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Down-regulation of β1,4-galactosyltransferase V is a critical part of etoposide-induced apoptotic process and could be mediated by decreasing Sp1 levels in human glioma cells

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β1,4-Galactosyltransferase V (β1,4GalT V; EC 2.4.1.38) is considered to be very important in glioma for expressing transformation-related highly branched *N*-glycans. Recently, we have characterized β1,4GalT V as a positive growth regulator in several glioma cell lines. However, the role of β1,4GalT V in glioma therapy has not been clearly reported. In this study, interfering with the expression of β1,4GalT V by its antisense cDNA in SHG44 human glioma cells markedly promoted apoptosis induced by etoposide and the activation of caspases as well as processing of Bid and expression of Bax and Bak. Conversely, the ectopic expression of β1,4GalT V attenuated the apoptotic effect of etoposide on SHG44 cells. In addition, both the β1,4GalT V transcription and the binding of total or membrane glycoprotein with *Ricinus communis* agglutinin-I (RCA-I) were partially reduced in etoposide-treated SHG44 cells, correlated well with a decreased level of Sp1 that has been identified as an activator of β1,4GalT V transcription. Collectively, our results suggest that the down-regulation of β1,4GalT V expression plays an important role in etoposide-induced apoptosis and could be mediated by a decreasing level of Sp1 in SHG44 cells, indicating that inhibitors of β1,4GalT V may enhance the therapeutic efficiency of etoposide for malignant glioma.

Key words: apoptosis/etoposide/glioma/Sp1/β1,4-galactosyltransferase V

Introduction

Malignant gliomas, the most common subtype of primary brain tumors, are considered one of the deadliest of human cancers (Maher *et al.*, 2001). The poor outcome for patients with malignant glioma is mainly attributed to two characteristics of the tumor: one is invasiveness, which makes total resection less feasible, and the other is drug resistance, resulting in unsuccessful chemotherapy (Weller *et al.*, 1998; DeAngelis, 2001). Because most anticancer agents exert effects via activation of apoptotic pathways common to many cellular stresses, any gene alteration disrupting the intrinsic pathways to execute physiological cell death during tumor development can also make malignant cells resistant to chemotherapy (Bogler and Weller, 2002; Johnstone *et al.*, 2002). Such anti-apoptotic alterations are routinely observed in malignant gliomas, including both the functional loss of tumor suppressors and the deregulated hyperfunction of oncogenic proteins (Johnstone *et al.*, 2002; Steinbach and Weller, 2004).

In this report, we have focused on β1,4-galactosyltransferase V (β1,4GalT V) as a potential regulator of apoptosis in glioma cells. Among seven members of β1,4GalT family that transfer uridine-5-diphosphate-galactose to their respective substrates (Guo *et al.*, 2001; Sato *et al.*, 2001), the β1,4GalT V preferentially galactosylates the GlcNAcβ1→6Man arm of the highly branched *N*-glycans (Sato *et al.*, 1998), which are characteristic of glioma (Yamamoto *et al.*, 2000). A series of results acquired with great efforts of both our laboratory and others have testified that β1,4GalT V is essential for glioma to express this transformation-related oligosaccharide (Arango and Pierce, 1988; Shirane *et al.*, 1999; Sato *et al.*, 2000; Xu *et al.*, 2001), indicating a role of β1,4GalT V in glioma malignancy. More recently, we have reported that β1,4GalT V performs as a positive growth regulator in several glioma cell lines through Ras/mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling pathways (Jiang *et al.*, 2006). Given the intimate relations between the pathways of cellular proliferation and apoptosis (Harrington *et al.*, 1994), these findings shed light on the role of β1,4GalT V in the apoptotic response of glioma to chemotherapeutic drugs.

Etoposide (VP16), a topoisomerase-II-inhibitor agent, is one of the most common chemotherapy drugs used for the treatment of malignant glioma, but similar to other agents, its usage is limited by drug resistance (Nagane *et al.*, 1999). The present studies are undertaken to examine the role of β1,4GalT V in the sensitivity of SHG44 glioma cells to etoposide-induced apoptosis. Our results reveal that the down-regulation of β1,4GalT V transcription in an Sp1-mediated way is an integral and pivotal part of the etoposide-induced apoptotic process in SHG44 cells, suggesting β1,4GalT V inhibitors in combination with etoposide as a potent modality to treat patients with malignant glioma.

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Results

Interference of β1,4GalT V expression by transfection with β1,4GalT V antisense cDNA could promote apoptosis induced by etoposide in SHG44 cells

To evaluate the role of in anticancer agent-induced apoptosis, its antisense cDNA construct or control vector was stably transfected into human glioma cell line SHG44, as previously described (Xu *et al.*, 2002). As shown in Figure 1A, reduction in the β1,4GalT V expression sensitized SHG44 cells to etoposide-induced apoptosis as indicated by fragmented and condensed nuclei, suggesting a pro-apoptotic effect of decreasing β1,4GalT V expression on glioma cells. This conclusion was further supported by FACS assay, as the percentage of apoptotic cells in antisense-transfected SHG44 cells was increased compared with that of the controls (data not shown).

To identify the proteins responsible for the enhanced apoptotic response in antisense-transfected SHG44 cells, we explored whether β1,4GalT V has influence on the activation of Caspase-9 or Caspase-3, both of which have been proved to mediate etoposide-induced apoptosis in a variety of cell lines (Yin *et al.*, 2000). As described in Figure 1b, suppression of β1,4GalT V expression markedly increased the cleavage of Caspase-9 and Caspase-3. Consistent with this, the expression of Bax and Bak, two important promoters of Caspase-9 activation (Wei *et al.*, 2001; Degenhardt *et al.*, 2002), was significantly up-regulated in antisense-transfected SHG44 cells. Moreover, reduced β1,4GalT V expression enhanced Bid cleavage that is mainly executed by active Caspase-8 and then stimulates Caspase-9 processing together with Bax or Bak (Luo *et al.*, 1998). These data suggested that interference with β1,4GalT V expression might promote etoposide-induced apoptosis by enhancing the activation of Caspase-9 and Caspase-3.

