Suppression of Integrin Expression and Tumorigenicity by Sulfation of Lactosylceramide in 3LL Lewis Lung Carcinoma Cells

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To investigate the cellular functions of sulfated glycosphingolipids, we introduced the cerebroside sulfotransferase (CST) gene into J5 cells, a subclone of 3LL Lewis lung carcinoma cells. The J5 cells lack acidic glycosphingolipids but accumulate their common biosynthetic precursor, lactosylceramide. We established the stable CST transfectants, J5/CST-1 and J5/CST-2 clones, highly expressing sulfated lactosylceramide (SM3). Both clones exhibited more spherical morphology in comparison to mock transfectant, and their adhesiveness to fibronectin and laminin was significantly lower. The loss of cell-substratum interactions in these SM3-expressing cells could be attributed to decreased expression of integrins (α5, α6, and β1) on the cell surface and their whole cellular levels. However, the levels of H-2Kb and H-2Db antigens in J5/CST-1 and -2, but there was no change in the levels of normal SM3 negatively regulates the cell-substratum interaction, which is essential for the development of malignant tumors in vivo. Thus, we showed the evidence that cellular SM3 negatively regulates the cell-substratum interaction, resulting in the loss of tumorigenicity.

Sulfatides are biosynthesized from the acceptor substrates, glycosphingolipids (GSLs), and the sulfate donor, 3′-phosphoadenosine 5′-phosphosulfate, by the action of cerebroside sulfotransferase (CST, EC 2.8.2.11) (1). CST transfers the sulfate group to the 3-position of the galactose in lactosylceramide (LacCer) and galactosylceramide (GalCer) and forms galactosylsulfatide (SM4) and lactosylsulfatide (SM3), respectively (2). CST did not show sulfotransferase activity toward the oligosaccharides that had been used as substrates in the previous studies on the glycoprotein β-Gal-3′-sulfotransferase (3). Northern blot analysis showed that CST gene is preferentially transcribed in stomach, small intestine, brain, kidney, lung, and testis, in that order (4).

The physiological functions of sulfatides have long been noted for their unique property of having a strong negative electronic charge in the molecule. Sulfatides interact with various biomolecules especially in cell adhesion, differentiation, and signal transduction (reviewed in Refs. 5 and 6). In the case of cell-substratum adhesion, SM4 and SM3 have been found to bind several proteins such as laminin and thrombospondin (7). When exogenous SM4 was incorporated into SMKT-R3 cells (human renal cell carcinoma), their attachment to laminin, but not to fibronectin, was enhanced (8). However, it was uncertain whether the data obtained from these experiments truly reflect the cellular functions of endogenous sulfatides.

The purpose of the present study was to investigate the functional role of endogenous sulfatide in cell adhesion. We employed a subclone of the mouse 3LL Lewis lung carcinoma cell line, 3LL-J5, which has high LacCer content but no galactosylceramide and SM3 (9, 10), to introduce the recently cloned CST gene (1). The stable CST transfectants expressing SM3 at a high level exhibited decreased adhesive abilities to both fibronectin and laminin. We demonstrated here an inverse (or negative) relationship between the level of cellular SM3 and cell adhesive ability as well as tumorigenicity.

EXPERIMENTAL PROCEDURES

Materials—For the GSL analysis, DEAE-Sephadex A-25 was purchased from Amersham Pharmacia Biotech, and Sep-Pak C18 and silica gel HPTLC plates were from Waters Associates (Milford, MA) and Merck, respectively. pCDNA3.1/Zeo (+) and zeocin were from Invitrogen.

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(Carlsbad, CA), and LipofectAMINE PLUS™ Reagent was from Life Technologies, Inc. Human fibroblasts and mouse laminin were from Biomedic Technologies Inc. (Stoughton, MA). A cell counting kit based on a modified 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide method was employed (Dojindo, Kumamoto, Japan). For flow cytometry, live cells were recognized from dead cells using 7-AAD (BioSys, San Diego, CA); anti-\(\alpha_5\) (monoclonal antibody 1378) and \(\beta_1\) (monoclonal antibody 1997) integrins were from Chemicon International Inc. (Temecula, CA); anti-\(\alpha_5\) (11) was from Seikagaku Corp. (Tokyo, Japan); and fluorescent-conjugated anti-H-2D\(^+\) and H-2K\(^+\) were from Meiji Nygu Corp. (Tokyo, Japan) as the primary antibodies. Fluorescein-conjugated anti-mouse IgM from Vector Laboratories Inc. (Burlingame, CA) and fluorescent-conjugated anti-rat IgG as the secondary antibodies were from Immunotech (Marseille, France). For Western blotting of integrins, anti-\(\alpha_5\) integrin (antibody 1928) and anti-\(\beta_1\) integrin (monoclonal antibody 1997) were from Chemicon. For the chemolescence detection of glycolipids, horseradish peroxidase-conjugated anti-mouse IgM from Jackson ImmunoResearch (West Grove, PA) and the enhanced chemolescence system (ECL kit) from Amersham Pharmacia Biotech were used. Bicinchoninic acid reagent from Pierce was used for protein determination. All animal experiments were carried out in accordance with NIH Guide for Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee in Hokkaido University.

