^5 cells to 0.54 nmol/10^5 cells and increased the AMP/ATP ratio from 0.084 to 0.113, compared with the H1299 cells without oligomycin treatment (Fig. S3). As oligomycin decreased ATP production, oligomycin treatment alone enhanced AMPK activation and reduced the global *O*-GlcNAc levels (Fig. 4J). Furthermore, oligomycin abrogated the cisplatin-induced enhancement in the *O*-GlcNAc levels, suggesting that AMPK activation was downregulated by the cisplatin-induced decrease in the AMP/ATP ratio (Fig. 4J). Taken together, these findings demonstrated that cisplatin enhanced the intracellular UDP-GlcNAc level by modulating the AMP/AMPK/GFAT1 pathway.

Glucose consumption is not involved in the increase in cisplatin-induced O-GlcNAcylation. As GFAT1 is mostly activated by high glucose levels, the present study then investigated whether cisplatin promotes glucose uptake in H1299 cells. Intracellular glucose contents were measured after the cells were treated with cisplatin for 24 h. As shown in Fig. 5A, the glucose contents were unaltered following cisplatin treatment at 2, 8 and 16 μg/ml; however, treatment with 32 and 64 μg/ml cisplatin led to significant increases in the glucose contents. The intracellular glucose level did not exhibit any marked changes after the H1299 cells were exposed to 8 μg/ml CDDP for 0, 6, 12, 24, 48 and 72 h (Fig. S4). Subsequently, the effects of extracellular glucose on the intracellular UDP-GlcNAc level and protein *O*-GlcNAcylation in the H1299 cells were examined. As shown in Fig. 5B and C, the intracellular UDP-GlcNAc level and the protein *O*-GlcNAc levels were gradually enhanced when the cells were cultured in medium with increasing glucose concentrations. Cells cultured in 0 mM glucose exhibited the lowest UDP-GlcNAc levels, whereas those cultured in 25 mM glucose demonstrated 6.27-fold greater UDP-GlcNAc levels than the cells cultured in 0 mM glucose. The cells cultured in high glucose exhibited increased cisplatin-induced *O*-GlcNAc levels than the cells cultured in low glucose. The aforementioned results demonstrated that the upregulation of both GFAT1 and UDP-GlcNAc were not due to glucose consumption in H1299 cells.

Cisplatin-induced elevation of protein O-GlcNAc does not sensitize cancer cells to cisplatin in vivo. In order to examine whether protein *O*-GlcNAcylation affects the antitumor effect of cisplatin *in vivo*, the OGA inhibitor, PUGNAc, was selected to enhance the global protein *O*-GlcNAc levels. First, the concentrations and duration of PUGNAc administration were determined. In the preliminary experiment using nude mice bearing H1299 cells, the mice were sacrificed at 24, 48, 72 and 96 h following the injection of 1 and 2 mg/kg PUGNAc via the tail vein, and the O-GlcNAc levels in the tumors were measured. As shown in Fig. 6A, treatment with both 1 and 2 mg/kg PUGNAc significantly increased the global *O*-GlcNAc levels at 24 h, and these high levels were maintained for 72 h in the tumor cells, suggesting that PUGNAc had the potential to be used to enhance *O*-GlcNAc levels in mice. Subsequently, 1 mg/kg PUGNAc was administered via the tail vein on days 0, 4, 8 and 12. Cisplatin at a dose of 6 mg/kg body weight was administered on days 0, 5, 9 and 13. All nude mice were sacrificed on day 16. Consistent with the results observed *in vitro*, cisplatin treatment notably enhanced the OGT and OGA protein expression levels in the tissues of nude mice. Moreover, cisplatin decreased the AMPK phosphorylation levels (Fig. 6B). As compared with the PBS control group, the *O*-GlcNAc levels were significantly increased in the tumor, liver and lung tissues of the PUGNAc-treated group, as well as in the group treated with PUGNAc and cisplatin (Fig. 6B). Although PUGNAc promoted *O*-GlcNAcylation, PUGNAc alone did not affect the tumor growth and bodyweight of the mice. The dosage of 6 mg/kg cisplatin administered 4 times resulted in a 14.7% weight loss. Cisplatin treatment alone inhibited tumor growth by 37.5% and combined treatment with cisplatin and PUGNAc inhibited tumor growth by 41.8%, indicating that the enhanced *O*-GlcNAcylation did not affect the sensitivity of tumor cells to cisplatin *in vivo* (Fig. 6C-E).