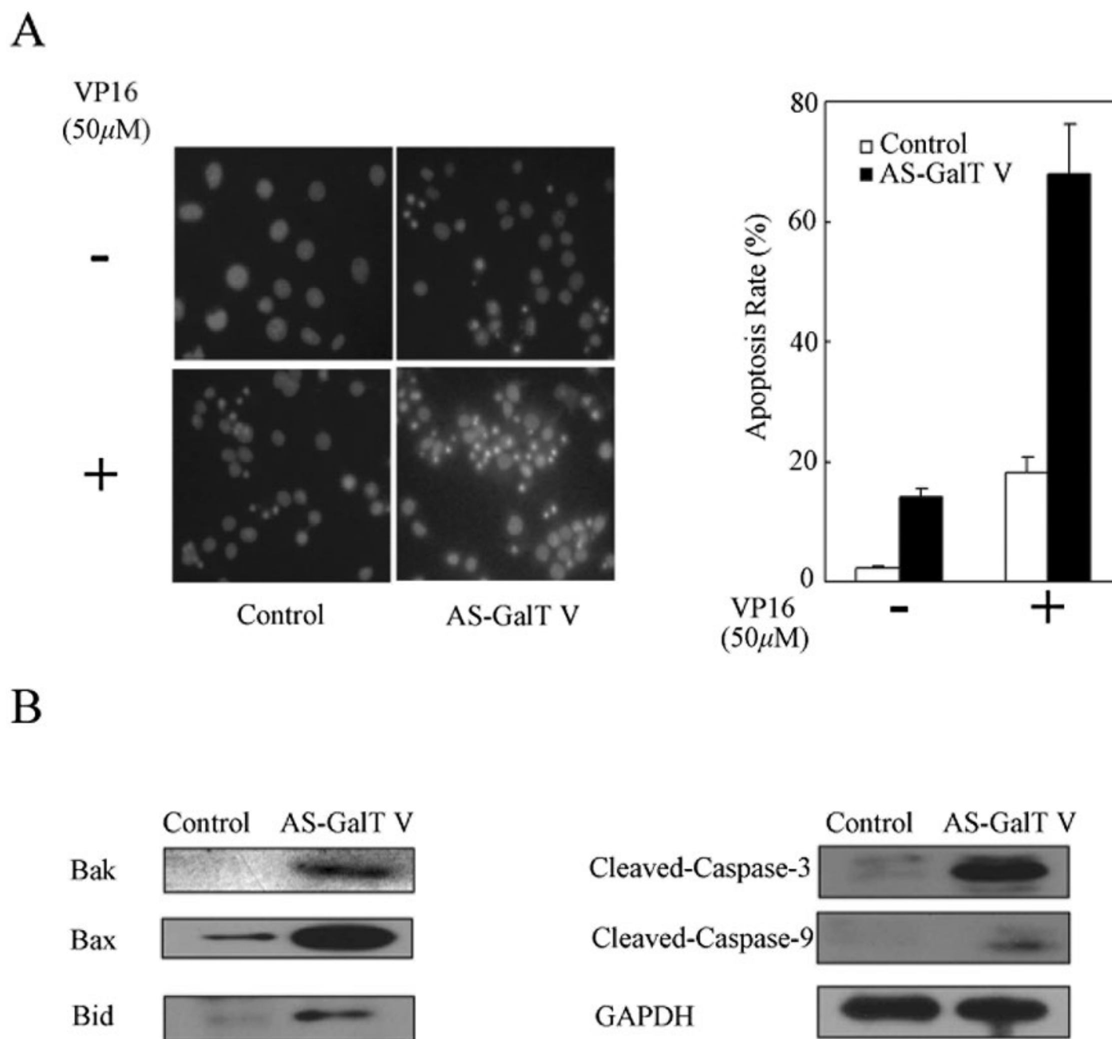

Fig. 1. Interference of β1,4GalT V expression by transfection with β1,4GalT V antisense cDNA could promote apoptosis induced by etoposide (VP16) in SHG44 cells. (**A**) Apoptosis was assessed morphologically. Hoechst33258 staining of nuclei from SHG44 cells stably transfected with mock (control) or antisense-β1,4GalT V (AS-β1,4GalT V) untreated or treated with VP16 (50μM) for 16h. Images were visualized at 200× using a fluorescent microscope (left panel). At least 300 cells were counted from three different microscope fields, and each value was the mean±SD of three independent experiments (right panel). (**B**) Cell homogenates from indicated cells were analyzed by western blot with anti-cleaved Caspase-3, anti-cleaved Caspase-9, anti-Bax, anti-Bak, and anti-Bid antibodies as indicated. The GAPDH western blot served as a loading control.

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The expression level of β1,4GalT V mRNA and N-glycans is decreased with etoposide treatment

To test the possibility that the expression of *β1,4GalT V* gene could be regulated by etoposide, we measured the contribution of etoposide in the expression level of β1,4GalT V mRNA by RT–PCR assay. As shown in Figure 2A, endogenous β1,4GalT V mRNA expression level was partially decreased in etoposide-treated SHG44 cells.