Line and Culture Conditions—A subclone of the murine 3LL Lewis lung carcinoma cell line, J5, was described previously (9). Cells were maintained in RPMI 1640 medium (Nissui, Tokyo, Japan) containing 10% (v/v) fetal calf serum (Sigma), 100 units/ml penicillin, 100 \(\mu\)g/ml streptomycin, 50 \(\mu\)M 2-mercaptoethanol, and 2 mM L-glutamine. Cells were cultured in a 100-mm dish were harvested with 0.1M NaOH for 1 h at 4 °C. The solution was neutralized with 1 M acetic acid in methanol, diluted with an equal volume of aqueous 50 mM NaCl, and applied to a Sep-Pak C\(_18\), reverse-phase cartridge. The cartridge was washed with 40 ml of water, and lipids were eluted with 10 ml of methanol and 10 ml of chloroform/methanol (1:1), successively. The eluate was evaporated to dryness, and the lipids were analyzed by HPTLC. The plates were then developed with chloroform/methanol/aqueous 12 m magnesium chloride (60:25:4, for neutral lipids) or chloroform/methanol/water (65:25:4, for acidic lipids). Glycolipids were visualized by spraying orcinol reagent and heating at 100 °C for 10 min and then quantified with a dual-wavelength flying spot scanner (CSS9300-PC, Shimadzu, Kyoto, Japan) in the reflectance mode at 500 nm with integrated areas.

Immunological Detection of SM4 and SM3—TLC immunoblotting was performed by the method of Taki et al. (12), slightly modified as follows. Acidic lipids were separated on a HPTLC plate with chloroform/methanol/water (65:25:4) and then immersed in a mixture of isoamyl alcohol/water (96:4) and 10\% \(\mathrm{NH}_4\)OH for 30 min. The plates were then washed and then dried. The PVDF membrane was agitated in 5% skim milk/TBS-T and dried. The PVDF membrane was then incubated with the appropriate primary antibodies (1:500 dilution) at 4 °C over-night. The PVDF membrane was then washed, and then incubated with the appropriate secondary antibodies (1:5000 dilution) for 1 h. After washing with TBS-T, the PVDF membrane was incubated with a 100 mM solution of D-glucosamine (For lactosyl sulfatide and lactosyl sulfatide) or anti-SM3 (for lactosyl sulfatide) (11) solution in 5% skim milk/TBS-T at 4 °C overnight. The PVDF membrane was washed with TBS-T and dipped and shaken in the secondary antibody, peroxidase-conjugated goat anti-mouse IgM, primary solution in 5% aqueous 0.2% calcium chloride/methanol (40:20:7) for 20 s. The plate was then covered with a polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore, Bedford, MA) and a glass microfiber filter (Atto Instruments, Tokyo, Japan). This was then pressed (level 8) for 50 s with TLC Thermal Blotter (Atto Instruments) at 180 °C, after which the PVDF membrane was separated from the plate and dried. The PVDF membrane was saturated in 5% skim milk/methanol (1:1) for 1 h and then incubated with the appropriate primary antibodies, washed, and then incubated with the appropriate secondary antibodies. The membranes were then washed with TBS-T and dried. The PVDF membrane was incubated with alkaline phosphatase-linked anti-mouse IgM (Promega, Madison, WI). The PVDF membrane was then washed, and then incubated with the appropriate secondary antibodies, washed, and then incubated with the appropriate secondary antibodies. The PVDF membrane was then washed with TBS-T and dried. The PVDF membrane was incubated with alkaline phosphatase-linked anti-mouse IgM (Promega, Madison, WI). The PVDF membrane was then washed, and then incubated with the appropriate secondary antibodies. The PVDF membrane was then washed, and then incubated with the appropriate secondary antibodies. The PVDF membrane was then washed, and then incubated with the appropriate secondary antibodies.
Flow Cytometry—Cells were detached from the culture surface by a 20-min incubation at 37 °C in 10 mM EDTA/PBS, pelleted, washed with FACS buffer (0.1% BSA and 0.1% NaN₃ in PBS) twice, and then resuspended in FACS buffer containing 10 μg/ml anti-α₅, α₆, β₁ integrin or anti-SM3 for 1 h at 4 °C. For the detection of whole cellular integrins, cells were permeabilized with 0.1% saponin containing FACS buffer for 15 min at 4 °C before adding the appropriate primary antibody. Then the cells were washed with FACS buffer three times, treated with fluorescein-conjugated anti-rg IgG or fluorescein-conjugated anti-mouse IgM for 1 h at 4 °C in the dark, and washed with FACS buffer as above. The intensity of cell fluorescence was determined by a FACScan cytometer (Becton Dickinson). For the detection of MHC, cells treated with or without saponin were stained with 100 μg/ml fluorescein-conjugated anti-SM3 for 1 h at 4 °C. 

