β1,4-Galactosyltransferase V Functions as a Positive Growth Regulator in Glioma*

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β1,4-galactosyltransferase V (GalT V; EC 2.4.1.38) can effectively galactosylate the GlcNAcβ1→6Man arm of the highly branched N-glycans that are characteristic of glioma. Previously, we have reported that the expression of GalT V is increased in the process of glioma. However, currently little is known about the role of GalT V in this process. In this study, the ectopic expression of GalT V could promote the invasion and survival of glioma cells and transformed astrocytes. Furthermore, decreasing the expression of GalT V in glioma cell lines promoted apoptosis, inhibited the invasion and migration, and the ability of tumor formation in vivo, and reduced the activation of AKT. In addition, the activity of GalT V promoter could be induced by epidermal growth factor, dominant active Ras, ERK1, JNK1, and constitutively active AKT. Taken together, our results suggest that GalT V functioned as a novel glioma growth activator and might represent a novel target in glioma therapy.

The carbohydrate moieties of cell surface glycoconjugates play an important role in cell adhesion and metastasis (1). One of the most prominent transformation-associated changes in the sugar chains of glycoproteins is an increase in the large N-glycans of cell surface glycoprotein (2). β1,4-galactosyltransferase (GalT) family are the enzymes responsible for the biosynthesis of N-acetyllactosamine on N-glycans by transferring UDP-galactose to the terminal N-acetylgalactosamine (N-GlcNAc) residues, and this family consist of seven members, from GalT I to GalT VII (3, 4).

GalT V, a member of β1,4-galactosyltransferase (GalT) family, could effectively galactosylate the GlcNAcβ1→6Man branch (5), which is a marker of glioma (6). The expression change of GalT V has been investigated using NIH3T3 and the highly malignant transformed cell line MTag. Northern blot analysis has revealed that the transcript of GalT V gene increases by 2–3-fold in the transformed cells (7). Similar results have been obtained in several human cancer cell lines (8). Consistently, our previous study has shown that the expression of GalT V is increased in the process of glioma development, with the highest level in grade IV glioma (9). Despite this knowledge, currently little is known about the role of GalT V in the process of glioma formation.

The experiments reported here were undertaken to further study the role of GalT V in glioma malignancy, including cell migration, invasion, growth, and survival. Our results indicate that GalT V functioned as a novel glioma growth activator and could represent a novel target in glioma therapy.

EXPERIMENTAL PROCEDURES

Materials—Restriction enzymes, bovine calf serum, fetal bovine serum (FBS), DMEM, RPMI 1640 medium, and TRIZol reagent were from Invitrogen. G418, phenylmethylsulfonyl fluoride, aprotinin, pepstatin, epithelial growth factor (EGF), etoposide (VP16), and toluidine blue O were from Sigma. [γ-32P]dATP and the ECL assay kit were from Amersham Biosciences. Sialidase was from Roche Applied Science. The following antibodies were purchased from Santa Cruz Biotechnology: anti-JNK1/2, anti-phospho-JNK, anti-ERK1/2, anti-phospho-ERK and anti-glyceraldehyde-3-phosphate dehydrogenase. Anti-Cyclin D1 Ab, anti-Cyclin D2 Ab, anti-Cyclin D3 Ab, and anti-E2F1 Ab were from Pharmingen. Biotinylated PHA-L or RCA-I was purchased from Vector Laboratories. Anti-AKT Ab, anti-phospho-AKT (Ser473) Ab, and anti-phospho-AKT (Thr308) Ab were purchased from Cell Signaling. FITC-conjugated streptavidin and horseradish peroxidase-conjugated streptavidin were purchased from Southern Biotechnonlogy Associates. Anti-HA Ab was purchased from Roche Applied Science. Normal human brain tissues and glioma tissues were obtained from Huashan Hospital, Shanghai, China. Other reagents were commercially available in China.