It has been reported that β1,4GalT V plays a critical role in the expression of β1,6-linked GlcNAc-bearing *N*-glycans, which is a marker of tumor progress in glioma (Sato and Furukawa, 2004). To examine whether etoposide has an effect on the expression of *N*-glycans in SHG44 cells, we performed lectin blotting analysis using horseradish peroxidase (HRP)-conjugated *Ricinus communis* agglutinin-I (RCA-I) that interacts with oligosaccharides terminating with the Galβ1→4GlcNAc group or biotinylated *Phaseolus vulgaris* leucoagglutinin (L-PHA) that interacts with highly branched *N*-glycans with the Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2)Man branch (Sato and Furukawa, 2004). As expected, a significant decrease of the binding of total glycoprotein with RCA-I (Figure 2B, left panel) or L-PHA (Figure 2B, right panel) was observed for several protein bands in cells exposed to etoposide compared with that in the controls. Furthermore, the treatment of SHG44 cells with etoposide decreased the binding with RCA-I on the cell surface (Figure 2C). Together, these observations suggested that etoposide might have a negative effect on β1,4GalT V expression in SHG44 cells, leading to a decreased level of Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2)Man branch and thus the highly branched *N*-glycans.

Etoposide down-regulates β1,4GalT V promoter activity through reducing the level of transcription factor Sp1

To confirm that the down-regulation of β1,4GalT V expression by etoposide was at the transcriptional level, we constructed the *β1,4GalT V* reporter construct pGL3 (–200/+120), which retained relatively strong promoter activity in cancer cells and contained one Sp1-binding site at nucleotide positions –81/–69 (Sato and Furukawa, 2004). As expected, the transient transfection of the construct pGL3 (–200/+120) into SHG44 cells showed a suppressed activity responding to etoposide treatment in a manner similar to that observed in RT–PCR assay (Figure 3A).

The general transcription factor Sp1 has been reported as an essential activator in the transcriptional regulation of human *β1,4GalT V* gene in cancer cells (Sato and Furukawa, 2004). To investigate whether the inhibitive effect of etoposide on *β1,4GalT V* promoter was mediated by Sp1, we introduced site-directed mutagenesis into the Sp1-binding site on the pGL3 (–200/+120) reporter plasmid, as previously described (Sato and Furukawa, 2004). It was found that the mutagenesis of this Sp1-binding site abolished the effects of etoposide on the *β1,4GalT V* promoter activity (Figure 3B). In addition, a lower level of Sp1 was observed in extracts prepared from etoposide-treated cells, whereas the level of E2F1, a pro-apoptotic protein, was increased (Figure 3C), suggesting that etoposide may induce apoptosis in glioma cells in part through down-regulating β1,4GalT V transcription via decreasing Sp1 level. This deduction was further supported in Figure 3d, as the stimulation of *β1,4GalT V* promoter activity by transient transfection with Sp1 expression plasmids was largely blunted after etoposide treatment.

