HNK-1 Glycan Functions as a Tumor Suppressor for Astrocytic Tumor

Misa Suzuki-Anekoji, Masami Suzuki, Tatsuya Kobayashi, Yoshiko Sato, Jun Nakayama, Atsushi Suzuki, Xingfeng Bao, Kiyohiko Angata, and Minoru Fukuda

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Abstract

Astrocytic tumor is the most prevalent primary brain tumor. However, the role of cell surface carbohydrates in astrocytic tumor invasion is not known. In a previous study, we showed that polysialic acid facilitates astrocytic tumor invasion and thereby tumor progression. Here, we examined the role of HNK-1 glycan in astrocytic tumor invasion. A Kaplan–Meier analysis of 45 patients revealed that higher HNK-1 expression levels were positively associated with increased survival of patients. To determine the role of HNK-1 glycan, we transfected C6 glioma cells, which lack HNK-1 glycan expression, with HNK-1 precursor glycan (15, 16). These results indicate that sulfated HNK-1 glycan plays a critical role in cell–cell interaction and in cell migration.

Expression of HNK-1 glycan is thought to vary during embryonic development, and the levels of HNK-1 have been examined in primary cancer and metastatic tumors. In neural tumors, the polylactosamine repeat containing HNK-1 glycan, SO$_4^-$GlcA$_3$Gal$_3$GlcNAc$_3$Glc$_3$ was the prominent form of glycolipid capped by HNK-1 glycan (24). HNK-1 glycan was also detected in small cell bladder carcinoma (25) and Ewing’s sarcoma (26). In cutaneous malignant melanoma, metastatic cells distant from the primary tumor express HNK-1 glycan, as does the primary tumor. By contrast, HNK-1 glycan was not detected in lymph node metastasis of cutaneous melanoma (22, 27). These results suggest that HNK-1 glycan expression may be regulated by the tumor microenvironment.
Among adult primary brain tumors, astrocytic tumor, including glioblastoma, is the most prevalent. Although there are reports of HNK-1 expression in glioma (28, 29), its role has not been extensively investigated. Here, we report that HNK-1 glycan expression is inversely correlated with astrocytic tumor aggressiveness in human patients. We then generated HNK-1 glycan-positive C6 glioma cells and found that C6-HNK-1 cells migrate more slowly in the presence of a laminin fragment than parental HNK-1-negative C6 cells. C6-HNK-1 cells implanted into mouse brain formed smaller tumors than HNK-1-negative parental C6 cells. Moreover, HNK-1-positive C6 cells grew more slowly and formed smaller colonies in anchorage-dependent and anchorage-independent growth conditions. This activity was associated with decreased activation of ERK kinase, but not with focal adhesion kinase (FAK), indicating that HNK-1 glycan functions as a tumor suppressor for astrocytic tumor.

**EXPERIMENTAL PROCEDURES**

**Patient Immunohistochemistry and Survival Study**—Formalin-fixed and paraffin-embedded tissue blocks of astrocytic tumors from 43 patients, whose diagnoses were followed for at least for 5 years after surgical resection of the tumor at Shinshu University Hospital, Matsumoto, Japan, were retrieved from the pathology file of the same hospital. According to the World Health Organization classification (30), tumors included pilocytic astrocytoma (four cases), diffuse astrocytoma (eight cases), anaplastic astrocytoma (16 cases), and glioblastoma (17 cases). In each patient, a representative portion of tumor was examined. All tissue samples were fixed in 20% formalin buffered with 0.1 M phosphate buffer (pH 7.4) at room temperature for 48 h and then embedded in paraffin. The experimental protocol was approved by the Ethical Committee of Shinshu University School of Medicine.

Expression of HNK-1 in astrocytic tumors was analyzed by immunohistochemistry using anti-HNK-1 antibody (mouse IgM) and Envision+ secondary antibody (Dako) as described previously (31). Counterstaining was carried out using hematoxylin. A control immunostaining was performed by omitting the primary antibody from the staining procedure, and no specific staining was found. Results of the immunostaining were scored based on the ratio of the number of immunoreactive cells to the number of examined total tumor cells, irrespective of staining intensity; *i.e.* negative (−, no positive cells), weak (+, less than one-third of tumor cells positive), moderate (+++, less than two-thirds of tumor cells positive), or strong (+++, more than two-thirds of tumor cells positive). The scoring data were collected blindly without knowing tumor grade and the patients’ survival period. The association between HNK-1 expression and tumor grade was statistically analyzed by Pearson test using JMP 7 software (SAS, Cary, NC). In addition, Kaplan-Meier analyses for HNK-1 expression and patients’ five-year survival were carried out by log-rank test with the same software. *p* values < 0.05 were considered statistically significant.