Discussion

Protein *O*-GlcNAc modification plays crucial regulatory roles in cellular signaling. Global protein *O*-GlcNAc levels are increased in response to numerous types of cellular stresses, including drug treatment (6). However, less is known concerning the underlying mechanisms through which chemotherapeutic agents affect the *O*-GlcNAcylation in cancer cells. The present study illustrated that cisplatin treatment elevated the global *O*-GlcNAc levels in lung cancer cells in a concentration-dependent manner by regulating the enzymatic activity of OGT and OGA, and increased the intracellular UDP-GlcNAc levels via the inhibition of AMPK activation. It was also demonstrated that alterations in *O*-GlcNAc levels via the inhibition of OGT and OGA did not affect the sensitivity of lung cancer cells to cisplatin.

In the present study, the effect on the levels of *O*-GlcNAc by CDDP was more evident in MCF-7 and HepG2 cell lines, than in H1299. However, mouse tumor xenograft models using mice injected with H1299 cells were constructed in advance; thus, only the H1299 cells were selected for use in subsequent experiments, which is a limitation of the present study. As another potential limitation, it would have been prudent to compare the *O*-GlcNAc level in cisplatin-sensitive cells and cisplatin-resistant cells. However, due to the unsuccessful development of cisplatin-resistant H1299 cells by the long-term exposure of H1299 cells to an increasing concentration of CDDP, the H1299/Taxol cell line was used in the experiments, which is multi-drug resistant cell line. In the future, the authors aim to focus on the effects on the *O*-GlcNAc levels between cisplatin-sensitive and cisplatin-resistant cells.

In the present study, the global *O*-GlcNAc levels were elevated upon the cisplatin treatment of H1299 lung cancer cells in a concentration- and time-dependent manner. Cisplatin enhanced the protein expression, mRNA levels and enzymatic activity of OGT. Although >1,000 proteins have been found to be modified with *O*-GlcNAc, only OGT and OGA are involved in the process to add or remove the moiety of *O*-GlcNAc. OGT is

directly regulated by the concentration of UDP-GlcNAc, with its activity increasing as UDP-GlcNAc levels increase. Moreover, the substrate specificity of OGT changes at different UDP-GlcNAc concentrations (24). In the present study, the amount of intracellular UDP-GlcNAc was gradually upregulated after the H1299 cells were exposed to cisplatin for different periods of time. These data demonstrated that the enhanced OGT activity was principally regulated by the elevation of UDP-GlcNAc. It is also important to note that the increase in intracellular UDP-GlcNAc levels is likely to be cell-dependent. The levels of intracellular UDP-GlcNAc are early markers of cisplatin treatment in brain tumor cells, while levels remain unaltered in resistant cells (25). Duarte *et al* reported that the treatment of lung cancer cells A549 with cisplatin for 48 h resulted in a 2-fold elevation of UDP-GlcNAc levels; however, this effect was not observed in osteosarcoma cells treated with a comparable dose of cisplatin (26,27). The induction of UDP-GlcNAc by other chemotherapeutic drugs remains to be demonstrated.

Notably, in the present study, cisplatin also increased the OGA protein and mRNA levels, but significantly decreased OGA activity. At present, little is known about the regulation of OGA, although it can be cleaved by caspase-3 and is *O*-GlcNAcylated, phosphorylated, ubiquitinated and acetylated. However, the impact of these modifications on the localization, substrate specificity or activity of OGA has not been reported (6).

GFAT is the first step of the HBP and a rate-limiting enzyme that plays a key role in the regulation of the glucose through the HBP. Among the three identified human GFAT isoforms, GFAT1 is the major form that is ubiquitously expressed. GFAT1 is overexpressed in various types of cancer, and GFAT1 overexpression predicts a worse progression and pathological outcomes in various types of cancer (28,29). The present study illustrated that the elevation in the UDP-GlcNAc level induced by cisplatin was a result of an enhanced GFAT1 activity, rather than changes in intracellular glucose. Cisplatin dose-dependently upregulated GFAT1 activity; moreover, cisplatin inhibited the phosphorylation of GFAT1. This result is consistent with the findings of previous studies, which demonstrated that GFAT1 phosphorylation downregulates its activity (16,20,30). Relatively, the regulation of GFAT1 is complex; it is known that it is phosphorylated by two kinases, cAMP-dependent protein kinase at serine 205 and by AMPK at serine 243. AMPK is a conserved sensor of cellular energy changes and is activated by increased AMP/ATP and/or ADP/ATP ratios. In the present study, cisplatin treatment resulted in an enhanced ATP content and a decreased AMP/ATP ratio, suggesting that decreased AMP/ATP inhibited AMPK activation.

In the present study, the inhibitor of GFAT1, DON, abrogated the cisplatin-induced elevation of UDP-GlcNAc; therefore, DON significantly prevented the increase in global *O*-GlcNAc levels induced by cisplatin in H1299 cells. A recent study demonstrated that the inhibition of GFAT1 activity by DON suppressed cell proliferation and exerted a synergic or additive effect with cisplatin in inducing cancer cell death (11). Another recent study revealed that treatment with DON sensitized pancreatic tumors cells to anti-PD1 therapy, resulting in tumor regression and prolonged survival (31). The role of GFAT1 in cancer has also drawn increasing attention in recent years. Recent data indicated that GFAT1 inhibitors were effective in cancer treatment, indicating that targeting GFAT1 may provide novel adjuvant approaches for the clinical treatment of cancer (32).