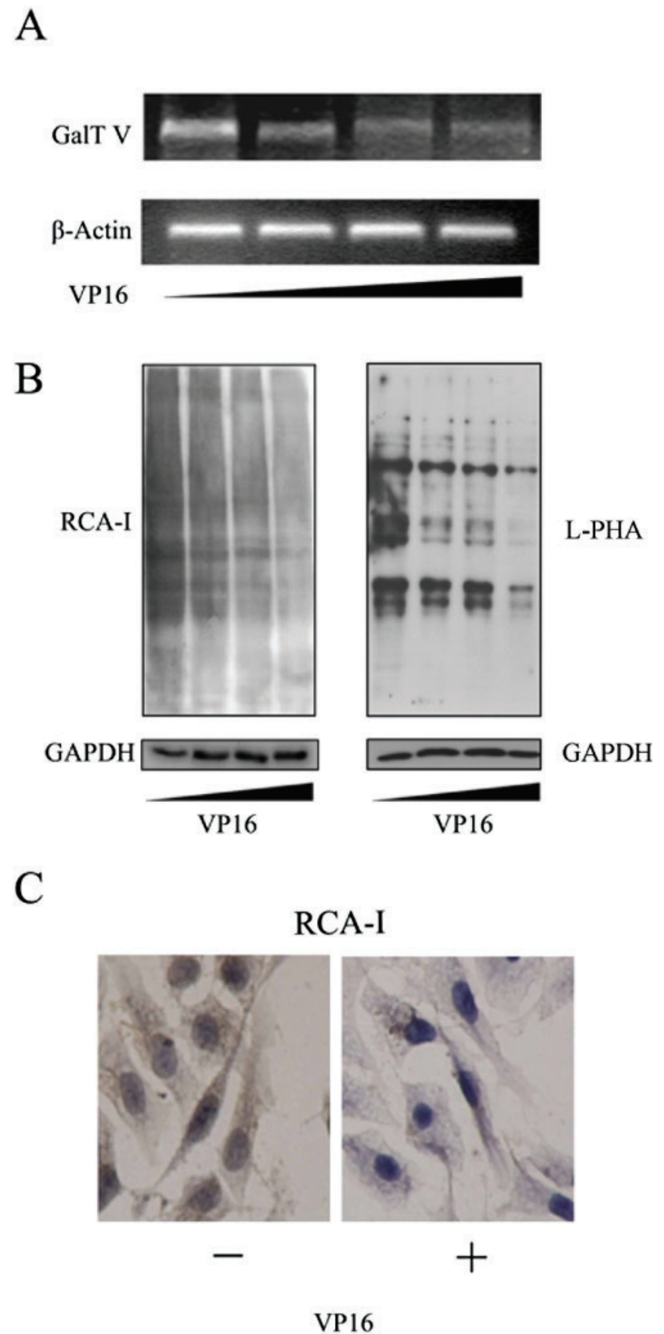

Fig. 2. The expression level of β1,4GalT V mRNA and Galβ1→4GlcNAc group is decreased with the treatment of etoposide. (**A**) RT–PCR analysis of endogenous β1,4GalT V mRNA expression level in SHG44 cells in the absence or presence of VP16 for 4h. The concentration of VP16 was 50μM, 100μM, or 200μM. The levels of β-actin mRNA expression were assessed as loading controls. (**B**) Proteins from SHG44 cells treated as described under Materials and methods were separated by SDS–PAGE, and the binding to RCA-I or L-PHA was analyzed by RCA-I-lectin (left panel) or L-PHA-lectin (right panel). The GAPDH western blot served as a control. (**C**) SHG44 cells were treated with vehicle (–) or VP16 (+) for 12h, and RCA-I lectin staining analysis was performed as described under *Materials and methods*.

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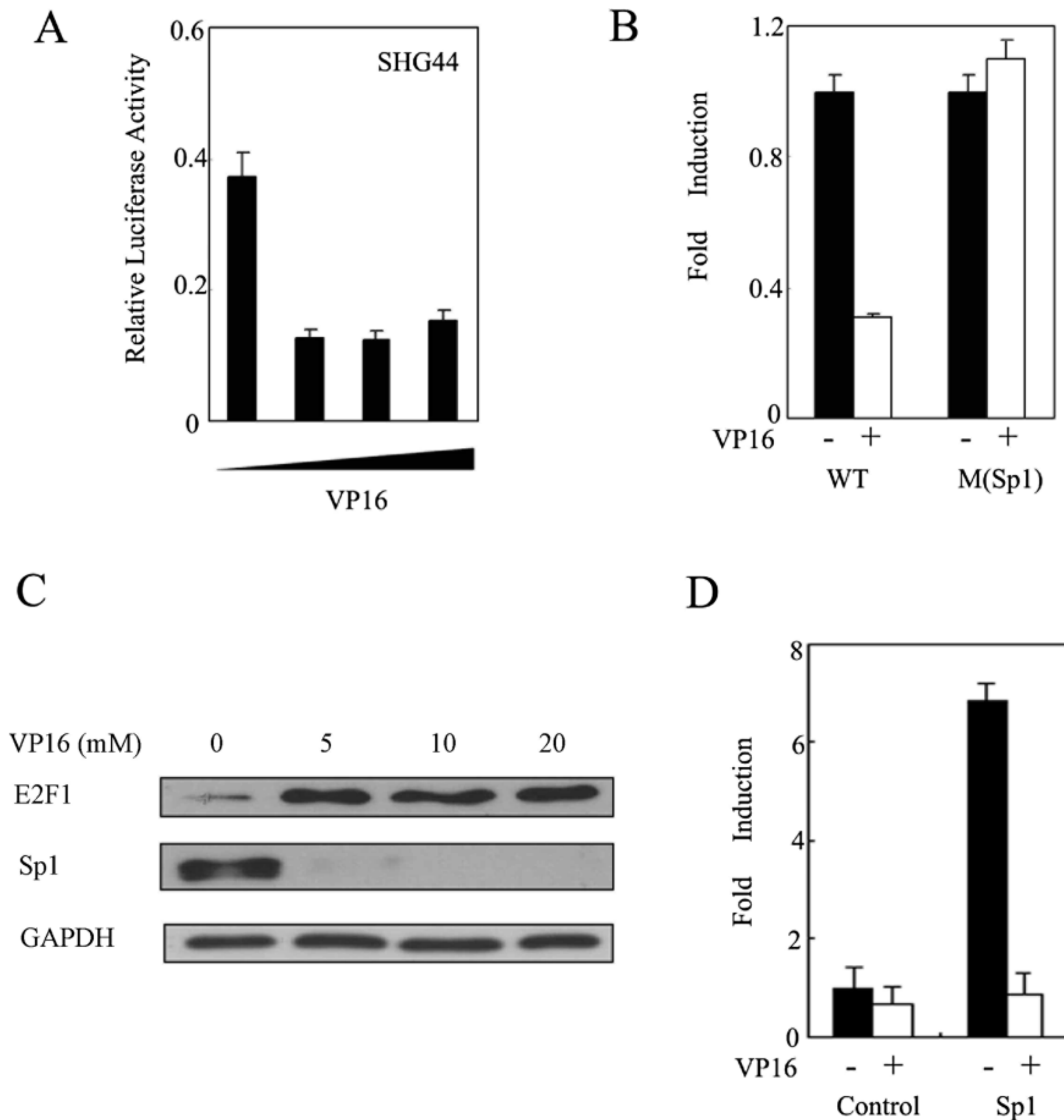

Fig. 3. Etoposide down-regulates β1,4GalT V promoter activity through reducing the level of transcription factor Sp1. (**A**) SHG44 cells were transiently transfected with β1,4GalT V promoter construct pGL3 (–200/+120). At 24h after transfection, cells were treated with vehicle or an increasing dose of VP16 for an additional 4h. The luciferase activity values were standardized to those observed in nontreated samples. Each value is the mean±SD of at least three independent experiments. (**B**) Reporter plasmids pGL3 (–200/+120) (WT) or its Sp1-binding site-mutated construct (M (Sp1)) were transfected into SHG44 cells. At 24h after transfection, cells were treated with vehicle (–) or 100 μM VP16 (+) for 4h. The luciferase activities were obtained and presented as described under Materials and methods. (**C**) SHG44 cells were treated for 4h with indicated dose of VP16, and cell extracts were subjected to immunoblot analysis using an anti-Sp1 antibody. The anti-E2F1 antibody was used as a positive control. (**D**) SHG44 cells were transiently cotransfected with pGL3 (–200/+120) construct and control vector or Sp1-expressing vector. At 24h after transfection, cells were treated with vehicle (–) or 100 μM VP16 (+) for 4h. Normalized luciferase activity was standardized to pGL3 (–200/+120) with control vector in untreated cells.