**Intracranial Injection of C6 Cells**—Control C6 cells and HNK-1-positive clone G14 and H1 cells were inoculated into the striatum of 8-week-old wild-type C57BL/6 male mice or C57BL/6 nude mice, as described previously (31). Subconfluent cells were cultured in 10% FBS-supplemented alpha modification of Eagle’s minimum essential medium, harvested, and suspended in PBS, and then 4.8 × 10⁶ cells were injected with a Hamilton syringe into the left striatum using a stereotaxic frame. Three weeks later, the brains were isolated and fixed in 4% paraformaldehyde. Frozen or paraffin sections were prepared from tumor-bearing specimens (+ 0.1 – + 1.2 mm anterior to the bregma), stained with anti-vimentin antibody to detect the tumor (31), and visualized by two methods, HRP-conjugated secondary antibody with 3,3′-diaminobenzidine or fluorescein-conjugated secondary antibody. Fluorescent images were assembled as a montage on Deneba Canvas 8.0 software. Relative tumor area versus total brain area was calculated by densitometry using ImageJ software and shown as a percentage with S.E. Eight mice were injected in a set of experiments repeated two to three times. In total, C6, G14, and H1 cells were injected into 29, 26, and 17 mice, respectively. A representative set of experiment is shown in Fig. 3. The protocol for animal use was approved by the Animal Research Committee at Sanford-Burnham Medical Research Institute in accordance with National Institutes of Health guidelines.

**Cell Migration Assay**—A cell migration assay was performed using the Boyden chamber cell culture insert system in a 24-well format (BD Biosciences) (32, 33) following the manufacturer’s protocol. The bottom surface of the insert membrane was coated with 10 μg/ml mouse laminin-111 (Invitrogen) or human fibronectin (Sigma) overnight at 4 °C. The bottom chamber was filled with serum-free alpha modification of Eagle’s minimum essential medium, the protein-coated insert was placed in chambers, and 1.25 × 10⁷ cells were applied on top of the insert. Cells were allowed to migrate for 20 h, and cells migrating to the bottom surface of the filter were visualized with 0.5% crystal violet and photographed under a microscope. Three to four fields per membrane and two membranes per each condition were evaluated. Cell numbers were counted manually using the Tally counter on Adobe Photoshop images. The counting error was assumed to be less than 10% when cell numbers exceeded 1000. The experiments were repeated two to five times for each condition.

Blocking antibodies such as HNK-1, β1-integrin antibodies, or laminin E3 peptide were mixed at a concentration of either 10 or 30 μg/ml, as indicated, with the cell suspension for 10 min prior to application. Cells were allowed to migrate in the antibody mixture. Mouse IgG solution (Sigma-Aldrich) or boiled mouse IgG solution (Sigma-Aldrich) or boiled HNK-1 antibody was used as a control.

**Cell Proliferation Assay**—In 6-well plates, 5 × 10⁴cells were plated on cover glasses that were collected every 24 h for 6 days, stained with Hoechst 33342 (Sigma) to detect nuclei or anti-NCAM antibody to visualize the cell surface, and photographed under a fluorescence microscope. The number of nuclei per field was counted by Tally counter.

**Anchorage-independent Colony Formation Assay**—Mono-dispersed C6, G14, and H1 cells (n = 20,000) were mixed with prewarmed 10% FBS-supplemented alpha modification of Eagle’s minimum essential medium, containing 0.375% top agar and layered on 0.5% base agar in the same medium in 35-mm dishes (32, 34). Cells were cultured for 4 weeks at 37 °C in 5% CO₂, fixed with 4% paraformaldehyde, and visualized with 0.005% crys-
tal violet. Colonies were photographed under a light microscope (Olympus DX50) with /H11003 40 magnification, and colony areas larger than 0.01 mm² were measured by ImageJ software. Six fields per dish and three to four dishes per condition were prepared, and the experiments were repeated four times.

