Journal of Neurochemistry, 2004, **89**, 1436–1444 doi:10.1111/j.1471-4159.2004.02435.x

Transfection of 2,6 and 2,3-sialyltransferase genes and GlcNAc-transferase genes into human glioma cell line U-373 MG affects glycoconjugate expression and enhances cell death

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Abstract

Human glioma cell line U-373 MG expresses CMP-NeuAc : Galβ1,3GlcNAc α2,3-sialyltransferase [EC No. 2.4.99.6] (α2,3ST), UDP-GlcNAc : β-D-mannoside β1,6-*N*-acetylglucosaminyltransferase V [EC 2.4.1.155] (GnT-V) and UDP-GlcNAc3: β-D-mannoside β1,4-*N*-acetylglucosaminyltransferase III [EC 2.4.1.144] (GnT-III) but not CMP-NeuAc : Galβ1,4GlcNAc α2,6-sialyltransferase [EC 2.4.99.1] (α2,6ST) under normal culture conditions. We have previously shown that transfection of the *α2,6ST* gene into U-373 cells replaced α2,3-linked sialic acids with α2,6 sialic acids, resulting in a marked inhibition of glioma cell invasivity and a significant reduction in adhesivity. We now show that U-373 cells, which are typically highly resistant to cell death induced by chemotherapeutic agents (< 10% death in 18 h), become more sensitive to apoptosis following overexpression of these four glycoprotein glycosyltransferases. U-373 cell viability showed a three-fold decrease (from 20 to 60% cell death) following treatment with staurosporine, C2-ceramide or etoposide, when either *α2,6ST* and *GnT-V* genes were stably overexpressed. Even glycosyltransferases typically raised in cancer cells, such as α2,3ST and GnT-III, were able to decrease viability two-fold (from 20 to 40% cell death) following stable overexpression. The increased susceptibility of glycosyltransferase-transfected U-373 cells to pro-apoptotic drugs was associated with increased ceramide levels in Rafts, increased caspase-3 activity and increased DNA fragmentation. In contrast, the same glycosyltransferase overexpression protected U-373 cells against a different class of apoptotic drugs, namely the phosphatidylinositol 3-kinase inhibitor LY294002. Thus altered surface protein glycosylation of a human glioblastoma cell line can lead to lowered resistance to chemotherapeutic agents.

Keywords: apoptosis, ceramide, etoposide, glioblastoma, glycoprotein, glycosyltransferases.

J. Neurochem. (2004) **89,** 1436–1444.

The structure of *N*-linked oligosaccharide chains on glycoproteins has been correlated with tumor cell adhesion to the extracellular matrix and the metastatic potential of tumors, for example in colon cancers and their derived cell lines (Demetriou *et al*. 1995) and malignant gliomas and their derived cell lines (Yamamoto *et al*. 1997b,a). Inhibition of *N*-glycosylation by treatment with 1-deoxymannojirimycin or tunicamycin has been reported to reduce both the adhesion and metastatic potential of colon carcinoma cells (von Lampe *et al*. 1993) and melanoma cells (Chammas *et al*. 1993), implying that reducing *N*-glycosylation should slow tumorigenesis. Likely targets for this altered glycosylation are the integrins, a family of sialoglycoproteins involved in cellular adhesion, whose ability to form functional dimers is dependent upon the presence of *N*-linked oligosaccharides (Zheng *et al*. 1994). For this reason there has been considerable interest in the role of glycosyltransferases in

Received December 12, 2003; revised manuscript received February 2, 2004; accepted February 5, 2004.

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Abbreviations used: α2,3ST, α2,3-sialyltransferase; α2,6ST, α2,6-sialyltransferase; DAP1, AcG-α-ketoamide-palmitoyl diamino proprionate; DAP-KA, DAP1 ketoamide; DMSO, dimethylsulfoxide; GnT-III, *N*-acetylglucosaminyltransferase III; GnT-V, *N*-acetylglucosaminyltransferase V; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3-kinase; PPT, palmitoyl : protein thioesterase.

tumorigenesis (e.g. Couldrey and Green 2000), but the diagnostic, therapeutic and prognostic potential has never been realized. We therefore attempted to expand these studies to CNS tumors.