Cell Attachment Assay—100 μl of fibronectin or laminin (1, 5, 10, and 25 μg/ml) in PBS were added to each well of 96-well plates, incubated overnight at room temperature, and removed. The coated wells were further incubated with 100 μl of 0.1% BSA in PBS at room temperature for 1 h and washed with PBS three times. Each well was incubated with 50 μl of 0.01% BSA in RPMI 1640 medium at 37 °C for 1 h. A 50-μl suspension of J5/CST-1, J5/CST-2, or mock cells (5 × 10⁵) in 0.01% BSA/RPMI 1640 was added to the fibronectin- or laminin-coated wells and incubated for 30 min. Non-adherent cells were removed by inverting the plate, and each well was gently washed with 100 μl of serum-free RPMI 1640 medium. To each well were added 100 μl of the same medium followed by 10 μl of cell counting kit. After incubation at 37 °C for 2 h, the absorbance (450 nm) of formazan generated in the wells was measured with a dual-wavelength flying spot scanner (CS9300-PC, Shimadzu, Kyoto, Japan). The attachment ability was expressed as the percentage of attached cells (absorbance of attachment cells in the well/absorbance of total cells added to the well × 100).

RESULTS

Establishment of the Stable CST Transfectants Highly Expressing SM3—Previously, we subcloned the J5 clone, which lacked acidic GSLs and accumulated LacCer, from the wild type of murine 3LL Lewis lung carcinoma cells (9). The CST gene was introduced into the J5 clone to generate sulfatide-expressing clones. A total of 24 transfectant clones were finally obtained by limited dilution, and 2 clones (J5/CST-1 and J5/CST-2) which expressed relatively high levels of CST gene mRNA (Fig. 1A) and CST activity (Fig. 1B) were chosen for further study. The mRNA and the enzyme activity of CST appeared only in the clones into which the vector constructed with the CST gene had been introduced but not in the mock-transfected clones (Fig. 1, A and B). Because the J5 clone expresses only LacCer but not GalCer (9, 10), introduction of the CST gene into this clone should express only SM3 as a sulfatide. The GSL fractions were prepared from mock and J5/CST-1 and -2 cells, and the SM3 content in proportion to the CST activity was confirmed on HPTLC visualized with orcinol-sulfuric acid (acidic GSLs, left and neutral GSLs, right). D, GSLs in J5/CST-1, -2, and mock, corresponding to 0.1 mg of protein, were stained with an anti-SM3 antibody (top) or O4 antibody (bottom), as described. E, cell surface expression of SM3 by flow cytometry. Mock (top), J5/CST-1 (middle), and J5/CST-2 (bottom) were stained with an anti-SM3 antibody and fluorescein-conjugated anti-mouse IgM. The solid line indicates reactivity with anti-SM3; the dotted line shows secondary antibody alone.

Analyses of the neutral GSLs of J5/CST-1 and -2 cells showed that not only the contents of LacCer and its precursor GlcCer were decreased, but also that of the globo type GSL, Gb3 (Fig. 3C), indicative of weak adhesiveness of the SM3-expressing cells. Both J5/CST-1 and -2 cells exhibited a marked decrease in their adhesive abilities to the plastic coated with laminin and fibronectin (Fig. 3A). Moreover, the cells highly expressing SM3 were unable to spread on the surface coated with fibronectin even 2 h after seeding (Fig. 3B).