Details on cells and antibodies, HNK-1 overexpression in C6 cells, RT-PCR, immunoblot analysis, laminin overlay, the cell binding assay, and the microglia cytotoxicity assay are described in the supplemental information.

RESULTS

HNK-1 Glycan Expression Is Inversely Correlated with Progression of Human Astrocytic Tumors—Immunohistochemistry using anti-HNK-1 monoclonal antibody demonstrated that almost all brain tissues free from cancer cells were positive for HNK-1 glycan (Fig. 1). Representative staining of glioblastoma-negative (−) and weakly positive (+) for HNK-1 glycan, anaplastic astrocytoma moderately positive (++), and pilocytic astrocytoma strongly positive (+++) are shown (Fig. 1, A–D). HNK-1 glycan was detected on the surface of neurons and in the neuropil (Fig. 1E). It was also detected along the cytoplasmic process and in the cell body of tumor cells in 21 (46.6%) of 45 patients examined (Fig. 1C). Notably, moderate to strong expression of HNK-1 in tumor cells was seen in all of 12 patients with less aggressive tumors, such as pilocytic astrocytoma and diffuse astrocytoma. By contrast, in more aggressive forms of astrocytic tumors, six (37.5%) of 16 cases with anaplastic astrocytoma and four (23.5%) of 17 cases with glioblastoma were negative or weakly positive for HNK-1 (Fig. 1G). Interestingly, expression of HNK-1 in the tumor cells in the invasive front was less than those in the central portion of the tumor in 50% of glioblastoma patients (supplemental Fig. S1). Although a significant correlation was not observed between the HNK-1 expression level and tumor grade (p = 0.3929 by Pearson test), these results suggest that loss of HNK-1 expression in tumor cells is associated with the aggressiveness of astrocytic tumors.

Expression of HNK-1 in Astrocytic Tumors and Prognosis of Glioma Patients—To determine the relationship between HNK-1 expression in astrocytic tumors and patient prognosis, the five-year survival of 45 patients was statistically analyzed using the Kaplan-Meier method. HNK-1 glycan expression was positively correlated with increased survival with statistical significance (p = 0.0058 by log-rank test).

FIGURE 1. Expression of HNK-1 glycan is inversely correlated with progression of human astrocytoma. A–F, representative images of immunohistochemistry for HNK-1 glycan in various types of human astrocytic tumor. Immunostaining was scored based on the ratio of immunoreactive cells to total cells evaluated. No staining (−) (A, glioblastoma), weak staining (+) (B, glioblastoma), moderate staining (++) (C, anaplastic astrocytoma), and strong staining (+++) (D, pilocytic astrocytoma) are shown. In non-tumorous tissue, staining is seen on the surface of neurons and neuropil (E, cerebral cortex; the inset shows an enlargement of the indicated area). A control experiment using second antibody only is shown (F). The arrow in A indicates multinucleated tumor cells. Scale bar = 50 μm. G, expression of HNK-1 glycan related to patients’ prognosis. HNK-1 glycan is expressed at lower levels in aggressive tumors (anaplastic astrocytoma and glioblastoma) than in less aggressive tumors (pilocytic astrocytoma and diffuse astrocytoma). H, five-year survival of 45 patients was statistically analyzed using the Kaplan-Meier method. HNK-1 glycan expression was positively correlated with increased survival with statistical significance (p = 0.0058 by log-rank test).
associated with better survival with statistical significance \((p = 0.00581\) by log-rank test) (Fig. 1H). This correlation was observed regardless of the type of astrocytic tumor.