Many distinct sialyltransferases have been described (Kitagawa and Paulson 1994) and their differential level of expression has been associated with tumorigenesis (Hakomori 1996). For example, CMP-NeuAc : Galβ1,4GlcNAc α2,6-sialyltransferase (α2,6ST) has a wide range of expression, including normal mature astrocytes (Le Marer and Stehelin 1995) but not tumors, whereas CMP-NeuAc : Galβ1,3(4)GlcNAc α2,3-sialyltransferase (α2,3ST) is less common in mature neural cells but is highly expressed in malignant gliomas and fetal astrocytes. We have shown previously that overexpression of α2,6ST in U-373 glioma cells (which lack α2,6ST but express a 2,3ST) produces a marked decrease in both adhesion and metastatic potential (Yamamoto *et al*. 1997a,b). A likely target of the α2,6ST in U-373 gliomas is the major α3β1 integrin, which mediates the adhesion of U-373 cells to collagen or fibronectin matrices (Yamamoto *et al*. 1997a, 2000). Thus, previous studies have shown that increased sialylation of the β1 integrin subunit might be correlated with decreased adhesiveness and decreased metastatic potential (Kawano *et al*. 1993), possibly through lack of mitogen-activated protein (MAP) kinase activation (Faraldo *et al*. 2000). In an *in vivo* model, *α2,6ST*-transfected glioma cells produced no intracranial tumors in severe combined immunodeficient mice, whereas parental U-373 MG cells, the vector-transfected control cells and *ST3Gal III*-transfected U-373 MG cells all produced tumors (Yamamoto *et al*. 2000). These results suggest that both the linkage and expression levels of the terminal sialic acids of α3β1 integrin *N*-glycans play an important role in glioma cell–extracellular matrix interactions.

The metastatic potential of tumor cells has also been shown to be correlated with the expression of highly branched tri- and tetra-antennary β1,6-GlcNAc-bearing *N*-glycans (Dennis *et al*. 1987; Dennis and Laferté 1989) and this has be used as a marker of tumor progression in human breast and colon cancers. The expression of UDP-GlcNAc : β-D-mannoside β1,6-*N*-acetylglucosaminyltransferase V (GnT-V) mRNA, which is responsible for the biosynthesis of these β1,6-GlcNAc-bearing *N*-glycans, was found to be high in glioma cell lines and low in normal astrocytes and brain (Demetriou *et al*. 1995). This was confirmed by transfecting *GnT-V* into human glioma U-373 MG cells and showing a marked increase in glioma invasivity *in vitro* (Yamamoto *et al*. 2000). Other evidence such as the ability of *Phaseolus vulgaris* erythroagglutinating lectin (which binds to bisecting β1,4-GlcNAc-bearing *N*-glycans) to strongly inhibit cell migration suggests that the branching of complex type *N*-glycans plays a major role in glioma invasivity and tumorigenesis (Rebbaa *et al*. 1997). In contrast, UDP-GlcNAc3: β-D-mannoside β1,4-*N*-acetylglucosaminyltransferase III (GnT-III), the key transferase that inhibits the extension of *N*-glycans by introducing a bisecting *N*-acetylglucosamine residue and prevents GnT-II, -IV and -V from acting, can suppress metastasis (Yoshimura *et al*. 1995). However, other studies show that GnT-III overexpression is associated with increased metastasis, for example in B16 melanomas. More recently, its overexpression has been shown to inhibit hydrogen peroxide-induced C-Jun NH_2 terminal kinase (JNK1) activation, leading to suppression of protein kinase C (PKC)-δ and the inhibition of apoptosis (Shibukawa *et al*. 2002).

Despite the many studies on glycosylation and tumor proliferation, very little attention has been paid to the effects of cell surface glycosylation on neurotumor cell death. We now report that alteration of cell surface oligosaccharides by overexpression of four different glycosyltransferases leads to increased susceptibility to cell death induced by commonly used chemotherapeutic agents such as etoposide.

Experimental procedures

Stable transfection of the genes into U-373 cells

The human glioma cell line U-373 MG (American Type Culture Collection, Rockville, MD, USA) was used for stable transfection with glycosyltransferases such as GnT-III, GnT-V, α2,6ST and α2,3ST. As described previously, the cDNA encoding the open reading frame was inserted into a pcDNA3 expression vector (Invitrogen, San Diego, CA, USA) at suitable restrictions sites, e.g. *Eco*RI and *Xba*I for *GnT-III* and *Kpn*I and *Xba*I sites for *GnT-V*. The vector alone or the pcDNA3–glycosyltransferases construct was then transfected into U-373 MG cells using a cationic liposome system, N-[1-(2,3-Dioleoyloxy)]-N,N,N-trimethylammonium propane methylsulfate (DOTAP) (Boehringer Mannheim, Indianapolis, IN, USA) according to the manufacturer's instructions. Transfectants were then selected by antibiotic resistance in cell culture medium containing 800 μg/mL G418 (Life Technologies, Inc., Grand Island, NY, USA). All cell lines and transfectants were maintained in Dulbecco's modified Eagle's medium (containing 4.5 g/L glucose) supplemented with 10% heat-inactivated fetal bovine serum (Whittaker BioProducts, Walkersville, MD, USA) at 37°C in a humidified 10% CO_2 incubator, and glycosyltransferase mRNA expression was verified as described previously (Yamamoto *et al*. 1997a).