Decreased Integrin Expression in CST Transfectants—The expression of integrin molecules on the cell surface involved in the recognition of fibronectin (α₅ and β₁) and laminin (α₆ and β₁) was analyzed by flow cytometry (Table I). All of the integrins examined here exhibited a marked decrease (around 32–43% in both J5/CST-1 and -2 cells). After cell permeabilization with saponin, almost similar decreases in the cellular integrins were observed, demonstrating that the decreases in cell surface integrins in the SM3 highly expressing cells were due to their cell proliferation under the normal culture conditions on plastic plates (Fig. 2A). On the other hand, there was a significant increase of the spherical form in the CST transfectants (Fig. 2B), indicative of weak adhesiveness of the SM3-expressing cells. Both J5/CST-1 and -2 cells exhibited a marked decrease in their adhesive abilities to the plastic coated with laminin and fibronectin (Fig. 3A). Moreover, the cells highly expressing SM3 were unable to spread on the surface coated with fibronectin even 2 h after seeding (Fig. 3B).
of each clone was measured every day up to 4 days by the modified
J5/CST-1, -2, and mock were plated in 96-well plates, and the growth
tants.

in CST transfectants originated from the decreased cellular
tants—

in CST transfectants was decreased to around 50% in compar-
A

the precursor form (Fig. 4

CST transfectants was mainly due to the dramatic decrease of
integrin expression in the CST transfectants may

decrease of integrin mRNA in both SM3 high expressing cells, J5/CST-1 and J5/CST-2, but only a slight decrease in the SM3 low expressing J5/CST-3 cells, showing the inverse relationship between the levels of cellular SM3 and β1 integrin mRNA content (Fig. 6, A versus C). The levels of α5 transcript both in mock and CST transfectants were essentially the same (Fig. 6B). These results dem-

strate the selective transcriptional down-regulation of β1 integrin by endogenous SM3.

Loss of Tumorigenicity—Since the tumorigenic and meta-
static potentials of tumors are greatly affected by the expres-
sion levels of integrins (17–20), we were interested in the behavior of the SM3-expressing cells in vitro. When the mock and the CST transfectants were inoculated subcutaneously into syngeneic C57BL/6 mice, and then examined for their tumor growth, a remarkable decrease or even no sign of tumor growth was observed (Fig. 7).

Since no difference in in vitro cell growth in culture plastic
between the mock and the CST transfectants was observed (Fig. 2A), the loss of tumorigenicity in vivo could be due to the global loss of integrin functions that are essential for malignant tumor growth.

DISCUSSION

LacCer is the common precursor of numerous GSLs con-
sisting of six groups classified as the ganglio, globo, isoglob, neolacto, lacto, and sulfo series. Their expression is primarily
determined by cell type-specific expression of enzymes at the luminal side of Golgi membranes responsible for either glyco-
sylation (21) or sulfation (22, 23) of branch point LacCer.

Since the composition and distribution of LacCer-derived GSLs are known to be greatly altered during development and oncogenic transformation (reviewed in Ref. 24), the elucidation of the biological significance of LacCer branching in various cellular functions is one of the most important issues of glycobiology. The cDNAs of the branching enzymes have

been extensively cloned recently (1, 25–30), and it is now possible to clarify the functions of GSLs utilizing these syn-

thase genes.

As our first trials to study the biology of LacCer branching,
we were able to show the functioning of cellular SM3 in cell-
substratum adhesion using genetically manipulated SM3-ex-
pressing cells, containing the CST gene in the J5 clone of 3LL
Lewis lung carcinoma cells (9). Significant findings demon-

strated here are as follows. 1) The transfectants expressing
SM3 at a relatively high level exhibited decreased adhesion to
both fibronectin and laminin substrates. 2) The defect in cell
adhesion could be attributed to decreased expression of inte-
grin proteins, including α5, αc, and β1-subunits, on the cell
surface as well as their whole cellular levels. Although there
might be a general decrease of cellular integrins in the CST
transfectants, HIC expression was, however, not altered sig-
ificantly, suggesting a selective action of cellular SM3 on inte-
grin expression. 3) Integrin expression is regulated at both
transcriptional and post-transcriptional levels, since only β1
integrin mRNA but not αc and α5 mRNAs decreased in SM3-
expressing cells. 4) Although the rates of cell proliferation in vitro were similar for the mock and the CST transfectants,
tumorigenicity of the SM3-expressing cells in vivo was dramat-
ically lower, probably due to the global loss of the efficient cell-matrix interactions in vivo.