**Establishment of C6 Glioma Cells Expressing HNK-1 Glycan—** To directly evaluate the role of HNK-1 glycan, we first established C6 glioma cells expressing HNK-1 glycan by transfection with cDNA encoding GlcAT-P. C6 glioma cells express endogenous HNK-1ST but not GlcAT-P. Consequently, GlcAT-P cDNA transfection enabled expression of HNK-1 glycan on transfected cells (Fig. 2, A–C). It is noteworthy that other cells, such as COS cells also lack GlcAT-P and are negative for expression of HNK-1 glycan (Fig. 2, A–C). Between two HNK-1-positive clones, G14 and H1, the amount of HNK-1 expression was slightly more in H1 than G14. No difference in the proteins carrying HNK-1 was observed in G14 and H1 clones (Fig. 2D). Transfected C6 cells displayed HNK-1 glycans on several glycoproteins, including NCAM, but not on \(\beta 1\) integrin (Fig. 2D). A protein around 90 kD (Fig. 2D, arrowhead) might be myelin-associated glycoprotein (35). Although HNK-1 glycan is likely attached to glycolipids, we did not characterize those in this work.

**Tumor Invasion of C6 Glioma Cells Expressing HNK-1 Glycan—** Parental or mock-transfected C6 glioma cells barely express HNK-1 glycan or polysialic acid, thus allowing us to determine the effect of HNK-1 glycan on tumor cell invasion independent of polysialic acid. Mock-transfected C6 and C6-HNK-1 cells were inoculated into the striatum of C57BL/6 mice, as described previously (31). Mock-transfected C6 cells were highly invasive and spread almost exclusively in the same hemisphere of the brain where they were inoculated. The size of tumors formed by HNK1-positive H1 and G14 cells was much smaller than tumors formed by control C6 cells, a statistically significant finding (Fig. 3, A and B). To evaluate whether differences in tumor growth may differ in different host mice, C6 cells were inoculated into nude mice. C6-HNK-1 cells again produced smaller tumors than did mock-transfected C6 cells (Fig. 3C). Consistently, a proliferation marker Ki67 was detected much less in HNK-1 glycan-positive cells than the mock-transfected cells (Fig. 4A). These results indicate that HNK-1 glycans on C6 glioma cells attenuate tumor growth. HNK-1 expression, however, had a minimal effect on apoptosis (Fig. 4B).

**HNK-1-positive C6 Cells Migrate More Slowly on the Laminin E3 Fragment—** HNK-1 is reportedly a ligand for laminin (36). To elucidate whether HNK-1 expression affects C6 cell migration, migration of mock-transfected C6 and C6-HNK-1 cells was assayed using a Boyden chamber. When laminin was coated in the bottom surface of the chamber,
C6-HNK-1 cells migrated slightly more slowly than did mock-transfected C6 cells (Fig. 5, A and C). On the other hand, C6-HNK-1 cells migrated slightly faster than control C6 cells toward fibronectin (Fig. 5, A, B, and D). These migrations were stopped by adding laminin protein or fibronectin to the upper chamber (Fig. 5A). We then added a laminin E3 fragment to the upper chamber. E3 binds various proteins, including α-dystroglycan modified with unique glycans, and binding of E3 to α-dystroglycan reduces cell migration (32, 37). C6-HNK-1 cells migrated more slowly on the E3 fragment than C6 cells in a dose-dependent manner, suggesting that HNK-1 is recognized by E3 (Fig. 5B). Indeed, C6-HNK-1 cells bound much more to laminin and the E3 fragment than mock-transfected C6 cells (supplemental Fig. S2).