Cell viability assay

The modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done in 24-well culture dishes, each well containing 0.5 mL medium (Wiesner *et al*. 1997). After treatment, 50 μL stock MTT [5 mg/mL in sterile phosphate-buffered saline (PBS)] was added, incubated for 45 min at 37°C and 500 μL 10% sodium dodecyl sulfate in 0.01 M HCl added. After incubating overnight to dissolve the membranes, the absorption value at 570 nm was determined with a Hitachi spectrophotometer (Hitachi, Conroe, TX, USA). We have previously demonstrated that MTT

data correlate well with cell counts and DNA fragmentation characteristic of apoptosis (Wiesner *et al*. 1997).

DNA fragmentation assay using Hoechst Fluorescence

Cells (10^6) were lysed in 0.1% Triton X-100/5 mM Tris/20 mM EDTA, pH 8.0 (0.5 mL). A 200-μL aliquot of the supernatant (or 200 μL of centrifuged culture medium) was added to 2 mL TNE (100 mM Tris, 10 mM EDTA, 2 M NaCl, pH 7.4) and 0.1 ng/mL Hoechst 33285 dye added for fluorescence measurement. Fluorescence of the soluble DNA (apoptotic) fragments was measured in a Varian fluorometer (Perkin-Elmer, Oakbrook, IL, USA) at excitation wavelength of 365 nm and emission wavelength of 460 nm. DNA values were calculated by comparison with a standard curve of calf thymus DNA (Wiesner *et al*. 1997).

Assay of caspase 3 activity

Cells were treated with drugs at concentrations and times indicated, harvested, washed with PBS and the pellets resuspended in 25 mM HEPES (pH 7.4) containing 2 mM dithiothreitrol, 5 mM EDTA and 10 mM digitonin (Goswami *et al*. 1999). Hydrolysis of the DEVD 7-aminotrifluoromethylcoumarin (AFC) substrate (release of fluorescent AFC) was followed for up to 2 h by fluorometry (excitation 400 nm, emission 505 nm) and activity was calculated from the slope.

Statistical analysis

Data represent the average ± SE of two independent experiments with each sample done in triplicate. Caspase 3 assays were done twice in triplicate. Statistical analyses were performed by Student's *t*-test and $p < 0.05$ was considered statistically significant.

Results

Increased glycosylation increases cell death induced by staurosporine

All four glycosyltransferase transfections, previously shown to result in increased glycosylation (Rebbaa *et al*. 1997; Yamamoto *et al*. 1997a, 2000), resulted in increased cell death following treatment with the caspase 8 activator staurosporine. The order was GnT-V > α2,6ST > GnT-III > α2,3ST (Fig 1) based on cell death at 100 nM staurosporine, increasing from 10% in parental or vector-transfected U-373 cells to 40–50% for *GnT-V* and *α2,6ST* transfectants. Staurosporine was originally described as an inhibitor of protein kinases but it is known to activate caspase 8, a key activator of the apoptosis cascade (Lee *et al*. 2002) and has been previously used to study mechanisms of cell death in glioma cells (Yamasaki *et al*. 2003). Caspase 8 then activates a neutral sphingomyelinase activity to generate the pro-apoptotic lipid ceramide (Tepper *et al.* 1999); the site of this activation appears to be in the Raft fraction of the plasma membrane (Kilkus *et al*. 2003; Testai *et al*. 2004). How plasma membrane oligosaccharide chain composition affects caspase 8 activation is not known, but isolation of the Raft fraction from these cells by Triton X-100 insolubility at 4°C and sucrose density gradient ultracentrifugation (Kilkus *et al*.

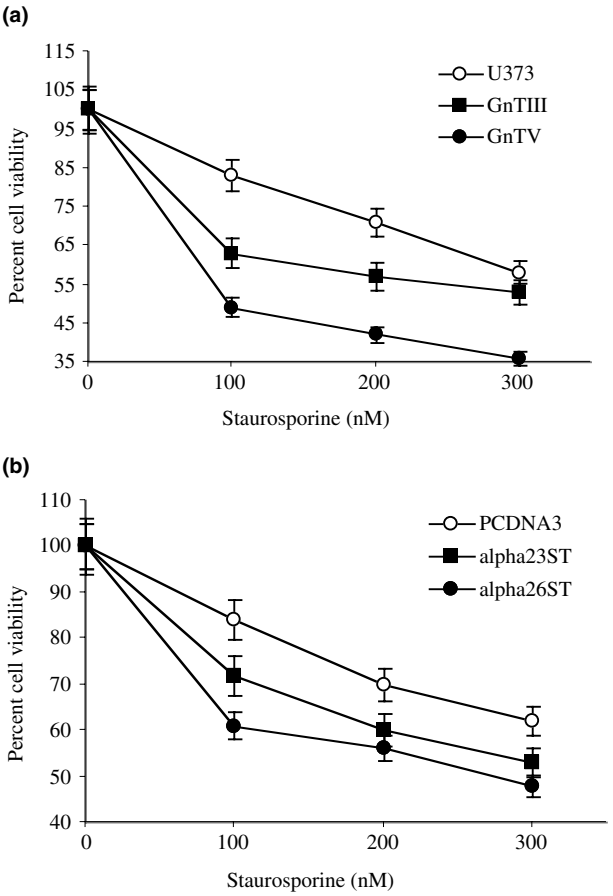

Fig. 1 Decreased resistance to staurosporine following glycosyltransferase transfection. (a) Response of U-373 cells and U-373 following stable transfection of GnT-III and GnT-V to increasing staurosporine concentration. (b) Response of U-373 cells and U-373 following stable transfection of α2,6ST and α2,3ST to increasing staurosporine concentration. Cell death was measured by the MTT method. Results are average ± SE. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