Forced expression of β1,4GalT V could protect SHG44 cells from apoptosis induced by etoposide

To further determine the role of β1,4GalT V in etoposide-induced apoptosis, we stably transfected SHG44 cells with hemagglutinin (HA)-β1,4GalT V constructs (Figure 4A) (Jiang *et al*., 2006). As expected, the ectopic expression of β1,4GalT V resulted in a remarkable reduction in the number of apoptotic cells in etoposide-contained conditions relative to the control cells (Figure 4B), pointing out that a decreased level of β1,4GalT V is important for etoposide to induce apoptosis in glioma cells. Similar results were obtained in FACS assay (data not shown).

Discussion

Results from previous studies have demonstrated that β1,4GalT V plays an important role in glioma biology, including growth, invasiveness, and expression of abnormal *N*-glycans (Shirane *et al*., 1999; Xu *et al*., 2002; Sato and

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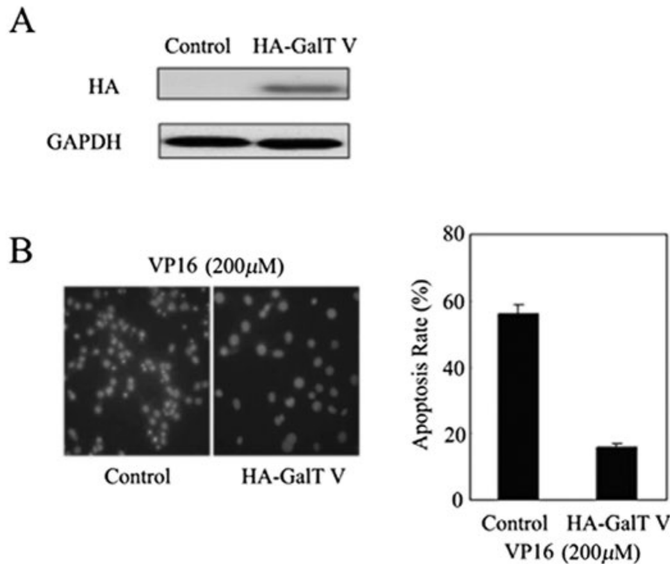

Fig. 4. Forced expression of β1,4GalT V could protect SHG44 cells from apoptosis induced by etoposide. (**A**) Western blot assay demonstrated HA-β1,4GalT V expression in the SHG44 cells stably transfected with HA-β1,4GalT V construct using anti-HA antibody. (**B**) Mock- (control) or HA-β1,4GalT V-transfected SHG44 cells were treated with VP16 (200μM) for 16h. The apoptotic percentages were assayed by Hoechst33258 staining, as described under Materials and methods.

Furukawa, 2004; Jiang *et al.*, 2006). In this study, we explored the relationship between β1,4GalT V and the sensitivity of glioma cells to etoposide-induced apoptosis. As shown here, morphological and nuclear changes were inhibited, the loss of cell viability was prevented, and the cells in sub-G1 were reduced when β1,4GalT V expression was suppressed in etoposide-treated SHG44 cells, indicating that β1,4GalT V might serve as a negative regulator in the apoptotic response of glioma to etoposide.

The activation of caspases plays a critical role in apoptosis (Budihardjo *et al.*, 1999), and two main pathways have been identified to lead to it: one is the extrinsic pathway initiated by death-receptor activation and the other is the intrinsic pathway triggered by various stress signals, including DNA damage and acting through mitochondria (Zimmermann *et al.*, 2001). Crosstalk can occur between these two pathways, resulting in the amplification of mitochondrial release of cytochrome *c*, which in turn leads to the enhanced activation of Caspase-9 and downstream executioner caspases that are responsible for the cleavage of specific cellular substrates (Zimmermann *et al.*, 2001). To investigate the mechanism by which β1,4GalT V affected etoposide-induced apoptosis, we examined the potential influence of β1,4GalT V on the caspase activity. As expected, SHG44 cells transfected with antisense β1,4GalT V cDNA displayed an enhanced activation of Caspase-9 and Caspase-3, indicating that decreasing β1,4GalT V expression might sensitize SHG44 cells to etoposide-induced apoptosis via augmenting the transduction of signals leading to the sequential activation of Caspase-9 and Caspase-3. This hypothesis was supported by our observations that the expression of Bax and Bak, two pro-apoptotic Bcl-2-like proteins reported to be essential for mitochondrial dysfunction in response to diverse apoptotic stimuli (Wei *et al.*, 2001), was significantly increased in antisense-transfected SHG44 cells. Furthermore, the cleavage of Bid, another member of the Bcl-2 family, was increased by suppression of β1,4GalT V expression. Bid is believed to stand at the crossroads of mitochondria and death receptor, as it is cleaved by active Caspase-8 to form truncated Bid (tBid), which in turn stimulates Bax- or Bak-mediated cytochrome *c* release (Luo *et al.*, 1998; Broaddus *et al.*, 2005). As a DNA topoisomerase-II inhibitor, etoposide causes an accumulation of protein-linked DNA double-strand breaks (Dolega, 1998) and thus induces apoptosis chiefly through mitochondrial pathway with little influence on the activation of Caspase-8 or Bid (Miao *et al.*, 2003; Broaddus *et al.*, 2005). In this background, our observations suggest that interference with β1,4GalT V expression may complement and reinforce the apoptotic effect of etoposide on glioma via augmenting the signal transduction in both extrinsic and intrinsic pathways, leading to the enhanced activation of caspases.