To determine how HNK-1 glycan alters cell migration on laminin, cells were pretreated with anti-HNK-1 glycan antibody or anti-β1 integrin antibody. Anti-HNK-1 antibody efficiently inhibited migration of C6-HNK-1 but not mock-transfected C6 cells (Fig. 5C). Surprisingly, anti-β1 integrin antibody had no effect on C6-HNK-1 migration, whereas the same antibody significantly inhibited migration of parental C6 cells (Fig. 5D) (38). These results suggest that migration of C6-HNK-1 cells is facilitated by interaction of laminin and HNK-1 glycan but is independent of β1 integrin-mediated migration signals. Because HNK-1 glycan is attached to many glycoproteins (Fig. 2D), the binding of anti-HNK-1 glycan antibody apparently inhibits the migration signal exhibited by these proteins. Hyaluronic acid is also abundant in brain, thus we tested the migration of C6 cells on hyaluronic acid. Although the hyaluronic acid facilitates C6-HNK-1 cell migration, the migration was observed only in a very high concentration of hyaluronic acid (supplemental Fig. S3), indicating that hyaluronic acid is likely a minor adhesion molecule for C6 and C6-HNK-1 cells.
To determine whether HNK-1 glycans are recognized by microglial cells, which are the major cytotoxic cells of the brain immune system (39, 40), we incubated C6 or C6-HNK-1 cells with BV2 microglial cells that had been stimulated with either IFN-γ or LPS (40). The number of lysed cells did not differ between the two cell types (supplemental Fig. S4), excluding the possibility that C6-HNK1 cells are more sensitive to cytolysis by microglial cells than parental cells.
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**FIGURE 6. Laminin-binding glycans and HNK-1 glycan are attached to different populations of α-dystroglycan.** A–C, immunoblot analysis (IB) of α-dystroglycan-IgG-Fc released from C6 and C6-HNK-1 cells (G14). α-dystroglycan-expressing HNK-1 glycan is larger in the presence of LARGE (L). HNK-1 glycan was detected by anti-HNK-1 glycan antibody (A), whereas laminin-binding glycans were detected by IIH6 antibody (B) and laminin overlay (Lam O/L) (C). D, cell migration assay to laminin. Treatment of anti-laminin-binding glycan IIH6 antibody slightly facilitates migration of both C6-HNK-1- and mock-transfected C6 cells. The experiments were carried out three times. The arrows in B and C indicate IIH6-positive α-dystroglycan. The error bars indicate S.D.

**HNK-1 Glycan and Laminin-binding Glycans Are Attached to α-Dystroglycan**—The α-dystroglycan in brain is suggested to carry two kinds of glycans, laminin-binding glycan and HNK-1 (7). We reported previously that laminin-binding glycan is a strong tumor suppressor inhibiting integrin-mediated migration signaling in prostate cancer (32). The above results suggest that C6 cells expressing HNK-1 glycan enhance laminin-binding, possibly by adding to the terminal of laminin-binding glycans. C6 cells expressed endogenous α-dystroglycan, which is weakly modified by laminin-binding glycan (IIH6 antibody–reactive) and that glycan modification was enhanced by transfection of LARGE (supplemental Fig. S5, A–C). HNK-1 was also positive on α-dystroglycan immunoprecipitated from G14 cell lysate upon immunoblot analysis (supplemental Fig. S6B). We then expressed α-dystroglycan-IgG-Fc chimeric protein in C6 and G14 cells, and α-dystroglycan-IgG-Fc purified from culture supernatant was subjected to immunoblot analysis. First, we found that the majority of HNK-1 glycan is attached to α-dystroglycan-IgG-Fc with a molecular mass of approximately 102 kDa and 135 kDa (Fig. 6A), similar to α-dystroglycan in the mouse brain (41). In cotransfection of LARGE, released α-dystroglycan is shifted to heterogeneous and larger molecules with a molecular mass of approximately 150 to > 250 kDa (Fig. 6, A and B, right lanes). The molecular weight of α-dystroglycan in the presence of LARGE was, however, not increased by addition of HNK-1 glycan (Fig. 6, A and B). These results suggest that the presence of HNK-1 glycan does not contribute to the elongation of glycans synthesized by LARGE but rather inhibit it. The laminin overlay result shows that laminin binds to α-dystroglycan–Fc produced in G14 cells slightly more than that in C6 and in the presence of LARGE much more significantly than those produced in the absence of LARGE (Fig. 6C). It has been shown that laminin binds to LARGE-synthesized glycans on α-dystroglycan and that this interaction attenuates β1-integrin-mediated migration (32). It has also been demonstrated that treatment with IIH6 antibody, which recognizes laminin-binding glycans on α-dystroglycan, enhances cell migration, as this antibody neutralizes interactions between laminin and glycans on α-dystroglycan (32). Indeed, both mock-transfected C6 and C6-HNK-1 cells migrated faster after IIH6 antibody treatment (Fig. 6D). On the other hand, anti-β1 integrin-antibody treatment did not reduce cell migration of C6-HNK-1 cells, although HNK-1 glycan is not capped on β1-integrin of C6-HNK-1 cells (Figs. 2D and 5D). Overall, these findings show that HNK-1 glycan changes cell migration properties by a mechanism different from that described in previous reports of the laminin-binding glycans and integrin (32).