2003) showed a 1.7-fold increase in the ceramide : cholesterol ratio in *α2,6ST*- and a 2.4-fold increase in *GnT-V*-transfected U-373 cells. This suggests a mechanism involving ceramide-induced cell death (Wiesner *et al*. 1997; Kolesnick and Hannun 1999; Tepper *et al*. 1999; Pettus *et al*. 2002).

Increased glycosylation increases cell death induced by C2-ceramide

Staurosporine typically increases the cell content of ceramide (Wiesner *et al*. 1997; Tepper *et al*. 1999) in the lipid-rich domain or Raft fraction of the plasma membrane (Kilkus *et al*. 2003). Ceramide in turn may initiate cell death, by activating a phosphatase (Chalfant *et al*. 1999) to dephosphorylate the pro-apoptotic protein Akt (protein kinase B) (Goswami *et al*. 1999; Goswami and Dawson

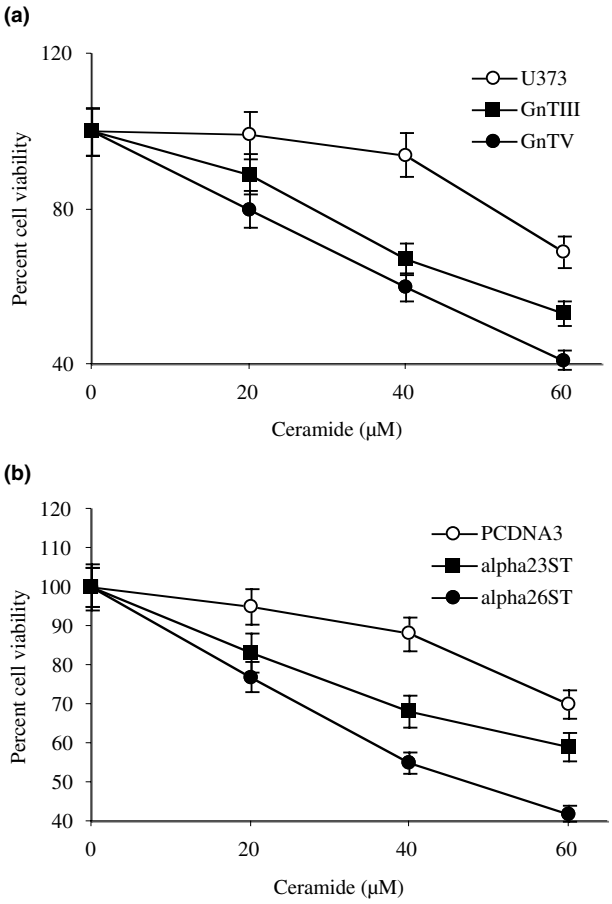

Fig. 2 Decreased resistance to ceramide following glycosyltransferase transfection. (a) Response of U-373 cells and U-373 following stable transfection of GnT-III and GNT-V to increasing C2-ceramide concentration. (b) Response of U-373 cells and U-373 following stable transfection of α2,6ST and α2,3ST to increasing staurosporine concentration. Cell death was measured by the MTT method. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