Cell Culture and Transfection—Human glioma cell lines SHG44 (10), U87, and U251 were cultured in RPMI 1640 medium or DMEM containing 10% bovine calf serum, 100 units/ml penicillin, and 50 μg/ml streptomycin at 37 °C in a humidified CO2 incubator (5% CO2, 95% air). Transfected astrocytes C8-D30 (American Type Culture Collection) were cultured in DMEM containing 10% fetal bovine serum, 1.5 g/liter streptomycin and 4.5 g/liter glucose. SHG44 cells stably transfected with pcDNA3.0 or GalT V antisense cDNA have been described previously (11). HA-GalT V- and HA-tagged mutant format of GalT V- or mock-transfected stable cells were generated by transfection with pcDNA3.0 or HA-GalT V or the HA-tagged mutant format of GalT V, followed by selection in G418. Individual clones were picked and analyzed. Cell transfection was performed with Lipofectamine (Invitrogen) according to the manufacturer’s instructions. Cells were harvested and measured by flow cytometry 24–48 h after transfection, as reported previously (12).

Plasmids—Expression constructs for HA-pcDNA3.0, Ras DA, Ras DN, ERK1, JNK1, pGL3-SV40, pGL3-Basic, constitutively active AKT (Gag-AKT), HA-GalT V, pSilencer-2.0, and pRL-CMV have been described previously (11, 13, 14). GalT V site-directed mutagenesis con-
Structs were derived from HA-GalT V by PCR amplification using TakaRa MutanBEST mutagenesis kit and specific primers (Y268G forward, 5'-H11032-TATCTGCTTCCTGGCACCGAGTT-3'; H11032; reverse 5'-H11032-ACATATACTTATCCAATTTGGTT-3'; W294G forward, 5'-H11032-TAATGCTTTTCGGCGGTTGGGGTGG-3'; reverse, 5'-AGGAAAGCCATTGATT TTCCGA-3'). Mutated constructs were sequenced, and the correct ones were selected for further experiments. A 320-bp fragment (containing nucleotides from -200 to +120 relative to the transcription

**FIGURE 1. Effects of reduction in the GalT V expression on glioma cell SHG44 invasiveness and growth.**

A, RT-PCR analysis of GalT V mRNA expression level in normal brain and multiple grade I–IV brain tumors. B, transfection with GalT V antisense cDNA reduced the expression of GalT V in SHG44 cells using RT-PCR analysis. C, cell morphology of mock- (Control) or antisense-transfected SHG44 cells. D, when cells were grown to confluence in RPMI 1640 medium containing 10% FBS, photographs were taken. E, cell migration assay of mock- (Control) or antisense-transfected SHG44 cells. Wound healing assay was prepared as described under “Experimental Procedures” (left panel). And, the wound-induced migration of cells was measured after 24 h (right panel). F, decreasing the GalT V expression in SHG44 cells inhibited the invasive ability assayed in a modified Boyden chamber (p < 0.05, n = 3). G, nude mice were injected with either mock- (Control) or antisense-transfected SHG44 cells. 3 weeks later, photographs were taken (right panel, middle panel). Tumors were removed and weighted. Results were shown as mean ± S.D. of tumor weights (left panel).
start) of the GalT V promoter was prepared by PCR amplification of genomic DNA using a sense primer containing a XhoI restriction site and an antisense primer containing a HindIII restriction site (sense, 5′-CTCGAGAGGGTCGGCGGCGAGC-3′; antisense, 5′-ATAAGCTTCCAGGCGGCCGCTAGAGA-3′) as reported previously (15).

Following digestion with restriction enzymes, the GalT V promoter fragment was directionally cloned into the pGL3-Basic (Promega). To prepare site-mutated promoter, the putative Sp1-binding site CCCCCG between nucleotide positions 78 and 71 was changed to CCCCCAAA and named M (Sp1). The mutation was created from pGL3 (200/120) by PCR using a TakaRa MutanBEST mutagenesis kit. Mutated constructs were sequenced, and the correct ones were selected for further experiments.