**C6-HNK-1 Cells Proliferate More Slowly Than Do Mock-transfected C6 Cells**—We then asked if proliferation of mock-transfected C6 and C6-HNK-1 cells differed. Cell counting analysis showed that C6-HNK1 cells proliferate more slowly than do mock-transfected C6 cells when cultured on plates (Fig. 7A). Moreover, C6-HNK1 cells produced much smaller colonies in agar than did mock-transfected C6 cells (Fig. 7B). These results combined indicate that HNK-1 glycan expression decreases proliferation in both anchorage-dependent and -independent conditions. Interestingly, similar results were obtained from a different human astrocytic tumor cell line, U87. HNK-1 positive clones of U87, clones 2 and 10, proliferated slower than mock-transfected control U87 cells (supplemental Fig. S6), suggesting a common mechanism involving HNK-1 attenuated proliferation of these astrocytoma cell lines, C6 and U87.

**Activation of ERK1/2 Is Attenuated in C6-HNK-1 Cells**—To determine how HNK-1 glycan affects downstream signaling, phosphorylation of ERK1/2 and FAK upon laminin-111 stimulation was measured. FAK phosphorylation was essentially equivalent in C6-HNK-1 cells and mock-transfected cells (Fig. 8B). By contrast, phosphorylation of ERK1/2 was significantly attenuated in C6-HNK1 cells compared with mock-transfected C6 cells (Fig. 8A). Consistent with smaller colony formation by H1 clone, ERK1/2 activation was even weaker by H1 than G14.
Because ERK1/2 activation reflects signaling governing cell migration and proliferation, these results indicate that expression of HNK-1 glycan specifically attenuates MAP kinase signaling, resulting in reduced cell migration and proliferation in vitro and in vivo. These results are consistent with the finding that tumors formed by C6 cells expressing HNK-1 glycans are smaller than tumors formed by the mock-transfected C6 cells.

**DISCUSSION**

This study demonstrates that HNK-1 glycan is expressed in normal adult brain and that its expression is decreased in patients with aggressive forms of astrocytic tumors, such as anaplastic astrocytoma and glioblastoma. This finding is consistent with the recent report that glioblastoma stem cell (GSC11) up-regulates GlcAT when the cells are differentiated and decreases tumorigenesis (42). A Kaplan-Meier analysis showed that the five-year survival rate of the patients expressing significant amounts of HNK-1 glycan was high. Notably, this correlation is observed regardless of histological grade of astrocytic tumor. Previously, it was reported that polysialic acid expression is highly correlated with a poor prognosis of astrocytoma (31, 43–45). These combined results indicate that HNK-1 glycan expression is an efficient and favorable prognosis marker for patients with astrocytoma.

We observed a dramatic difference in tumor growth between C6-HNK-1 and C6 mock-transfected cells when these lines were inoculated into mouse brain. This difference may be due to several factors. When we assayed migration and invasion of C6-HNK-1 and control cells, C6-HNK-1 cells migrated and invaded slightly more slowly than did C6-mock cells on laminin. Notably, this decrease was observed more clearly when the laminin E3 fragment was used in the assay. Previously, we reported that laminin E3 fragment suppressed prostate and breast carcinoma migration because of binding to the laminin-binding glycans of α-dystroglycan expressed in these cells (32). Indeed, we found that C6 glioma cells endogenously express α-dystroglycan, although the laminin-binding glycans are only moderately expressed on C6 cells. Our study revealed that C6-HNK1 cells bind more efficiently to laminin than do mock-transfected C6 cells. However, laminin-binding glycans on C6 cells still attenuate C6 cell migration regardless of HNK-1 because IIH6 antibody, which neutralizes laminin-binding to α-dystroglycan, increased cell migration of both cell lines.

In this study, we identified a specific suppressive role of HNK-1 glycans in laminin-mediated MAP kinase ERK1/2 phosphorylation but not FAK phosphorylation. FAK and ERK phosphorylation play critical roles in extracellular matrix-induced cell adhesion, migration, and proliferation processes related to integrin pathways (46). FAK is directly linked to integrin, whereas ERK is rather downstream, closer to the control of cell proliferation. The observation that laminin-111- and fibronectin-mediated cell migration of mock-transfected C6 cells was completely inhibited by anti-β1 integrin is consistent with previous reports that β1 is the dominant integrin receptor for laminin-111 and fibronectin (32, 47, 48). In contrast, C6-HNK-1 cell migration was not altered by anti-β1 integrin antibody. Because HNK-1 is not attached to β1 integrin (Fig. 2D), anti-β1 integrin antibody should not interfere with the function of β1 integrin in C6-HNK-1 cells. More likely, HNK-1-fi-
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expression of HNK-1 glycan can reduce cell proliferation and thereby antagonize tumor growth in vivo.

