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Synthetic glycolipids for glioma growth inhibition developed from neurostatin and NF115 compound

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ABSTRACT

Neurostatin, a natural glycosphingolipid, and NF115, a synthetic glycolipid, are inhibitors of glioma growth. While neurostatin shows high inhibitory activity on gliomas its abundance is low in mammalian brain. On the contrary NF115 exhibits less inhibitory activity on gliomas, but could be prepared by chemical synthesis. In this study we describe synthetic compounds, structurally related to NF115, capable of inhibiting glioma growth at low micromolar range. We used DNA microarray technology to compare the genes inhibited in U373-MG human glioma cells after treatment with the natural or synthetic inhibitor. New synthetic compounds were developed to interact with the product of Rho GDP dissociation inhibitor alpha gene, which was repressed in both treatments. Compounds that were inhibitors of glioma cell growth in assays for [3H]-thymidine incorporation were then injected in C6 tumor bearing rats and the tumor size in each animal group were measured. The GC-17, GC-4 and IG-5 are new compounds derived from NF115 and showed high antiproliferative activity on tumor cell lines. The GC-17 compound inhibited U373-MG glioblastoma cells (3.2 μM), the effects was fifty times more potent than NF115, and caused a significant reduction of tumor volume ($P < 0.05$) when tested in Wistar rats allotransplanted with C6 glioma cells.

Glycolipids are sugar-containing lipid molecules with asymmetric membrane distribution, found in the noncytosolic monolayer of the lipid bilayer. Gangliosides are sialic acid-containing glycosphingolipids (GSLs), present in the outer leaflet of the cell membranes. Specific types of gangliosides, detectable in normal cells, show higher expression in tumors.[1] Tumor progression and metabolism are associated to changes in ganglioside composition and various functional roles have been ascribed to these glycolipids.[2] Some gangliosides have been shown to be adhesion molecules, involved in tumor cell metastasis, as well as modulators of the transduction of the signals that control tumor cell growth and motility.[1] As a result, attempts have been made to use synthetic as well as natural gangliosides for cancer therapy. On the other hand, tumor-associated ganglioside antigens have been used in the development of antitumor vaccines and in the diagnosis of cancer.[3]

Neurostatin, an O-acetylated GD1b present in mammalian brain,[4,5] is a natural inhibitor of astroblast and astrocytoma division. Neurostatin is cytostatic for rat astroblasts, C6 glioma cells and various human astrocytoma lines grades III and IV, but does not affect the division of either primary or transformed fibroblasts.[6,7] NF115, a synthetic octyl *N*-acetylglucosaminide derivative with a pentaerythritol chain at position 6,[4] was cytotoxic for cultured human astroblastoma, obtained after surgical biopsy, and destroyed human neuroectodermic tumors implanted in rats and human astrocytoma implanted in immunodeficient mice.[8] Neurostatin was sensitive to deacetylation, producing inactive or even mitogenic gangliosides.[4,9] New synthetic inhibitory compounds in the low micromolar range, with higher chemical and biological stability, have been described recently by our group.[10,11]

To find whether neurostatin and NF115 affected common biological pathways, we treated the U373-MG human glioblastoma line with both inhibitors and used DNA microarray screening to examine those genes whose expression was inhibited by both treatments. Derivatives of NF115 were designed and synthesized to interact with Rho GDP dissociation inhibitor alpha protein (RhoGDIα), because the respective ARHGDIA gene was inhibited

Abbreviation: GSL, glycosphingolipid.

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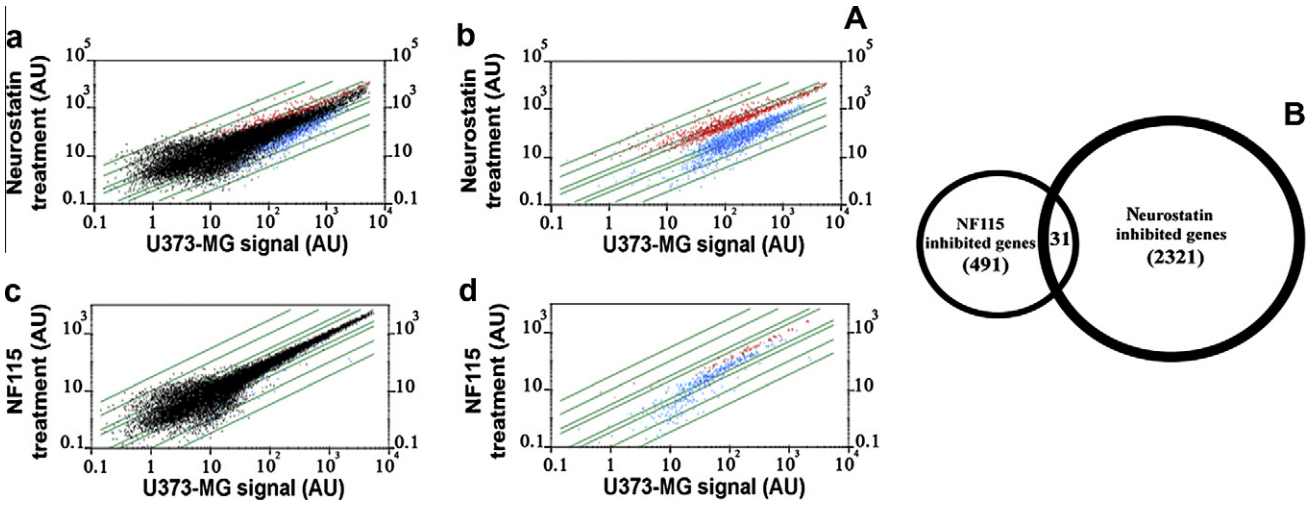