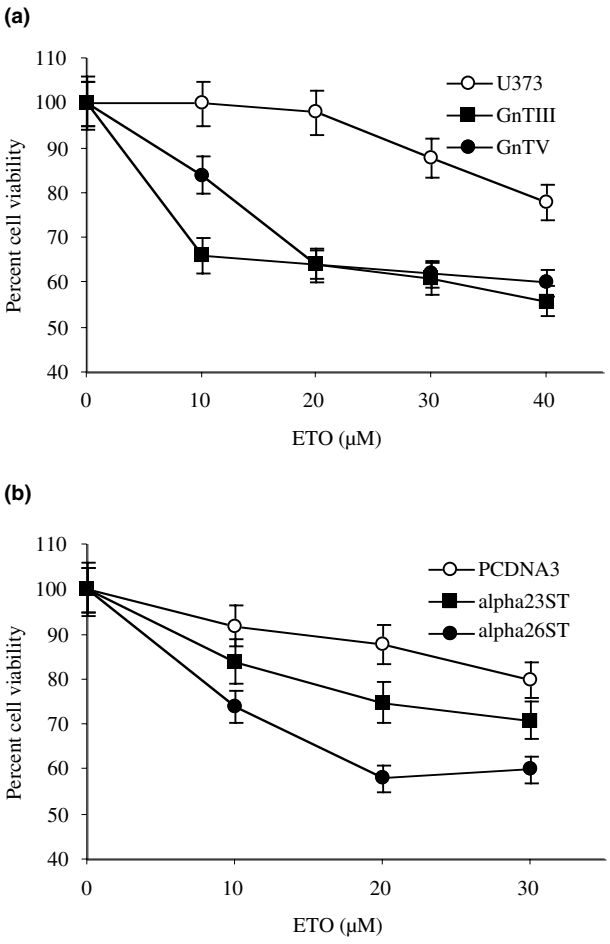

Fig. 3 Decreased resistance to etoposide (ETO) following glycosyltransferase transfection. (a) Response of U-373 cells and U-373 following stable transfection of GnT-III and GnT-V to increasing etoposide concentration. (b) Response of U-373 cells and U-373 following stable transfection of α2,6ST and α2,3ST to increasing staurosporine concentration. Cell death was measured by the MTT method. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

2000). By using the water-soluble ceramide analog C2-ceramide we were able to show increased cell death in all four transfected cell lines. The order of increased cell death with C2-ceramide was very similar to that observed with staurosporine, with *α2,6ST* and *GnT-V* transfectants being more sensitive to killing than *α2,3ST* and *GnT-III* transfectants(Fig. 2).

Increased glycosylation increases cell death induced by etoposide

To determine whether the increased susceptibility to cell death was specific or general, we used the chemotherapeutic agent etoposide, which targets DNA but has also been shown to increase ceramide levels in neuronal cells (Dawson 2000; Toman *et al*. 2002). Once again all four transfections resulted in increased cell death in response to etoposide and the order of increased cell death correlated with that observed with staurosporine and ceramide, namely α2,6ST > GnT-V > α2,3ST = GnT-III, based on 10 μM etoposide (Fig. 3). Thus three diverse drugs with the common ability to raise the ceramide level in the cell membrane (Pettus *et al*. 2002) were able to kill more effectively when cell surface glycoproteins in the glioma were hyperglycosylated, especially with 2,6-sialic acid on terminal galactose residues and *N*-acetylglucosmine on the biantennary mannose residues.

Increased glycosylation decreases cell death induced by phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002

To further investigate the mechanism of this increased susceptibility we tried a different class of pro-apoptotic

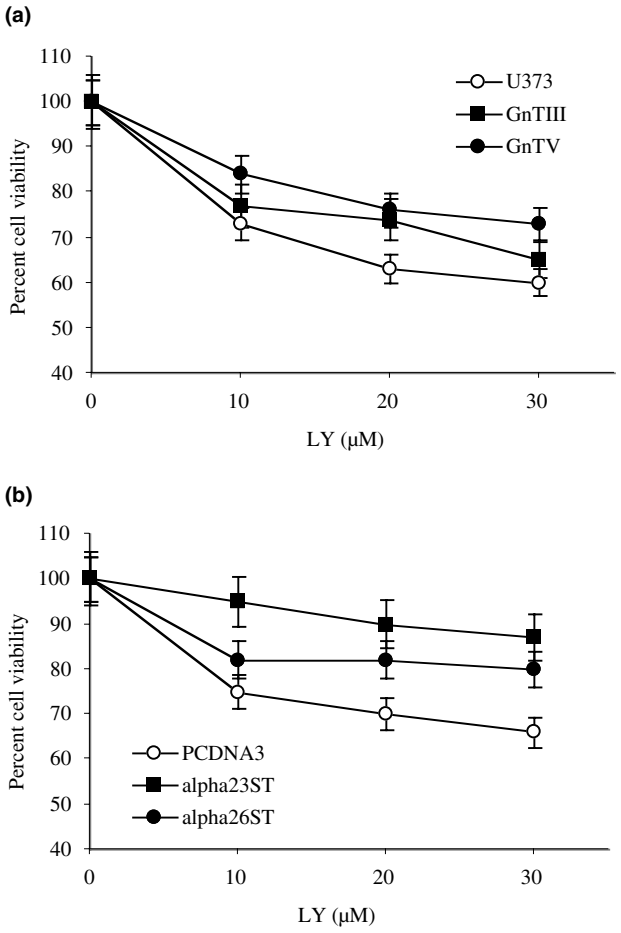

Fig. 4 Increased resistance to PI3K inhibitors following glycosyltransferase transfection. (a) Response of U-373 cells and U-373 following stable transfection of GnT-III and GnT-V to increasing LY294002 (LY) concentration. (b) Response of U-373 cells and U-373 following stable transfection of α2,6ST and α2,3ST to increasing LY294002 concentration. Cell death was measured by the MTT method. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

agent, the PI3 kinase inhibitors exemplified by LY294002. Surprisingly, with PI3K inhibitors we observed protection against apoptosis in all four transfected glioma cells (Fig. 4). Thus with LY294002, both U-373 and the vector-transfected cells underwent 30% cell death at 10 μM LY294002 whereas the *α2,3ST*-transfected cell line was totally resistant to cell death at 10 μM.