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REFERENCES

1. Schachner, M., and Martini, R. (1995) Trends. Neurosci. 18, 183–191
2. Abo, T., and Balch, C. M. (1981) J. Immunol. 127, 1024–1029
3. Chou, D. K., Ilyas, A. A., Evans, J. E., Costello, C., Quarles, R. H., and Jungalwalla, F. B. (1986) J. Biol. Chem. 261, 11717–11725
4. Ariga, T., Kohriyama, T., Fredro, L., Latov, N., Saito, M., Kon, K., Ando, S., Suzuki, M., Hemling, M. E., and Rinehart, K. L., Jr. (1987) J. Biol. Chem. 262, 848–853
5. Yanagisawa, M., Oka, K., Nakamura, K., Ariga, T., and Yu, R. K. (2005) J. Neurochem. 95, 1311–1320
6. Kleene, R., and Schachner, M. (2004) Nat. Rev. Neurosci. 5, 195–208
7. Yuen, C. T., Chai, W., Loveless, R. W., Lawson, A. M., Margolis, R. U., and Feizi, T. (1997) J. Biol. Chem. 272, 8924–8931
8. Fukui, S., Feizi, T., Galustian, C., Lawson, A. M., and Chai, W. (2002) Nat. Biotechnol. 20, 1011–1017
9. Kruse, J., Mailhammer, R., Wernecke, H., Faissner, A., Sommer, I., Goridis, C., and Schachner, M. (1984) Nature 311, 153–155
10. McGarry, R. C., Helfand, S. L., Quarles, R. H., and Roder, J. C. (1983) Nature 306, 376–378
11. Gennarini, G., Rougon, G., Vitiello, F., Corsi, P., Di Benedetto, C., and Goridis, C. (1989) J. Neurosci. Res. 22, 1–12
12. Abbott, K. L., Matthews, R. T., and Pierce, M. (2008) J. Biol. Chem. 283, 33026–33035
13. Morita, I., Kakuda, S., Takeuchi, Y., Itoh, S., Kawasaki, N., Kizuka, Y., Kawasaki, T., and Oka, S. (2009) J. Biol. Chem. 284, 30209–30217
14. Bleckmann, C., Geyer, H., Lieberoth, A., Splitsstoesser, F., Liu, Y., Feizi, T., Schachner, M., Kleene, R., Reinhold, V., and Geyer, R. (2009) J. Biol. Chem. 284, 627–645
15. Martini, R., Xin, Y., Schmitz, B., and Schachner, M. (1992) Eur. J. Neurosci. 4, 628–639
16. Kümemund, V., Jungalwalla, F. B., Fischer, G., Chou, D. K., Keilhauer, G., and Schachner, M. (1988) J. Cell Biol. 106, 213–223
17. Terayama, K., Oka, S., Seiki, T., Miki, Y., Nakamura, A., Kozutsumi, Y., Takio, K., and Kawasaki, T. (1997) Proc. Natl. Acad. Sci. U.S.A. 94, 6093–6098
18. Ong, E., Yeh, J. C., Ding, Y., Hindsgaul, O., and Fukuda, M. (1998) J. Biol. Chem. 273, 5190–5195
19. Bakker, H., Friedmann, I., Oka, S., Kawasaki, T., Nifant’ev, N., Schachner, M., and Mantei, N. (1997) J. Biol. Chem. 272, 29942–29946
20. Yoshihara, T., Sugihara, Y., Kizuka, Y., Oka, S., and Asano, M. (2009) J. Biol. Chem. 284, 12550–12561
21. Yamamoto, S., Oka, S., Inoue, M., Shimuta, M., Manabe, T., Takahashi, H., Miyamoto, M., Asano, S., Sakagami, J., Sudo, K., Iwakura, Y., Ono, K., and Kawasaki, T. (2002) J. Biol. Chem. 277, 27227–27231
22. Senn, C., Kutsche, M., Saghatelian, A., Bösl, M. R., Löhler, J., Bartsch, U., Morellini, F., and Schachner, M. (2002) Mol. Cell. Neurosci. 20, 712–729