Another important finding of this study is that the expression of *β1,4GalT V* gene was partially down-regulated in etoposide-treated SHG44 cells in an Sp1-mediated way, which was suggested by several evidences. (1) The expression level of β1,4GalT V mRNA was down-regulated with the treatment of etoposide, correlated with a lower level of Sp1. (2) The transient transfection of the *β1,4GalT V* reporter construct into SHG44 cells showed a suppressed activity responding to etoposide, which was abolished by the mutagenesis of the Sp1-binding site on the construct. (3) The positive effect of increasing Sp1 expression on *β1,4GalT V* promoter activity could be blocked by etoposide treatment. Consistently, the level of highly branched *N*-glycans with the Galβ1→4GlcNAcβ1→6(Galβ1→4GlcNAcβ1→2)Man branch which has been reported to play a major role in glioma invasivity (Yamamoto *et al.*, 2000) was decreased in etoposide-treated cells, indicating that etoposide may down-regulate β1,4GalT V transcription as an integral part of its apoptotic action to inhibit the expression of transformation-related highly branched *N*-glycans. In addition, it has been pointed out that β1,4GalT V functions as a positive growth regulator in glioma via contributing to the activation of AKT and MAPK pathways (Jiang *et al.*, 2006), both of which are important for facilitating tumor cell proliferation, inhibiting apoptosis, and maintaining the tumor phenotype (Jetzt *et al.*, 2003; Shi *et al.*, 2004; Nakada *et al.*, 2005). Taking all these facts into account, we conclude that etoposide might induce apoptosis in SHG44 cells in part through inhibiting the pro-survival and anti-apoptotic effect of β1,4GalT V by down-regulating β1,4GalT V transcription in an Sp1-mediated way.

Furthermore, the forced expression of β1,4GalT V rendered SHG44 cells considerably resistant to even high-dose etoposide, suggesting that a decreased level of β1,4GalT V expression is not only an integral part but also an essential condition of etoposide-induced apoptosis in glioma. The detailed mechanism underlying the interaction between β1,4GalT V and etoposide seems to associate with the complex network of multiple interrelated pathways regulating the proliferation and death of glioma cells and demands further research.

Thus, all data presented here suggest that (1) the down-regulation of β1,4GalT V expression is an integral and essential part of etoposide-induced apoptosis in glioma and

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could be mediated by decreasing Sp1 level and (2) interfering with β1,4GalT V expression could synergistically and additively augment the apoptotic effect of etoposide on SHG44 cells. Our findings may provide some clinical significance in the killing of malignant glioma cells, as combined treatment with β1,4GalT V inhibitors and DNA-damaging agents will help achieve more effective therapy with less toxicity by using a lower dose of cytotoxic drugs.

Materials and methods

Plasmids

Expression constructs for HA-pcDNA3.0, pGL3 (–200/+120) and M (Sp1) have been previously described (Xu *et al.*, 2002; Jiang *et al.*, 2006). PEVR2-Sp1 (human) was a generous gift from Prof. Dr. Guntram Suske (Marburg, Germany). PRL-CMV was purchased from Promega Corporation (San Luis Obispo, CA).

Cell culture

Human glioma cell line SHG44 and SHG44 cells stably transfected with pcDNA3.0, HA-β1,4GalT V, or β1,4GalT V antisense cDNA were previously described (Xu *et al.*, 2002; Jiang *et al.*, 2006). All these cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum, 100U/mL of penicillin and 50μg/mL of streptomycin at 37°C in a humidified CO_2 incubator (5% CO_2, 95% air).

Antibodies and reagents

Bovine calf serum, DMEM, TRIzol reagent and LipofectAMINE reagent were purchased from Invitrogen (Carlsbad, CA). Phenylmethylsulfonyl fluoride, aprotinin, pepstatin, dithiothreitol, Hoechst33258, dimethyl sulfoxide, and etoposide (VP16) were from Sigma Chemical (Saint Louis, MO). Sialidase was from Boehringer Mannheim (Mannheim, Germany). Anti-Sp1 antibody was purchased from Active Motif (Carlsbad, CA). Anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) and anti-E2F1 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-cleaved Caspase-3, anti-cleaved Caspase-9, anti-Bax, anti-Bak, and anti-Bid antibodies were purchased from Cell Signal Technology (Boston, MA). Anti-HA antibody was from Roche Applied Science (Indianapolis, IN). Anti-mouse-HRP secondary antibody and anti-rabbit-HRP secondary antibody were purchased from New England Biolabs (Ipswich, MA). HRP-conjugated RCA-I was from EY Laboratory (San Mateo, CA). Biotinylated L-PHA was purchased from Vector Laboratories (Burlingame, CA). HRP-conjugated Streptavidin was from Southern Biotechnology Associates (Birmingham, AL).

Analysis of nuclear morphology by fluorescence staining

Cells grown on the glass coverslips were treated with different doses of etoposide as indicated for 16h, and then the fluorescence staining was performed as previously described (Li *et al.*, 2005).

Western blot analysis

Total cell lysates from mock- or antisense-transfected SHG44 cells were analyzed by western blot with indicated antibodies as previously described (Li *et al.*, 2005), using an antibody to the GAPDH to ensure equivalent loading.