Reverse Transcriptase (RT)-PCR—Total RNA (1 μg) extracted was used as a template for cDNA synthesis, with a TaKaRa RNA PCR Kit and specific primers (GalT V forward, 5′-TGAGAACAATCGGTGCATCAG-3′; reverse, 5′-CTCGAGAGGGTCGGCGGCGAGC-3′; sense primer containing a XhoI restriction site and an antisense primer containing a HindIII restriction site (sense, 5′-ATCGAGAGGGTCGGCGGCGAGC-3′; antisense, 5′-ATAAAGCTTCCAGGCGGCCGCTAGAGA-3′) as reported previously (15). Following digestion with restriction enzymes, the GalT V promoter fragment was directionally cloned into the pGL3-Basic (Promega). To prepare site-mutated promoter, the putative Sp1-binding site CCCCCG between nucleotide positions 78 and 71 was changed to CCCCCAAA and named M (Sp1). The mutation was created from pGL3 (200/120) by PCR using a TakaRa MutanBEST mutagenesis kit. Mutated constructs were sequenced, and the correct ones were selected for further experiments.

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Implantation of Tumor Cells in Mice—Tumorigenicity assay was performed by inoculating 5 × 10⁶ cells of vector or full-length of GalT V (WT), or W294G-transfected cells. Wound healing assay was performed as described under “Experimental Procedures,” and the wound-induced migration of cells was measured after 24 h. E, Matrigel invasion assay was performed with cells stably transfected with mock (Control), HA-GalT V (WT), or W294G. Values were shown as mean ± S.D. of triplicates from two independent experiments.

Flow Cytometry of Glycan Level of Membrane Protein and Lectin Blotting—After grown to subconfluence, cells were treated with sialidase for 5 h. Endogenous peroxidase activity was blocked with 0.3% H₂O₂ for 30 min. After being washed with PBS, cells were harvested after treatment with EGTA (2 mM) and incubated with biotinylated PHA-L, or RCA-I (2 μg/μl) for 45 min at 4 °C. Then, the cells were washed with PBS and probed with FITC-conjugated streptavidin (1:128) for 30 min at 4 °C. Next, the cell samples were subjected to flow cytometry. Lectin blotting assay was performed as reported previously (13).

Analysis of Apoptosis by Flow Cytometry—Adherent and non-adherent cells were collected, washed twice in PBS, and fixed with ice-cold 70% ethanol for at least 1 h. The fixed cells were washed and stained with propidium iodide. After incubation for 45 min at 37 °C, the DNA content was determined by quantitative flow cytometry with standard optics of FACScan flow cytometer (BD Biosciences FACStar). The percentage of apoptotic cell was quantified from sub-G₁ events.

Implantation of Tumor Cells in Mice—Tumorigenicity assay was performed as described previously by Dong Xie et al. (19). At confluence, the cells were harvested and centrifuged, and then resuspended in a sterile solution of PBS at a final concentration of about 1.0 × 10⁷ cells/ml. A 100-μl aliquot of resuspended cells (about 1.0 × 10⁷ cells) was injected subcutaneously between the shoulder blade ~3 cm from the tail. After 3 weeks, photographs were taken, and tumors were harvested and individually weighed after mice were anesthetized. Data were presented as tumor weight (mean ± S.D.). Statistical analysis was performed by computer program software using the Student’s t test.

Statistics and Presentation of Data—All experiments were repeated three times. All numerical data were expressed as mean ± S.D. Data were analyzed using the two-tailed t test.

