Supplemental data

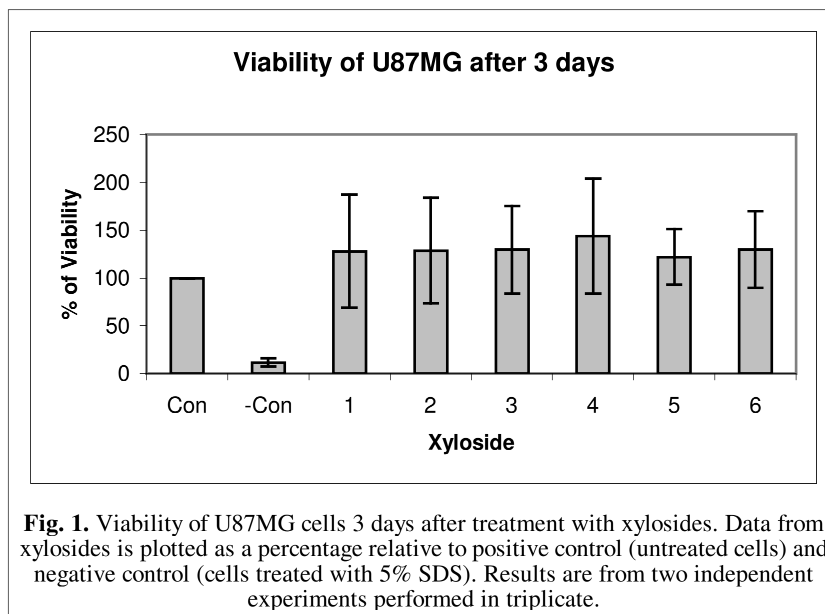


**Fig. 1.** Viability of U87MG cells 3 days after treatment with xylosides. Data from xylosides is plotted as a percentage relative to positive control (untreated cells) and negative control (cells treated with 5% SDS). Results are from two independent experiments performed in triplicate.

**Methods**

*Cell Culture:* U87MG human glioma cells (generously provided by Dr. Randy Jensen, University of Utah) were cultured in DMEM (Invitrogen) with 10% FBS, penicillin/streptomycin in a humidified incubator at 37 °C and 5 % $O_2$.

*Matrigel Invasion*: U87MG human glioma cells were plated at $1x10^5$ cells/well in the top well of a 6 well matrigel invasion chamber (BD Biosciences, San Jose, CA, USA). HAM S/ F-12 media with penicillin/streptomycin was added to the top well and HAMS/F-12 (Invitrogen) media with 10% FBS and penicillin/streptomycin (P/S) was added to the bottom well. Upon trypsinization (Tryp LE Express, Invitrogen Inc.), xyloside solutions (Table 1) were added to indicated final concentrations to both the top and bottom invasion wells. Invasion chambers were maintained in a humidified incubator at 37 $^o$C for 3 days. After this time period, supernatant from the top well was aspirated and cells with matrigel were removed using a cotton swab and the well was washed twice with PBS. Subsequently the PET membrades were cut from the well using a razor and placed in trypsin to remove cells that had invaded through and deposited on the bottom side of the PET membrane. Invaded cells were then counted using a hemocytometer.

*Priming activity:* 500 µl of LDEV-free Matrigel (BD Biosciences) was thawed overnight, added to 6 well plates, and then incubated at 37 °C for 1 hr. U87MG cells were then plated at $1x10^5$ cells per well in HamS/F-12 media supplemented with 10% dialyzed FBS and pennicilin/streptomycin. Xylosides and Sulfur-35 (Perkin Elmer) were added to wells to final concentration s of 100 µM and 10 µCi/ml, respectively. After 3 days, supernatants from the wells were collected. Subsequently, cell recovery solution was added at 2 ml/well and plates were stored at 4 °C for 1 hr. The solubilized matrigel was then centrifuged at 1000 x g for 10 min to remove cell debris. Both the supernatants (before and after cell recovery) were then combined and diluted 1:2 with 0.016% Triton X-100. The diluted supernatant mix was then loaded onto a DEAE Sepharose column (0.5 ml) pre-equilibriated with 10 column volumes of wash buffer (20 mM NaOAc buffer (pH 6.0), 0.1 M NaCl, 0.01% Triton X-100). The column was then washed with 30 column volumes of wash buffer. Bound HS/CS chains were then eluted with 6 column volumes of elution buffer (20 mM NaOAc,1 M NaCl, pH 6.0). Priming activity was determined as the counts per minute detectable in 50 µl of eluant.

*Cell Viability:* U87MG cells were tested for viability in the presence of xylosides using Cell Titer Blue reagent (Promega). Cells were seeded into triplicate wells of a 96 well plate at 2.5 x $10^4$ cells/well in 125 µl of Ham's F-12 media +10% FBS +P/S. Xylosides were added to final concentrations of 100 µM per well and cells were incubated in a humidified incubator for 3 days. After the incubation period, negative controls were treated with 50 µl of 3% SDS solution for 10 minutes. Subsequently 25 µl of Cell Titer-Blue reagent was added as per manufacturer's protocol. Cells were incubated in incubator for 2 hrs and fluorescence substrate generation was then stopped by addition of 50 µl of 3% SDS to all wells. The fluorescence was measured using a Spectra-Max M5 microplate reader (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 560 nm and an emission wavelength of 590 nm


This journal is © The Royal Society of Chemistry [year]