GnT-V transfection leads to enhanced caspase 3 in response to staurosporine, suggestive of increased apoptosis

Caspase 3 is generally considered a major executioner caspase and is downstream of both caspase 8 and ceramide (Tepper *et al.* 1999). *GnT-V* transfectants gave the most robust enhanced cell death response to staurosporine and we

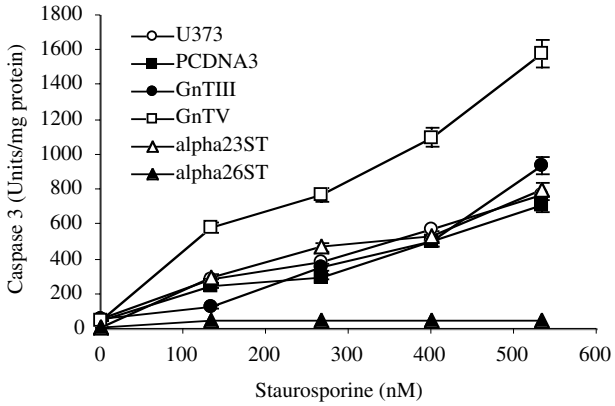

Fig. 5 Altered caspase 3 activity following glycosyltransferase transfection. Caspase 3 increase in response to increasing staurosporine concentration in U-373 cells and U-373 following stable transfection with vector alone, GnT-III, GnT-V, α2,6ST and α2,3ST. Caspase activity was measured by a fluorescence method. Results are the mean of three determinations. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

observed that this transfection greatly enhanced the caspase-3 response to staurosporine (Fig. 5). Surprisingly there was essentially no caspase 3 response at all in *α2,6ST*-transfected cells, whereas the response in *GnT-III*- and *α2,3ST*-transfected cells (which are killed less potently with staurosporine) was indistinguishable from that in U-373 cells.

GnT-V transfection leads to enhanced DNA fragmentation in response to staurosporine, suggestive of increased apoptosis

Caspase 3 activation leads to DNA fragmentation in many neurodegenerative disease situations (Goswami *et al.* 1999; Akbar *et al.* 2003). We observed that activation of *GnT-V*-transfected cells with either staurosporine or C2-ceramide also resulted in enhanced DNA fragmentation (Fig. 6) and this could be blocked by the caspase 3 inhibitor YVAD-fluoromethyl ketone (YVAD-fmk, data not shown). Once again, the response of the other transfected cell lines was hard to distinguish from that of controls and wild-type U-373 cells.

Cell death induced by etoposide is enhanced by co-addition of palmitoyl : protein thioesterase (PPT) 1 inhibitors

Protein palmitoylation is a signal for proteins to translocate to Rafts (El-Din El-Husseneini and Bredt 2002) and inhibition of PPT1 leads to activation of the death pathway (Dawson *et al.* 2002). Each of the transfected cell lines was subjected to etoposide (10 μM) together with either AcG-α-ketoamide-palmitoyl diamino proprionate (DAP1)(Fig. 7a) or its ketoamide (DAP-KA) (Fig. 7b), and the enhanced cell death was shown by the MTT method.

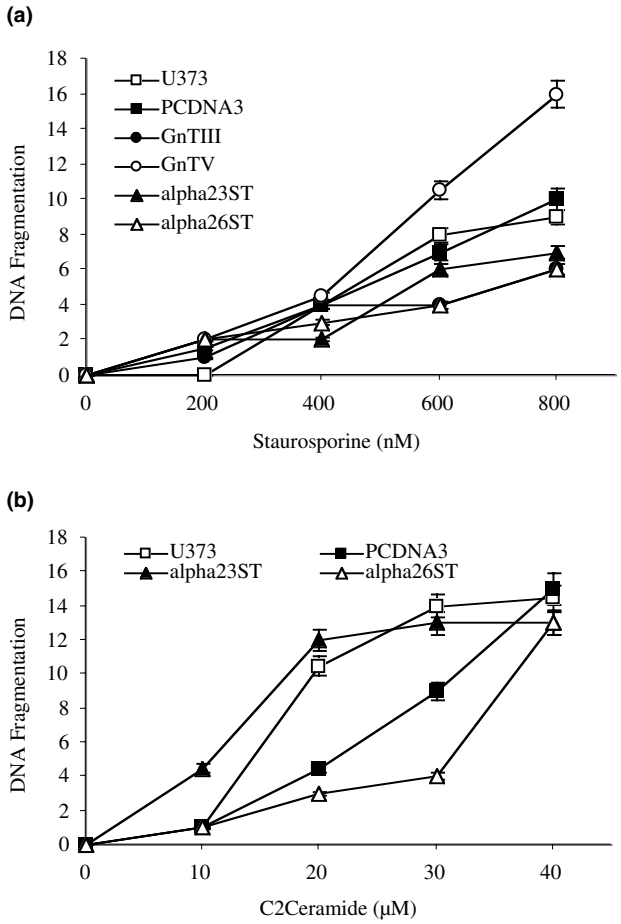

Fig. 6 Increased DNA fragmentation following glycosyltransferase transfection. (a) Response of U-373 cells and transfected U-373 cells to increasing staurosporine concentration. (b) Response to increasing C2-ceramide concentration. DNA fragmentation was measured by a fluorescence method. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