RESULTS

We adopted a semiquantitative RT-PCR to analyze the expression of GalT V mRNA in normal brain tissues and glioma tissues. Consistent with our previous study (9), GalT V mRNA expression was correlated with staging to the glioma tumor (Fig. 1A). To elucidate the biological significance of GalT V in glioma, the GalT V antisense cDNA construct was stably transfected into glioma cell line SHG44 (Fig. 1B) (11). In culture, the control SHG44 cells showed invasive growth with spindle-shaped morphology (Fig. 1C, left panel) and grew in an actinomorphic manner (Fig. 1D, left panel). However, antisense-transfected SHG44 cells exhibited a round morphology (Fig. 1C, right panel) and grew in a ramble way (Fig. 1D, right panel). To examine the property of GalT V in glioma, we studied the effects of reduction in GalT V expression on the motility of SHG44 cells, assayed by wound healing and Boyden chamber assays. As depicted in Fig. 1, E and F, reduction in GalT V expression resulted in a significant decrease in cell migration in vitro and the ability to migrate through Matrigel-coated 8-μm pore size membranes. Similar results were obtained in agarose drop explant assay (data not shown).
Moreover, reduction in the expression of GalT V resulted in a total suppression of glioma growth in vivo, compared with the controls (Fig. 1C).

To evaluate the relationship between GalT V overexpression and tumor behavior, transformed astrocytes C8-D30 and glioma cell lines U87 and U251 were stably transfected with HA-GalT V (Fig. 2A). GalT V overexpression in glioma cells U87 and U251 and transformed astrocytes C8-D30 resulted in a striking increase of cell migration (Fig. 2B), an almost 3-fold increase in vitro invasiveness through a reconstituted Matrigel basement membrane (Fig. 2C), a great increase in colony number (data not shown) and greater numbers of viable cells in serum-free conditions relative to the control cell lines (Fig. 2D). To examine the effect of GalT V overexpression on the ability of tumor formation in vivo, HA-GalT V- or vector-transfected glioma cells were injected subcutaneously into nude mice. As depicted in Fig. 2E, the HA-GalT V-transfected cells developed tumors with a markedly large size during the 3 weeks of observation compared with the control cells. Collectively, these experiments demonstrated that GalT V could promote glioma cell invasiveness and survival.

The GalT V protein consists of a short NH2-terminal cytoplasmic domain, a stem region, and a catalytic domain, which contains two conserved residues (Tyr268/Trp294) which are important for the galactosylation activity of GalT V, HA-tagged point mutants of GalT V (Y268G/W294G) were constructed and transiently transfected into U87 cells (Fig. 3A). As depicted in Fig. 3B, Trp294 was involved in the galactosyltransferase activity of GalT V. To investigate the contribution of the GalT V galactosylation activity in its tumorigenic effects, the mutation construct W294G was stably transfected into transformed astrocytes C8-D30 and glioma cell lines U87 and U251 (Fig. 3C). As shown in Fig. 3, D and E, the point mutation (W294G) abolished the ability of GalT V to promote the migration and invasive potential of glioma cells, indicating that an intact catalytic domain might be essential for GalT V tumorigenic function in glioma. This conclusion was further supported by clonogenic assay and tumorigenic assay in vivo (data not shown).

The expression of β1,6-linked GlcNAc-bearing N-glycans has been reported as a marker of tumor progress in glioma (6). To assess the contribution of GalT V in the expression of N-glycan of cell membrane protein, flow cytometry analysis was performed using FITC-conjugated RCA-1 which interacts with oligosaccharides terminating with the Galβ1→4GlcNAc group or FITC-conjugated PHA-L, which interacts with highly branched N-Glycans with the Galβ1→4GlcNAcβ1→6-(Galβ1→4GlcNAcβ1→2)Man branch (15). As expected, reduction in the expression of GalT V decreased the binding with RCA-I or PHA-L on the cell surface (Fig. 4A). Consistent with this, a significant decrease of the binding of total glycoprotein with RCA-I (Fig. 4B, left panel) or PHA-L (Fig. 4B, right panel) was observed for 50–80-kDa and 80–100-kDa protein bands in the antisense-transfected cells compared with the mock-transfected cells.