RT–PCR

Total RNA 1μg extracted from cells treated with different concentrations of etoposide for 4h was analyzed by RT–PCR as our previous report with following primers: β1,4GalT V forward 5′-TGAGAACAATCGGTGCATCAG-3′ and β1,4GalT V reverse 5′-CTCAATCCGCCAAATAACTC-3′ (Xu *et al.*, 2001). The PCR products for β1,4GalT V were 657bp.

Lectin blotting and RCA-I lectin staining analysis

RCA-I staining analysis was performed basically as previously described (Xu *et al.*, 2002). Briefly, cells coated on the glass coverslips were treated with vehicle or etoposide (200μM) for 12 h and then were fixed with 4% paraformaldehyde/phosphate-buffered saline (PBS) for 30min. To eliminate terminal sialic acid moieties, we treated cells with sialidase (0.03U/mL) for 5h at 37°C. Endogenous peroxidase activity was blocked with 0.3% H_2O_2/methanol for 30min. To minimize nonspecific binding reactions, we covered specimens for 30 min with 1% bovine serum albumin in Tris-buffered saline (TBS). Following this, cells were incubated at 37°C for 2h in the presence of HRP-conjugated RCA-I (4μg/μL). After rinsing the cells thoroughly in PBS, they were stained by treating the coverslips with 3,3′-diaminobenzidinetetra hydrochloride (DAB) solution for 3min. Finally, the samples were dehydrated, cleared, and mounted.

Total extracts from cells treated with different doses of etoposide for 4h were analyzed by lectin blotting assay with RCA-I or L-PHA (Zhu *et al.*, 2005). The GAPDH western blot served as a loading control.

Dual luciferase assay

Cells seeded in 24-well plates were transfected (Zhu *et al.*, 2005). After 24h, cells were treated with vehicle or etoposide for 4h. Then, cell lysates were prepared, and the luciferase activities were measured using a Dual Luciferase Reagent kit (Promega) and a LB 9507 luminometer (Berthold GmbH, Wildbad, Germany).

Statistics and presentation of data

All experiments were repeated three times. All numerical data were expressed as mean±SD. Data were analyzed using the two-tailed *t*-test.

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Conflict of interest statement

None declared.

Abbreviations

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GlcNAc, *N*-acetylglucosamine; HA, hemagglutinin; HRP, horseradish peroxidase; MAPK, mitogen-activated protein kinase; PHA, *Phaseolus vulgaris* leucoagglutinin; RCA, *Ricinus communis* agglutinin; VP16, etoposide; β1,4GalT V, β1,4-galactosyltransferase V.

References

Arango, J. and Pierce, M. (1988) Comparison of N-acetylglucosaminyltransferase V activities in Rous sarcoma-transformed baby hamster kidney (RS-BHK) and BHK cells. *J. Cell Biochem.*, **37**, 225–231.

Bogler, O. and Weller, M. (2002) Apoptosis in gliomas, and its role in their current and future treatment. *Front Biosci.*, **7**, e339–e353.

Broaddus, V.C., Dansen, T.B., Abayasiriwardana, K.S., Wilson, S.M., Finch, A.J., Swigart, L.B., Hunt, A.E., and Evan, G.I. (2005) Bid mediates apoptotic synergy between tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and DNA damage. *J. Biol. Chem.*, **280**, 12486–12493.

Budihardjo, I., Oliver, H., Lutter, M., Luo, X., and Wang, X. (1999) Biochemical pathways of caspase activation during apoptosis. *Annu. Rev. Cell Dev. Biol.*, **15**, 269–290.

DeAngelis, L.M. (2001) Brain tumors. *N. Engl. J. Med.*, **344**, 114–123.

Degenhardt, K., Sundararajan, R., Lindsten, T., Thompson, C., and White, E. (2002) Bax and Bak independently promote cytochrome C release from mitochondria. *J. Biol. Chem.*, **277**, 14127–14134.

Dolega, A. (1998) [Cytotoxic mechanism and antineoplastic action of etoposide]. *Postepy Hig. Med. Dosw.*, **52**, 67–87.

Guo, S., Sato, T., Shirane, K., and Furukawa, K. (2001) Galactosylation of N-linked oligosaccharides by human beta-1,4-galactosyltransferases I, II, III, IV, V, and VI expressed in Sf-9 cells. *Glycobiology*, **11**, 813–820.

Harrington, E.A., Fanidi, A., and Evan, G.I. (1994) Oncogenes and cell death. *Curr. Opin. Genet. Dev.*, **4**, 120–129.

Jetzt, A., Howe, J.A., Horn, M.T., Maxwell, E., Yin, Z., Johnson, D., and Kumar, C.C. (2003) Adenoviral-mediated expression of a kinase-dead mutant of Akt induces apoptosis selectively in tumor cells and suppresses tumor growth in mice. *Cancer Res.*, **63**, 6697–6706.

Jiang, J., Chen, X., Shen, J., Wei, Y., Wu, T., Yang, Y., Wang, H., Zong, H., Yang, J., Zhang, S., and others (2006) Beta 1,4-galactosyltransferase V functions as a positive growth regulator in glioma. *J. Biol. Chem.*, **281**, 9482–9489.

Johnstone, R.W., Ruefli, A.A., and Lowe, S.W. (2002) Apoptosis: a link between cancer genetics and chemotherapy. *Cell*, **108**, 153–164.

Li, Z., Wang, H., Zong, H., Sun, Q., Kong, X., Jiang, J., and Gu, J. (2005) Downregulation of beta1,4-galactosyltransferase 1 inhibits CDK11(p58)-mediated apoptosis induced by cycloheximide. *Biochem. Biophys. Res. Commun.*, **327**, 628–636.