The flux through the HBP and thus the synthesis of UDP-GlcNAc is regulated mainly due to the metabolism of glucose (33). The present study found that cisplatin at concentrations of 2, 8 and 16 μg/ml did not alter intracellular glucose consumption; however, the global *O*-GlcNAc levels were increased in a concentration-dependent manner upon cisplatin treatment at the same concentrations in H1299 cells. Compared with cells cultured in low glucose, the cisplatin-induced *O*-GlcNAc levels were higher when the H1299 cells were cultured in medium containing a higher glucose concentration. Moreover, a higher glucose concentration resulted in both increased UDP-GlcNAc and protein *O*-GlcNAcylation than the lower glucose concentration. The aforementioned results confirmed that the cisplatin-induced increase in the protein *O*-GlcNAc level was not related to glucose consumption. A previous study demonstrated that treatment with 0.6 μg/ml cisplatin for 48 h resulted in a 1.5-fold increase in glucose uptake in cisplatin-sensitive cells and no changes in cisplatin-resistant cell lines (34). However, another study reported that cisplatin decreased glucose uptake and 5-fluorouracil upregulated glucose metabolism in lung cancer A549 cells (35). Collectively, glucose uptake is cell-specific in response to the treatment with chemotherapeutic agents, including cisplatin. In the present study, glucose consumption was not involved in the upregulation of *O*-GlcNAcylation.

A number of studies have suggested that global protein *O*-GlcNAc levels are transiently elevated in response to moderate stress stimuli and the elevated *O*-GlcNAc levels promote cell survival (24,36). By contrast, decreased *O*-GlcNAc levels of sensitize cells and tissues to apoptosis and necrosis (8). In the present study, the data demonstrated that the changes in *O*-GlcNAc levels via the inhibition of OGT or OGA did not affect the sensitivity of H1299 cells to cisplatin *in vitro* and *in vivo*. Alloxan, an inhibitor of OGT, significantly counteracted the cisplatin-induced increase in *O*-GlcNAc levels; however, the changes in *O*-GlcNAc levels did not affect the growth inhibitory effect of cisplatin on H1299 cells. At the concentrations tested in the present study, the OGA inhibitor, PUGNAc, was non-toxic and had no effect on the growth rate of H1299 cells. It was also previously reported that PUGNAc did not affect OGT activity and UDP-GlcNAc levels (37). Although PUGNAc markedly enhanced the cisplatin-induced *O*-GlcNAc level in the present study, combined treatment with cisplatin and PUGNAc inhibited cell growth at the same rate as treatment with cisplatin alone in H1299 cells. Similarly, in nude mice injected with H1299 cancer cells, the group treated with both cisplatin and PUGNAc exhibited no changes in tumor growth inhibition compared with the group treated with cisplatin alone. The aforementioned results demonstrated that the turnover of *O*-GlcNAc was not essential for tumor growth, and the changes in *O*-GlcNAc levels did not affect the sensitivity of H1299 cells to cisplatin. The study by Zhou *et al* demonstrated that the downregulation of OGT increased cisplatin resistance in ovarian cancer, but had no effect on the efficacy of paclitaxel (12). By contrast, another study revealed that reducing hyper-*O*-GlcNAcylation by OGT knockdown facilitated the chemosensitivity of bladder cancer cells to cisplatin (38). These results indicated that the effects

of *O*-GlcNAc on the sensitivity of cells to chemotherapeutic agents need to be further explored in the future.

Taken together, the present study demonstrated that cisplatin augmented the global protein *O*-GlcNAc levels by altering the enzymatic activity of OGT and OGA. Cisplatin-reduced AMPK activation prevented GFAT1 phosphorylation and then promoted the activity of GFAT1. Cisplatin-induced GFAT1 activation improved production of the donor substrate UDP-GlcNAc through the HBP. The alteration of *O*-GlcNAcylation did not affect the sensitivity of lung cancer cells to cisplatin *in vitro* and *in vivo*. These findings may prove to be useful in enhancing the current understanding of the roles of *O*-GlcNAcylation in chemotherapy.

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Availability of data and materials

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

Authors' contributions

DiW and YS designed the study, planned the experiments, analyzed the data and wrote the manuscript. DiW, JW, DaW, XH and NZ performed the experiments. All authors have read and approved the final manuscript. DiW and JW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All experimental protocols were approved by the Institutional Review Board of the Department of Laboratory Animal Science of Shandong University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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