Furthermore, reduction in the expression of GalT V inhibited cell cycle progression (Fig. 5A, left panel) and reduced the endogenous expression of Cyclin D1, Cyclin D3, and E2F1 (Fig. 5A, right panel), which are important regulators of cellular proliferation and highly expressed in glioma (23–25). In addition, suppression of GalT V expression promoted apoptosis induced by chemotherapeutic agent etoposide (VP16), which was widely used for treatment of malignant gliomas (26) (Fig. 5B, left panel), and inhibited the ability to form invisible clones with x-irradiated treatments (Fig. 5B, right panel). We next examined the contribution of GalT V in the activation of AKT and MAPK kinase, which have shown to associate with glioma invasiveness (18, 27–29). As shown in Fig. 5C, reduction in the expression of GalT V led to a reduction of the levels of phospho-AKT (Ser473/Thr308) and phospho-JNK1/2 (Thr183/Tyr185) status. In summary, these data strongly suggest that GalT V could represent a novel target in glioma therapy.

We next explored the possible relationship between Ras/MAPK and PI3K/AKT signaling pathways and GalT V expression. GalT V mRNA expression levels in serum-starved and serum- or EGF-stimulated SHG44 cells were assessed by RT-PCR analysis. As shown in Fig. 6A, endogenous GalT V mRNA expression was markedly induced by serum (Fig. 6A, upper panel) or EGF (Fig. 6A, lower panel), indicating the contribution of serum or EGF in the transcription regulation of the GalT V gene. To adequately address this question, we constructed the
GalT V reporter construct pGL3 (−200/+120), which retained relative strong promoter activity in cancer cells and contained one Sp1-binding site at nucleotide positions −81/−69 (15). As expected, transient transfection of the GalT V reporter plasmid pGL3 (−200/+120) into SHG44 cells showed a dose-dependent reporter gene activity in response to serum (Fig. 6B) or EGF stimulation (Fig. 6C). To determine whether Ras signaling pathway was involved in the GalT V transcription activation, we transiently cotransfected reporter plasmid pGL3 (−200/+120) and either the dominant negative expression construct Ras-DN or the constitutively active expression construct Ras-DA into SHG44 cells. As expected, expression of Ras-DN decreased the GalT V promoter activity in a dose-dependent manner, whereas expression of Ras-DA caused a similarity dependent activation (Fig. 6D), indicating a role of Ras/MAPK signaling pathway in the transcription regulation of GalT V. Consistent with this, transient overexpression of ERK1 or JNK1 into SHG44 cells led to a significant increase in the GalT V promoter activity (Fig. 6E, lane 1–5). As the PI3K-AKT pathway represents one of the most potently pro-survival signaling pathways frequently activated in malignant gliomas (27), we explored the effect of AKT on the GalT V promoter activity. As expected, cotransfection of pGL3 (−200/+120) and constitutively active AKT (Gag-AKT) construct increased the promoter activity in a dose-dependent manner (Fig. 6E, lanes 1, 6, and 7). Sp1, which plays an essential role in the GalT V transcription in cancer cells (15), has been well defined as a nuclear target factor of pro-growth and pro-survival signal transduction pathways in tumor cells, including Ras/MAPK and PI3K/AKT signaling pathways (30, 31). To elucidate the contribution of this Sp1-binding site in the activation of the GalT V promoter by Ras/MAPK and PI3K/AKT signaling pathways, we introduced site-directed mutagenesis into this Sp1-binding site (M (Sp1)). It was found that the mutagenesis of this Sp1-binding site abolished the effects of Ras-DA, Ras-DN, ERK1, JNK1, or Gag-AKT on the GalT V promoter activity (Fig. 6F).

**DISCUSSION**

Malignant gliomas are the most common primary brain tumors and one of the deadliest human cancers (32). They develop as the result of stepwise accumulations of multiple genetic alterations, which result in the activation of oncogenes and/or the inactivation of tumor suppressor
genes (33). Our early studies have demonstrated that GalT V is highly expressed in glioma. Moreover, the expression of GalT V increases upon malignant transformation of cells and is correlated with staging to the glioma tumor (9). These findings motivate us to examine the function of GalT V protein in glioma.