Luo, X., Budihardjo, I., Zou, H., Slaughter, C., and Wang, X. (1998) Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. *Cell*, **94**, 481–490.

Maher, E.A., Furnari, F.B., Bachoo, R.M., Rowitch, D.H., Louis, D.N., Cavenee, W.K., and DePinho, R.A. (2001) Malignant glioma: genetics and biology of a grave matter. *Genes Dev.*, **15**, 1311–1333.

Miao, L., Yi, P., Wang, Y., and Wu, M. (2003) Etoposide upregulates Bax-enhancing tumour necrosis factor-related apoptosis inducing ligand-mediated apoptosis in the human hepatocellular carcinoma cell line QGY-7703. *Eur. J. Biochem.*, **270**, 2721–2731.

Nagane, M., Asai, A., Shibui, S., Oyama, H., Nomura, K., and Kuchino, Y. (1999) Expression pattern of chemoresistance-related genes in human malignant brain tumors: a working knowledge for proper selection of anticancer drugs. *Jpn. J. Clin. Oncol.*, **29**, 527–534.

Nakada, M., Niska, J.A., Tran, N.L., McDonough, W.S., and Berens, M.E. (2005) EphB2/R-Ras signaling regulates glioma cell adhesion, growth, and invasion. *Am. J. Pathol.*, **167**, 565–576.

Sato, T. and Furukawa, K. (2004) Transcriptional regulation of the human beta-1,4-galactosyltransferase V gene in cancer cells: essential role of transcription factor Sp1. *J. Biol. Chem.*, **279**, 39574–39583.

Sato, T., Furukawa, K., Bakker, H., Van den Eijnden, D.H., and Van Die, I. (1998) Molecular cloning of a human cDNA encoding beta-1, 4-galactosyltransferase with 37% identity to mammalian UDP-Gal:GlcNAc beta-1,4-galactosyltransferase. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 472–477.

Sato, T., Guo, S., and Furukawa, K. (2001) Occurrence of poly-N-acetyllactosamine synthesis in Sf-9 cells upon transfection of individual human beta-1,4-galactosyltransferase I, II, III, IV, V and VI cDNAs. *Biochimie*, **83**, 719–725.

Sato, T., Shirane, K., Kido, M., and Furukawa, K. (2000) Correlated gene expression between beta-1,4-galactosyltransferase V and N-acetylglucosaminyltransferase V in human cancer cell lines. *Biochem. Biophys. Res. Commun.*, **276**, 1019–1023.

Shi, Q., Bao, S., Maxwell, J.A., Reese, E.D., Friedman, H.S., Bigner, D.D., Wang, X.F., and Rich, J.N. (2004) Secreted protein acidic, rich in cysteine (SPARC), mediates cellular survival of gliomas through AKT activation. *J. Biol. Chem.*, **279**, 52200–52209.

Shirane, K., Sato, T., Segawa, K., and Furukawa, K. (1999) Involvement of beta-1,4-galactosyltransferase V in malignant transformation-associated changes in glycosylation. *Biochem. Biophys. Res. Commun.*, **265**, 434–438.

Steinbach, J.P. and Weller, M. (2004) Apoptosis in gliomas: molecular mechanisms and therapeutic implications. *J. Neurooncol.*, **70**, 245–254.

Wei, M.C., Zong, W.X., Cheng, E.H., Lindsten, T., Panoutsakopoulou, V., Ross, A.J., Roth, K.A., MacGregor, G.R., Thompson, C.B., and Korsmeyer, S.J. (2001) Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. *Science*, **292**, 727–730.

Weller, M., Rieger, J., Grimmel, C., Van Meir, E.G., De Tribolet, N., Krajewski, S., Reed, J.C., von Deimling, A., and Dichgans, J. (1998) Predicting chemoresistance in human malignant glioma cells: the role of molecular genetic analyses. *Int. J. Cancer*, **79**, 640–644.

Xu, S., Zhang, S., Chen, C., Yan, J., Cai, M., Zhu, X., and Gu, J. (2002) Over-expression of beta-1,4-galactosyltransferase V increases the growth of astrocytoma cell line. *J. Exp. Clin. Cancer Res.*, **21**, 409–414.

Xu, S., Zhu, X., Zhang, S., Yin, S., Zhou, L., Chen, C., and Gu, J. (2001) Over-expression of beta-1,4-galactosyltransferase I, II, and V in human astrocytoma. *J. Cancer Res. Clin. Oncol.*, **127**, 502–506.

Yamamoto, H., Swoger, J., Greene, S., Saito, T., Hurh, J., Sweeley, C., Leestma, J., Mkrdichian, E., Cerullo, L., Nishikawa, A., and others (2000) Beta1,6-N-acetylglucosamine-bearing N-glycans in human gliomas: implications for a role in regulating invasivity. *Cancer Res.*, **60**, 134–142.

Yin, D., Tamaki, N., and Kokunai, T. (2000) Wild-type p53-dependent etoposide-induced apoptosis mediated by caspase-3 activation in human glioma cells. *J. Neurosurg.*, **93**, 289–297.

Zhu, X., Jiang, J., Shen, H., Wang, H., Zong, H., Li, Z., Yang, Y., Niu, Z., Liu, W., Chen, X., and others (2005) Elevated beta1,4-galactosyltransferase I in highly metastatic human lung cancer cells. Identification of E1AF as important transcription activator. *J. Biol. Chem.*, **280**, 12503–12516.

Zimmermann, K.C., Bonzon, C., and Green, D.R. (2001) The machinery of programmed cell death. *Pharmacol. Ther.*, **92**, 57–70.

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