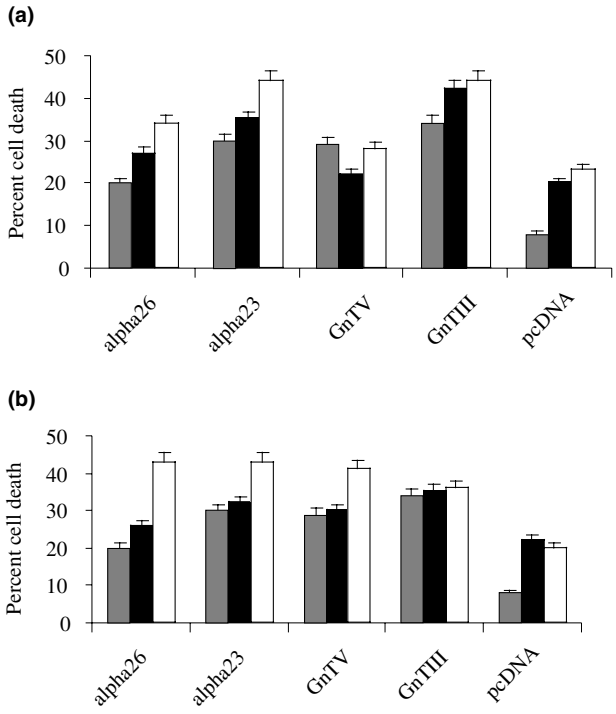

Fig. 7 Etoposide-induced cell death is enhanced by inhibitors of PPT in glycosyltransferase-transfected U-373 cells. Cells were incubated with etoposide (10 μM) in the presence of either (a), DAP1 (5 μM, 10 μM) or (b), DAP-KA (2.5 μM, 5 μM). Cell death was determined by the MTT method. ■, no drug; ■, 5 μM DAP1, 2.5 μM DAP1-KA; □, 10 μM DAPI, 5 μM DAP1-KA. Data represent the average ± SE of two independent experiments with each sample done in triplicate.

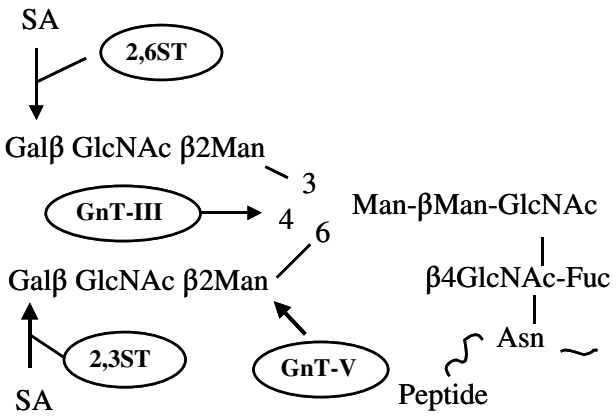

Fig. 8 Site of action of the four glysosyltransferases used in this study. Carbohydrate structure corresponds to IUPAC nomenclature.

Discussion

Protein glycosylation in the nervous system has been studied for many years but its function remains largely unknown. We have attempted to better understand the role of glycosylation by transfecting neural cells with genes for glysosyltransferases, a process that alters both the structure of the glycosyl chains (Fig. 8) and the overall level of glycosylation. Thus the stable transfection of *α2,6ST* resulted in the replacement of 2,3-linked sialic acids with 2,6-linked sialic acids on α3β1 integrin (Yamamoto *et al*. 1997a), an integrin that modulates cell migration (Manes *et al*. 2003). However, overall cellular 2,3-linked sialic acid expression was not diminished, suggesting that the glycoprotein substrates for α2,3ST and α2,6ST may be different, and that other factors, such as protein structure or substrate accessibility, may have an effect on terminal sialylation of *N*-glycans on glycoproteins. Similarly, alteration of *N*-glycan terminal sialylation by *α2,6STGal I* transfection resulted in marked changes in cell morphology, cell–cell interaction and adhesion-mediated signaling.

It has been suggested that integrin functions are modulated by not only *N*-glycosylation, but also by surrounding gangliosides and tetraspan membrane glycoproteins (Kazui *et al*. 2000; Claas *et al*. 2001). Further, gangliosides G_{T1b} and G_{D3} have been shown to inhibit keratinocyte adhesion to fibronectin by carbohydrate–carbohydrate interactions with integrin 5 (Wang *et al*. 2001). The major gangliosides in U-373 cells, G_{M3} and G_{M2} (Maeda and Hashi 1998), have been implicated in tumorigenesis (Deng *et al*. 2002) but others have shown G_{M3} to be toxic to gliomas (Noll *et al*. 2001). We did not observe any obvious differences in ganglioside content among the cell lines studied, whereas glycoprotein glycosylation increases have been well documented in the past (Rebbaa *et al*. 1997; Yamamoto *et al*. 1997a, 2000).