The mechanism by which the CST gene causes the selective decrease of $\beta_1$ integrin mRNA remains to be elucidated. Many cytokines are known to regulate integrin transcription (reviewed in Ref. 31). The initial signaling cascade of several cytokines may occur at lipid rafts, which are believed to function as signaling domains in the plasma membrane (reviewed in Ref. 32). GSLs including sulfatides are concentrated in the lipid rafts, which can be isolated as the detergent-insoluble microdomains (DIMs) (33). If the distinctive organization and functions of lipid rafts from the mock and CST transfectants can be elucidated, the information might explain the transcriptional suppression of $\beta_1$ integrin. We have obtained preliminary evidence regarding differences in the protein composition and patterns of tyrosine-phosphorylated proteins in the two types of DIMs.2

One could hypothesize the presence of an SM3-binding protein in the DIMs of 3LL Lewis lung carcinoma cell lines, since the sulfatide-binding proteolipid protein, the rat myelin and lymphocyte protein, has been identified in a detergent-insoluble complex obtained from oligodendrocytes and Schwann cells (33). The rat myelin and lymphocyte protein is a member of the tetraspanin membrane protein (TMP) group. Other TMPs, such as CD9 (35–38), CD53 (39), CD63 (36, 39, 40), CD81 (37, 39), and NAG-2 (41), have been known to associate with inte-

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**FIG. 3.** Cell adhesion and spreading on laminin and fibronectin. A, 96-well plates were coated with laminin and fibronectin, and cell attachment assay was performed as described under "Experimental Procedures." B, J5/CST-1 and mock cells were seeded on a plastic plate coated with laminin and fibronectin at 10 $\mu$g/ml, and 2 h later, the plates were washed with PBS, fixed with 2.5% glutaraldehyde, stained with Giemsa, and photographed ($\times$ 200). Bar = 100 $\mu$m.

**FIG. 4.** Decreased cellular integrin contents in CST transfectants. A, the cell lysates (20 $\mu$g of protein) from mock (lanes 1 and 4), J5/CST1 (lanes 2 and 5), and J5/CST2 (lanes 3 and 6) were subjected to Western blot analysis using anti-$\beta_1$ integrin antibody. Endoglycosidase H treatment of each cell lysate (lanes 4–6) was performed prior to SDS-PAGE. B, the cell lysates (20 $\mu$g of protein) from mock (lane 1), J5/CST1 (lane 2), and J5/CST2 (lane 3) were subjected to Western blot analysis using anti-$\alpha_5$ integrin antibody.

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| Table 1: Flow cytometric analysis of the amounts of cell surface and total cellular integrins and H-2 antigens | Mock | J5/CST-1 | J5/CST-2 |
|---------------------------------------------------------------|------|---------|---------|
| [Cell Surface]                                                |      |         |         |
| $\alpha_5$                                                    | 130.8| 87.9    | 77.8    |
| $\alpha_6$                                                    | 198.8| 113.1   | 114.1   |
| $\beta_1$                                                    | 199.1| 121.0   | 134.6   |
| H-2D$^b$                                                    | 24.1 | 34.5    | 33.9    |
| H-2K$^b$                                                    | 18.4 | 19.1    | 17.7    |
| [Total]                                                      | 243.3| 97.9    | 147.6   |
| $\alpha_5$                                                    | 563.5| 336.4   | 260.8   |
| $\alpha_6$                                                    | 327.1| 196.6   | 172.8   |
| H-2D$^b$                                                    | 111.0| 98.0    | 88.1    |
| H-2K$^b$                                                    | 227.6| 194.8   | 232.1   |
| H-2D$^b$                                                    | 80   |         |         |
| H-2K$^b$                                                    | 130  |         |         |
| [Total]                                                      | 130  |         |         |

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2 S. Uemura, K. Kabayama, Y. Igarashi, and J. Inokuchi, unpublished observations.
SM3 Downregulates Integrin Expression

The effects of SM3 on integrin expression and cell adhesion were studied. SM3 was found to downregulate the expression of integrins (Fig. 5). This was confirmed by semi-quantitative RT-PCR for α5, α6, and β1 integrins, which showed a significant decrease in the expression of α5 and β1 integrins in SM3-expressing cells. Additionally, the comparison of SM3 contents among the CST transfectants by HPTLC (Fig. 5) demonstrated a strong inverse relationship between the levels of cellular SM3 and β1 integrin mRNA (Fig. 6). These findings suggest that SM3 downregulates integrin expression in a manner that might modulate integrin signaling (42).