Results from previous studies have shown that the expression of GlcNAcβ1→6Man-branched N-glycans play a major role in glioma malignant (6). This GlcNAcβ1→6Man structure is synthesized by N-acetylglucosaminyltransferase V, a key enzyme in the processing of asparagine-linked glycans during the synthesis of glycoproteins (34). As the reduction in the expression level of the GalT V gene in SH-SY5Y cells resulted in the decreased galactosylation of highly branched N-glycans, and the expression of GalT V in several human cancer cell lines was highly correlated with that of the N-acetylglucosaminyltransferase V gene (8, 15), the GalT V was considered to be involved in the expression of highly branched N-glycans. Consistently, reduction in the GalT V expression in SHG44 cells decreases the galactosyltransferase of the highly branched N-glycans, which leads to the reduced binding to L-PHA. Similar results are obtained in other cancer cell lines including U87 and HeLa cells (data not shown). Therefore, GalT V might be very important in glioma for expressing the highly branched N-glycans that are involved in glioma growth and metastasis (6).

In this study, the role of GalT V in glioma development has been evaluated in several experimental models. Its forced expression in U87 and U251 cells could markedly stimulate their growth and invasion and migration and significantly enhance their tumorigenicity in vivo. These cells developed larger tumors in nude mice. To further address the physiological functions of GalT V in glioma, we investigated the effect of
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Reduction in the GaIT V expression on glioma malignant. Reduction in the expression of GaIT V resulted in the changes in cell morphology, and decreasing the GaIT V expression in glioma cells promoted apoptosis. The mechanism of the transcription regulation of GalT V in glioma should be explored next.