In contrast to α2,6ST, overexpression of 2,3ST showed only a slight increase in 2,3-linked sialylation of total glycoproteins (specifically enhanced *Maackia amarensis* agglutinin (MAA) lectin staining of 3–1 integrin), suggesting that most *N*-linked glycoproteins in parental U-373 MG glioma cells already contain terminal 2,3-linked sialic acids. The *α2.3ST* transfectants were indistinguishable from parental or vector-transfected cells in morphology and proliferation, spreading, and adhesion-mediated protein tyrosine phosphorylation (data not shown) but are more invasive. A possible explanation for this is that the α2,3ST is an acceptor for polysialylation (Angata *et al*. 1998) and that polysialic acid chains, especially those on neural cell adhesion molecules (NCAM), may modulate cell interactions.

We also transfected U-373 cells with two other tumor-associated glycosyltransferase genes: *GnT-III* and *GnT-V*; GnT-III produces *N*-glycans with bisecting structures, whereas GnT-V increases β1,6 branching to create tri- and tetra-antennary structures (Fig. 8). The overexpression of bisecting β1,4-GlcNAc *N*-glycans following *GnT-III* gene transfection has been reported to suppress lung metastasis of B16 melanoma (Yamamoto *et al*. 2000). In contrast, overexpression of highly branched β1,6-GlcNAc (GnT-V) leads to decreased cell adhesion, resulting in an increase in cell motility and metastasis (Kawano *et al*. 1993). Thus a shift in the expression of normal 'brain type' bisecting β1,4-GlcNAc to highly branched β1,6-GlcNAc *N*-glycans may play an important role in modulating the function of cell surface glycoproteins involved in glioma invasivity (Rebbaa *et al*. 1997). For example, expression of tri- or tetra-antennary β1,6-GlcNAc-bearing *N*-glycans has been positively correlated with metastatic potential in rodent tumor models (Dennis *et al*. 1987, Dennis and Laferté 1989) and the knock-out of the *GnT-V* gene results in the suppression of both breast tumor formation and lung metastases in the null mouse (Dennis *et al*. 2002).

We have previously shown that both parental and vector-transfected U-373 MG cells formed large intracranial tumors, *α2,3ST*-transfected U-373 MG clones formed small tumors, whereas *a2,6ST*-transfected U-373 MG glioma clones formed virtually no tumors (Yamamoto *et al*. 2001). This is consistent with the cell death data reported in this paper. However, the marked differences in intracranial tumor formation by the differentially modified U-373 cells are probably attributable to differences in glioma cell–extracellular interactions rather than to differences in mere cell proliferation rate. Thus all the cultured U-373 glioma cells used in this study showed little variation in rates of cell proliferation (data not shown), suggesting that the ultimate decision of a cell to proliferate, differentiate or undergo apoptosis may be dependent on the cellular microenvironment. Our observation that hyperglycosylation increases susceptibility to drugs is encouraging in terms of potential therapy because the overexpression pattern that has the greatest metastatic potential also appears to bring with it the greatest susceptibility to chemotherapeutic agents.

The response of overglycosylated U-373 tumor cells to anticancer drugs may also depend on the membrane microenvironment or Raft as inhibitors of protein depalmitoylation such as DAP1 and DAP-KA (Dawson *et al*. 2002) increased the sensitivity to killing by etoposide. Overglycosylation was also associated with increased pro-apoptotic ceramide in Rafts, where multidrug receptors are localized (Lavie and Liscovitch 2000). Thus it is possible that enhanced glycosylation may inhibit the multidrug resistance associated with overexpression of *mdr1* gene product P-glycoprotein, the ATP-dependent drug efflux pump in Rafts. Several studies have recently shown that the drug efflux activity of P-glycoprotein is accompanied by a considerable depression of oligosaccharide and/or polysaccharide biosynthesis (Fiala *et al*. 2003; Liang *et al*. 2003), so increased glycosylation might reverse this and account for the increased cell death we observe.

Acknowledgements

The 1.2-kb rat *ST3Gal III* cDNA was kindly provided by Dr James Paulson, The Scripps Research Institue, La Jolla, CA, USA). DAP1 and DAP-KA were generous gifts from Dr Philip Dawson (The Scripps Research Institute). Some DAP-KA (NSC D725284) was obtained from the National Cancer Institute (NCI) as part of Rapid Access to NCI Discovery Resources (R*A*N*D) Grant 716553D. We would like to thank John Kilkus for excellent technical assistance in Raft isolation. Supported by United States Public Health Service (USPHS) Grant NS-36866 and the Falk Foundation.

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