Supporting Information

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SI Materials and Methods

Cell Lines and Culture. Normal mouse mammary gland epithelial cells (NMuMG) and human mammary carcinoma cells (MCF7) were from American Type Culture Collection. Human normal bladder epithelial HCV29 cells were originally established at the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (Wroclaw, Poland) (1), and were donated to us by the Department of Urology, Tohoku University (Sendai, Japan). NMuMG cells were cultured in DMEM containing 10% FBS (HyClone), 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 5% CO2. MCF7 and HCV29 were cultured in RPMI medium 1640 containing 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin, at 37 °C in 5% CO2.

Antibodies and Reagents. Antibodies. Mouse anti-GM3 IgG3 mAb DH2 (2) and mouse anti-Gg4 IgM mAb TKH7 (3) were established in this laboratory. Mouse anti-GM2 IgM mAb MK1-8 was kindly donated by Reiji Kannagi (Nagoya Cancer Research Center, Nagoya, Japan). Other antibodies were: mouse anti-E-cadherin IgG1 (BD Biosciences); mouse anti-N-cadherin IgG1 and rabbit anti-desmoplakin IgG (Santa Cruz Biotechnology); mouse anti-GAPDH mAb IgG2b (Chemicon); mouse anti-fibronectin mAb IgG (Calbiochem); mouse anti-vimentin IgG1, anti-β-actin IgG1, and anti-γ-tubulin (Sigma); HRP-labeled goat anti-mouse IgG (Southern Biotech); and FITC-labeled goat F(ab')2 anti-mouse IgG+IgG (Biosource).

Reagents. Reagents used were EtDO-P4, the P4 derivative (4, 5) kindly donated by J.A. Shayman, University of Michigan, Ann Arbor, MI); CTB conjugated with biotin (Sigma); TGF-β1 (BD Biosciences); asialo-GM1 (Gg4), GM1, GM2, GM3, and GD1a (Maturea); and mixtures of ganglio-series disialogangliosides and trisialogangliosides (Sigma). Gb3 and Gb4 were prepared from human erythrocytes in our laboratory. Other reagents were from Sigma unless stated otherwise.

Phagokinetic Gold Sol Assay for Cell Motility. Motility was determined by modification (6, 7) of the original method (8). Gold sol-coated plates (24-well) were prepared as described previously (9). Cells were detached with trypsin/EDTA, and 5 × 10⁶ cells in complete culture medium were seeded onto gold sol-coated wells and incubated for 18 h. Photos were taken, and the track area of 30 cells was measured by the Scion image program and expressed as square pixels.

GSL Extraction, Analysis, and Immunostaining. GSL extraction, HPTLC analysis, and immunostaining were performed as described previously (9). Briefly, cells (~2 × 10⁶) were harvested with a cell scraper after washing with PBS and were extracted with 2 mL of isopropanol/hexane/water (55:25:20) by vortexing and sonication. Extracts were evaporated to dryness under nitrogen stream. Phospholipids were hydrolyzed in 0.1 M NaOH in methanol at 40 °C for 2 h. The solution was neutralized with 1 M HCl, added to 2 mL of hexane, shaken, and allowed to stand to separate the upper hexane layer from the lower layer. This step was repeated one more time. The lower layer was evaporated and solubilized in 1 mL of distilled water placed in a water bath sonicator (1 min). The solution was applied to a Sep-Pak C18 cartridge (Varian) and washed with water. GSLs were eluted with 2 mL of chloroform/methanol (2:1), analyzed by using HPTLC plates (EMD Bioscience), and stained with 0.5% orcinol in 1 M sulfuric acid.

Total GSL fractions were further separated by using DEAE-Sephadex A25 (Sigma) column chromatography. Nonacidic, monosialosyl, disialosyl, and trisialosyl fractions were eluted by a stepwise procedure with 0 M, 0.03 M, 0.13 M, and 0.45 M ammonium acetate in chloroform/methanol/water (30:60:8), respectively (11). Monosialosyl, disialosyl, and trisialosyl fractions were dialyzed in Spectra-Por membrane (NMCO 3.5 kDa; Spectra-Por) against distilled water and lyophilized.

GSLs were further analyzed by HPTLC immunostaining. After development, plates were dried, fixed with 5% poly(isobutyl-metacrylate) in hexane/chloroform (9:1), and stained with 0.5% HRP-labeled goat anti-mouse IgG (Southern Biotech). GSLs were eluted with 2 mL of chloroform/methanol/water (30:60:8), respectively (11). Monosialosyl, disialosyl, and trisialosyl fractions were dialyzed in Spectra-Por membrane (NMCO 3.5 kDa; Spectra-Por) against distilled water and lyophilized.