REFERENCES

1. Halomori, S. (1996) Cancer Res. 56, 5309–5318
2. Coulter, C., and Green, J. E. (2000) Breast Cancer Res. 2, 321–323
3. Guo, S., Sato, T., Shirane, K., and Furukawa, K. (2001) Glycobiology 11, 813–820
4. Sato, T., Guo, S., and Furukawa, K. (2001) Biochimie (Paris) 83, 719–725
5. Sato, T., Furukawa, K., Bakker, H., Van den Eijnden, D. H., and Van Der, I. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 472–477
6. Yamamoto, H., Swoger, J., Greene, S., Saito, T., Hurh, J., Sweeney, C., Leestma, J., Mrkdichian, E., Cerullo, L., Nishikawa, A., Ibara, Y., Taniguchi, N., and Moskal, J. R. (2000) Cancer Res. 60, 134–142
7. Shirane, K., Sato, T., Segawa, K., and Furukawa, K. (1999) Biochem. Biophys. Res. Commun. 265, 434–438
8. Sato, T., Shirane, K., Kido, M., and Furukawa, K. (2000) Biochem. Biophys. Res. Comm. 276, 1019–1023
9. Xu, S., Zhu, X., Zhang, S., Yin, S., Zhou, L., Chen, C., and Gu, J. (2001) J. Clin. Oncol. 19, 502–506
10. Chen, Z. P., Remack, J., Brent, T. P., Mohr, G., and Panasci, L. C. (1999) Clin. Cancer Res. 5, 4186–4190
11. Xu, S., Zhang, S., Chen, C., Yan, J., Cai, M., Zhu, X., and Gu, J. (2002) J. Exp. Clin. Cancer Res. 21, 409–414
12. Ma, Y., Yuan, J., Huang, M., Jove, R., and Cress, W. D. (2003) J. Biol. Chem. 278, 16770–16776
13. Zhu, X., Jiang, J., Shen, H., Wang, H., Zong, H., Li, Z., Yang, Y., Niu, Z., Liu, W., Chen, X., Hu, Y., and Gu, J. (2005) J. Biol. Chem. 280, 12503–12516
14. Zhu, X., Chen, S., Yin, X., Shen, A., Ji, S., Shen, Z., and Gu, J. (2003) Biochem. Biophys. Res. Commun. 309, 279–285
15. Sato, T., and Furukawa, K. (2004) J. Biol. Chem. 279, 39574–39583
16. Albini, A., Iwamoto, Y., Kleinman, H. K., Martin, G. R., Aaronson, S. A., Kozlowski, J. M., and McEwan, R. N. (1987) Cancer Res. 47, 3239–3245
17. Hauck, C. R., Hsia, D. A., and Schlaepfer, D. D. (2000) J. Biol. Chem. 275, 41092–41099
18. Shi, Q., Bao, S., Maxwell, J. A., Reese, E. D., Friedman, H. S., Bigner, D. D., Wang, X. F., and Rich, J. N. (2004) J. Biol. Chem. 279, 52300–52309
19. Xie, D., Yin, D., Tong, X., O’Kelly, J., Mori, A., Miller, C., Black, K., Gui, D., Said, J. W., and Koeffler, H. P. (2004) Cancer Res. 64, 1987–1996
20. Aoki, D., Appert, H. E., Johnson, D., Wong, S. S., and Fukuda, M. N. (1990) EMBO J. 9, 3171–3178
21. Lo, N. W., Shaper, J. H., Pevner, J., and Shaper, N. L. (1998) Glycobiology 8, 517–526
22. Schwientek, T., Almeida, R., Levery, S. B., Holmes, E. H., Bennett, E., and Clausen, H. (2001) J. Biol. Chem. 276, 1987–1996
23. Alemany, R., Gomez-Manzano, C., Balague, C., Yung, W. K., Curiel, D. T., Kyritsis, A. P., and Fueyo, J. (1999) Exp. Cell Res. 252, 1–12
24. Arato-Ohshima, T., and Sawa, H. (1999) Int. J. Cancer 83, 387–392
25. Bacon, C. L., Gallagher, H. C., Haughey, J. C., and Regan, C. M. (2002) J. Neurochem. 83, 12–19
26. Nagane, M., Asai, A., Shibui, S., Oyama, H., Nomura, K., and Kuchino, Y. (1999) Jpn. J. Clin. Oncol. 29, 527–534
27. Jette, A., Howe, J. A., Horn, M. T., Maxwell, E., Yin, Z., Johnson, D., and Kumar, C. C. (1999) Cancer Res. 59, 6697–6706
28. Nakada, M., Niska, J. A., Tran, N. L., McDonough, W. S., and Berens, M. E. (2005) Am. J. Pathol. 167, 565–576
29. Perkins, E., Calvert, J., Lancran, J. A., Parent, A. D., and Zhang, J. (2003) Brain Res. Mol. Brain Res. 111, 42–51
30. Pore, N., Liu, S., Shu, H. K., Li, B., Haas-Kogan, D., Stokoe, D., Milanini-Mongiat, J., Pages, G., O’Rourke, D. M., Bernhard, E., and Maity, A. (2004) Mol. Biol. Cell 15, 4841–4853
31. Bonello, M. R., and Khachigian, L. M. (2004) J. Biol. Chem. 279, 2377–2382
32. DeAngelis, L. M. (2001) N. Engl. J. Med. 344, 114–123
33. Cavenne, W. K. (1992) Cancer 70, 1788–1793
34. Arango, J., and Pierce, M. (1988) J. Cell Biol. 107, 225–231
35. Revenu, H., Geiger, T., Geiger, B., and Levitzki, A. (2000) J. Cell Biol. 151, 1179–1192
36. Claes, P., Grobben, B., Van Kolen, K., Roymans, D., and Slegers, H. (2001) Br. J. Pharmacol. 134, 402–408
37. Xiao, A., Wu, W., Pandolfo, P. P., Louis, D. N., and Van Dyke, T. (2002) Cancer Cell 1, 157–168
38. Maier, D., Jones, G., Li, X., Schonthal, A. H., Gratzl, O., Van Meir, E. G., and Merlo, A. (1999) Cancer Res. 59, 5479–5482
39. Kurose, K., Zhou, X. P., Araki, T., Cannistra, S. A., Maher, E. R., and Eng, C. (2001) Am. J. Pathol. 158, 2097–2106