SM3 has been shown to have a suppressive effect on integrin expression in various cell types. For example, the suppression of integrin expression in J5/CST transfectants was observed, leading to a decrease in cell adhesion to laminin and other bioactive molecules under cell-free conditions (7). This was initially expected in our study, as SM3 is known to negatively regulate cell-substratum adhesion. However, this expectation was not corroborated in our cell adhesion assay due to the significant down-regulation of integrin expression in the SM3-expressing cells (Table I). We also observed a decrease in spontaneous cell motility in CST transfectants (data not shown). Therefore, we are currently studying the effects of endogenous SM3 on integrin biosynthesis and recycling by the metabolic labeling and pulse-chase experiments.

Since it is known that SM4 and SM3 can directly bind to laminin and other bioactive molecules under cell-free conditions (7), we initially expected an enhancement of cell adhesion to laminin in the SM3-expressing J5/CST-1 and -2 cells. However, this expectation was not corroborated in our cell adhesion assay due to the significant down-regulation of integrin expression, which occurred when SM3 was expressed endogenously by CST transfection. In other words, we present the evidence for the first time that endogenous sulfated GSLs, especially SM3, negatively regulate cell-substratum adhesion. If the other anionic GSLs, such as gangliosides, exhibit similar or opposite effect against cellular SM3, then our current approach to express SM3 alone by introducing CST gene into the cells lacking all anionic GSLs would be one of the most practical approaches to reveal and identify the functional role of individual anionic GSL molecule. Interestingly enough, when we transfected GM3 synthase gene (26) constructed with the same vector used in this paper into J5 cells to express GM3, the GM3-expressing cells exhibited the ability to attach and spread on fibronectin more efficiently. In contrast to the SM3-expressing cells, the β1 integrin mRNA content was elevated in the GM3-expressing cells. This finding would explain the distinct roles of sulfation and sialylation on carbohydrates as well as the significance of GSL function at the LacCer branching point (3). Further detailed study will be performed to verify whether or not the suppressive effect of SM3 on integrin expression is a physiologically and/or pathologically relevant event in cells originally expressing SM3, such as renal cell lines (50, 51) and in tissues expressing CST gene, such as stomach, small intestine, brain, kidney, lung, and testis (4).

Fig. 5. Selective decrease of β1 integrin mRNA in CST transfectants. Semi-quantitative RT-PCR for α5, α6, and β1 integrins was performed as described under “Experimental Procedures.”

Fig. 6. Inverse relationship between the levels of cellular SM3 and β1 integrin mRNA. Northern blot analyses of β1 integrin (A) and α5 integrin (B). Methylene blue staining of 18 S and 28 S rRNA in the same membrane. Comparison of SM3 contents among the CST transfectants by HPTLC (C).

Fig. 7. Loss of tumorigenicity in CST transfectants. Tumors were induced in C57BL/6 mice (8 weeks old, female) by subcutaneous injection of 2 × 10⁴ cells/0.1 ml in the axillary region by puncture in the inguinal region. Three weeks later, the tumors were excised and weighed. Mock, n = 15; J5/CST-1, n = 10; J5/CST-2, n = 15.

Intracellular degradation of α-subunits before reaching the plasma membranes. In fact, this idea is supported by our experimental data showing the parallel decreases on the cell surface expression between α-subunits and β1 integrin in the SM3-expressing cells (Table I). We also observed a decrease in spontaneous cell motility in CST transfectants (data not shown). Therefore, we are currently studying the effects of endogenous SM3 on integrin biosynthesis and recycling by the metabolic labeling and pulse-chase experiments.

Many cell types have a large intracellular pre-β1 integrin pool in endoplasmic reticulum (45–49) and form αβ1 heterodimers when they are still located in this compartment (45). It has been also suggested that the excess of pre-β1 in endoplasmic reticulum might be required for the efficient heterodimer formation (47). As demonstrated in Table I and Figs. 4–6, we observed a significant decrease in the pre-β1 pool correlated with the reduction of the β1-transcript in the SM3-expressing cells. Although the protein levels of α5 and α6 integrins were considerably diminished similar to that of the pre-β1, the α5- and α6-transcripts remained unaltered. An interesting hypothesis is that under conditions in which only a limited number of pre-β1-chains are available, the α-subunits inefficiently form αβ1 heterodimers, thereby leading the en-
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