23. Chou, D. K., Schachner, M., and Jungalwala, F. B. (2002) J. Neurochem. 82, 1239–1251
24. Ariga, T., Suetake, K., Nakane, M., Kubota, M., Usuki, S., Kawashima, I., and Yu, R. K. (2008) Neurosignals 16, 226–234
25. Soriano, P., Navarro, S., Gil, M., and Llombart-Bosch, A. (2004) Virchows Arch. 454, 292–297
26. Lizard-Nacol, S., Lizard, G., Justrabo, E., and Turc-Carel, C. (1989) Am. J. Pathol. 135, 847–855
27. Thies, A., Schachner, M., Berger, J., Moll, I., Schulze, H. J., Brunner, G., and Schumacher, U. (2004) J. Pathol. 203, 933–939
28. Reifenberger, G., Bilzer, T., Seitz, R. J., and Wechsler, W. (1989) Acta Pathol. Microbiol. Immunol. Scand. 97, 541–551
29. Reifenberger, G., Bilzer, T., Seitz, R. J., and Wechsler, W. (1989) Acta Pathol. Microbiol. Immunol. Scand. 97, 541–551
30. Kuhnemund, V., Jungalwalla, F. B., Fischer, G., Chou, D. K., Keilhauer, G., and Schachner, M. (1988) J. Cell Biol. 106, 213–223
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32. Bao, X., Kobayashi, M., Hatakeyama, S., Angata, K., Gullberg, D., Nakayama, J., Fukuda, M. N., and Fukuda, M. (2009) Proc. Natl. Acad. Sci. U.S.A. 78, 3053–3057
33. Zhu, W., Kanoh, M., Ye, P., Laszkiewicz, I., Royland, J. E., Wiggins, R. C., and Konat, G. (1992) J. Neurosci. Res. 31, 745–750
34. Blum, A., Vives-Dominguez, S., Eckert, T., Burg-Roderfeld, M., Wechselberger, R., Romanuk, J., Bachle, D., Kornilov, A. V., von der Lieth, C. W., Jiménez-Barbero, J., Nifantiev, N. E., Schachner, M., Sewald, N., Lütteke, T., and Siebert, H. C. (2010) J. Am. Chem. Soc. 132, 96–105
35. Andac, Z., Sasaki, T., Mann, K., Brancaccio, A., Deutzmann, R., and Timpl, R. (1999) J. Mol. Biol. 287, 253–264
36. Amoureux, M. C., Coulibaly, B., Chinot, O., Loundou, A., Metellus, P., Rougon, G., and Figarella-Branger, D. (2010) BMC Cancer 10, 91–102
37. Giancotti, F. G., and Ruoslahti, E. (1999) Science 285, 1028–1032
38. Klinowska, T. C., Soriano, J. V., Edwards, G. M., Oliver, J. M., Valentijn, A. J., Montesano, R., and Streuli, C. H. (1999) Dev. Biol. 215, 13–32
39. Paulus, W., Baur, L., Schuppan, D., and Roggendorf, W. (1993) Am. J. Pathol. 143, 154–163
40. Petridis, A. K., Wedderkopp, H., Hugo, H. H., and Maximilian Mehroor, H. (2009) Acta Neurochir. 151, 601–604
41. Amoureux, M. C., Coulhaly, B., Chinot, O., Loundou, A., Metellus, P., Rougon, G., and Figarella-Branger, D. (2010) BMC Cancer 10, 91–102
42. Giancotti, F. G., and Ruoslahti, E. (1999) Science 285, 1028–1032
43. Klinowska, T. C., Soriano, J. V., Edwards, G. M., Oliver, J. M., Valentijn, A. J., Montesano, R., and Streuli, C. H. (1999) Dev. Biol. 215, 13–32
44. Paulus, W., Baur, L., Schuppan, D., and Roggendorf, W. (1993) Am. J. Pathol. 143, 154–163