Figure 1. Gene expression in human U373-MG glioma cells treated with neurostatin or NF115. In A, genes unaffected (black dots) by both treatments (a and c) were subtracted. The over-expressed (red dots) and repressed genes (blue dots) are shown (b and d). The number of genes over-expressed (1054) and repressed (2321) was greater in neurostatin treated cells (b) in comparison to NF115 treatment with 76 up-regulated and 491 repressed genes (d). An important gene repression, and low gene over-expression, was observed in NF115 treatment (d). In B, gen repression caused by NF115 was compared to gen inhibition profile for neurostin treatment. The 31 area corresponds to the common sequences repressed by synthetic and natural inhibitors. AU, arbitrary units. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

by natural and synthetic inhibitors. The new compounds were tested for inhibition of glioma cell proliferation assays and reduction of glioma tumor growth in Wistar rats allotransplanted with C6 glioma cells. The relationship between the final structure of glycolipids and the increase on tumor growth inhibition is discussed.

The antiproliferative effect of neurostatin and NF115 were assessed in vitro in a cell line of human astrocytoma, U373-MG. Inhibition of proliferation was measured with [H3]-thymidine incorporation assays. The concentration that inhibited 50% incorporation was used for treatments. Human U373-MG cells were treated using the different inhibitor preparations and total gene expression was analyzed by U133A DNA chips from Affymetrix. When genes not affected by treatments were discarded, we observed that 2321 gene sequences were repressed and 1054 sequences were over-expressed after neurostatin treatment. By contrast, 76 genes were induced in NF115 treated cells, while 491 genes were repressed by this synthetic glycolipid (Fig. 1A).

Genes repressed by treatment with NF115 were compared to those repressed by neurostatin. From a total of 2812 inhibited transcripts only 31 specific mRNA sequences were repressed by both treatments (Fig. 1B). The genes repressed two fold or more with respect to control were selected. These genes were ordered by the repression intensity and finally five gene sequences repressed by both treatments were selected. Those five sequences represented 0.17% of total inhibited genes and the 0.022% of 22215 human transcripts analyzed (Table 1).

Neurostatin treatment of U373-MG cells caused more extensive and intensive gene repression than treatment with the compound NF115. Repression by neurostatin was greater than 3-fold, for the sequence AI571798, coding for Rho GDP dissociation inhibitor alpha gene (ARHGDIA). The ARHGDIA gene was also inhibited by NF115, but less intensely than by neurostatin (Table 1).

The natural ganglioside neurostatin is a GD1b ganglioside O-acetylated. The size and complexity of neurostatin, a ceramide joined to six sugars with various acetylations, contrasts with the relative simplicity of NF115 and related compounds (Fig. 2A). These structural differences were taken in account for design inhibitors of glioma cell growth.

New synthetic glycolipids based on NF115 structure were produced, and were named GC-17 ($C_{32}H_{62}NNaO_{12}S$), GC-4 ($C_{24}H_{47}NO_6$), and IG-5 ($C_{30}H_{59}NO_8$). The modifications included: an increase in aliphatic chain lengths from 7 to 15 methylene groups in GC-17, GC-4 and IG-5; an enlargement of the carbon chain of amide group for GC-17 and IG-5; and a substitution of hydroxyl group by a sodium sulfate in GC-17 to give it an amphiphilic character, like the natural inhibitor (Fig. 2A).

Table 1
Gene repression in human glioma cells (U373-MG) after incubation with neurostatin or NF115[a]

Gene bank	Gene symbol	Neurostatin[b]	NF115[b]	Description
AI571798	ARHGDIA	−3.1 ± 1.5	−0.3 ± 0.2	Rho GDP dissociation inhibitor (GDI) alpha
NM_003040.3	SLC4A2	−2.9 ± 1.9	−0.7 ± 0.3	Solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)
U28936.1	YWHAE	−2.5 ± 0.4	−0.5 ± 0.1	Human epsilon 14-3-3 protein
K03199.1	TP53	−2.3 ± 1.3	−0.5 ± 0.2	Tumor protein p53
U72398.1	BCL2L1	82.2 ± 0.9	−0.3 ± 0.2	Human Bcl-x beta

[a] Gene expression values relative to control U373-MG cells, not exposed to neurostatin or NF115 inhibitors.
[b] Fold change in gene expression ± SEM.

Growth inhibition assays were performed to compare the activity of NF115 and the new compounds using the same human astrocytoma cell line, U373-MG. The ID50 values obtained were in the low micromolar range in GC-17, GC-4 and IG-5 (Fig. 2B), quite lower if compared to those used for NF115 (180 μM).

C6 glioma cells were transplanted in rats, which developed a palpable tumor one week post-transplantation. To examine whether glioma growth in vivo was affected by the synthetic compounds, these were injected by intra-tumor way. Tumor volume increased exponentially in all cases (Fig. 3a, b and c). Compound GC-17, however, reduced tumor growth from the beginning of the exponential tumor growth phase (Fig. 3a). During all the study