The occurrence of polysialogangliosides in a human trophoblast cell line

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Received 25 October 1982; revision received 22 December 1982

Abstract not supplied

Polysialoganglioside *Human* *Choriocarcinoma* *BeWo* *Cell* *Chromatography*

1. INTRODUCTION

Highly sialylated gangliosides containing 4 or more sialic acid residues per lipid backbone are primarily found in brains of teleost fishes and amphibians [1–4] where they contribute 40–50% of total ganglioside-bound sialic acid. Tetrasialogangliosides are present in mammalian brain [2,5–7], retina [5], and several established cell lines [8,9], but are relatively minor components. Comparisons of adult brain ganglioside composition in lower and higher vertebrates have led to the conclusion that polysialoganglioside content decreases relative to other gangliosides during vertebrate evolution [2,3]. The discovery of structural differences between tetrasialogangliosides of fish and mammalian brains [10,11] led to the proposal of a novel biosynthetic pathway for polysialogangliosides in fish brain [12], and provided a plausible biosynthetic rationale for their abundance in brains of fishes and perhaps other lower vertebrates.

In 1980, Rosner presented evidence for the stage-specific accumulation of polysialogangliosides in developing optic lobes and cerebrum of the chick, and suggested that these gangliosides might play a role in cellular differentiation and morphogenesis of the brain [13]. Recent studies [14] suggest that chick brain polysialogangliosides are probably synthesized by the 'b' pathway [11,15,16] which is ubiquitously found in neural and extraneural tissues of higher vertebrates.

Here, we report the occurrence of a high proportion of polysialogangliosides in BeWo, a human cell line of trophoblastic origin. Although BeWo was established from a gestational trophoblastic tumor [17], its morphological and biochemical properties closely resemble those of normal early placental trophoblast, making it a useful in vitro model for the study of selected aspects of trophoblastic behavior [18].

This is the first report of polysialogangliosides present in high proportions in a mammalian cell type. Trophoblast is the earliest cell lineage to emerge during mammalian development and it is possible that polysialogangliosides may play a role in implantation of the blastocyst and/or subsequent events leading to the formation of the fetal placenta.

2. MATERIALS AND METHODS

2.1. *Cell culture*

BeWo choriocarcinoma cells were cultured at 37°C in RPMI 1640 (Gibco) + 10% fetal calf se-

* A portion of this work was presented at the 12th International Congress of Biochemistry, Perth [28]

+ Gangliosides are named according to Svennerholm [29]

Published by Elsevier Biomedical Press
00145793/83/0000–0000/$3.00

rum (Flow labs) in a 95% air–5% CO_2 atmosphere. Near-confluent monolayers were dissociated with 0.25% trypsin–1 mM EDTA in NKT buffer (138 mM NaCl, 5.3 mM KCl, 1 mM Tris, pH 7.2) and plated for experiments at 8×10^5 cells/75 cm^2 tissue culture flask. Medium was changed daily to avoid nutrient depletion artifacts.

2.2. *Ganglioside labeling*

^{14}C-Labeled gangliosides were prepared by adding D-[1-^{14}C]galactose (Amersham, 56.6 mCi/mmol, 0.5–1.0 μCi/ml) to cultures in complete growth medium for periods of 24–96 h. Steady state labeling (cpm incorporated/mg cell protein) was achieved for most of the gangliosides by 48–72 h.

2.3. *Extraction and fractionation of gangliosides*

Cell monolayers were washed 3 times with NKT, scraped with a rubber policeman, and pelleted in a low speed centrifuge. When necessary, pellets were frozen at −80°C prior to extraction of total cell lipids with $CHCl_3$/MeOH (20 vol. $CHCl_3$/MeOH, 2:1 (v/v); 10 vol. $CHCl_3$/MeOH, 1:2 (v/v) + 5% H_2O; 20 vol. $CHCl_3$/MeOH, 2:1 (v/v) [19]. Extracted pellets were solubilized in alkali and assayed for protein content as in [20] using BSA as a standard. Ganglioside fractions were obtained by fractionating lipid extracts on silicic acid columns as in [21]. These were lyophilized and redissolved in $CHCl_3$/MeOH for chromatographic separation on silica gel hptlc plates (Merck) in the following solvent systems:

(i) I. $CHCl_3$; II. $CHCl_3$/MeOH/H_2O (70:30:4 by vol.); III. $CHCl_3$/MeOH/0.25% KCl (60:35:8 by vol.) [22];
(ii) I. $CHCl_3$/MeOH/aqueous 0.02% $CaCl_2$ (55:45:10 by vol.); II. $CHCl_3$/MeOH/2.5 N NH_4OH (60:35:8 by vol.) (modified from ref. [7]);
(iii) I. $CHCl_3$/MeOH/12 mM $MgCl_2$/15 N NH_4OH (60:35:7.5:3 by vol., at 37°C); II. $CHCl_3$/MeOH/12 mM $MgCl_2$ (58:40:9 by vol., at 37°C) [23].

All chromatographic solvents were of analytical reagent grade and were redistilled before use. Ganglioside standards were purchased from Sigma (bovine brain gangliosides, type II) and Supelco (GT1b + GD1b, GD1a, GM1). GM2 was provided by courtesy of Dr Robert Yu.

2.4. *Detection and quantitation of gangliosides*

Radioactive gangliosides fractionated on thin layers were detected by autoradiography, using Kodak Blue Brand film. The radioactive bands were scraped into scintillation vials, hydrated with 1 ml H_2O, and counted in 10 ml of Triton-toluene PPO-POPOP liquid scintillation mix (1:4 v/v).

Unlabeled gangliosides were stained with resorcinol reagent [24] as in [7], and scanned on a Beckman DU-8 spectrophotometer (absorbance mode) at 580 and 450 nm. After correcting for 450 nm absorbance of interfering sugars [25] the relative proportions of sialic acid in each peak were calculated from the integrated scans.

2.5. *Digestion of cells with neuraminidase*

Cells labeled for 3 days with [^{14}C]galactose (0.5 μCi/ml) were dissociated with trypsin-EDTA, replated at a subconfluent density in 60 mm tissue culture dishes in growth medium minus isotope, and allowed to attach for 2.5 h. Medium was removed and the monolayers were washed 3-times with NKT buffer adjusted to pH 5, then incubated for 1 h at 37°C in 2 ml NKT, 10 mg/ml BSA, 1 mM $CaCl_2$, pH 5 (control) or the same solution containing 5 units of *Clostridium perfringens* neuraminidase (Sigma Type VI). Cells remained attached to the dishes during this treatment and cell lysis was not detected. Cells were then harvested for extraction and fractionation of gangliosides in solvent system C as previously described.

3. RESULTS

BeWo cultures incorporated [^{14}C]galactose into a broad spectrum of gangliosides both at early and later times after plating (fig.1). Of particular interest is a cluster of gangliosides whose mobility is greatly retarded on silica gel 60 thin layers chromatographed in commonly used solvent systems. In solvent system A, shown here, these gangliosides separate into two peaks of radioactivity which travel between the origin and the trisialoganglioside GT1b. 25% of total ganglioside radioactivity was present in this fraction at the 27–70-h labeling period. When cultures were labeled continuously after plating and analyzed at 48, 72 and 96 h for [^{14}C]galactose incorporation into each of the ganglioside fractions, the low mobility gangliosides incorporated label at a faster rate and to a greater ex-

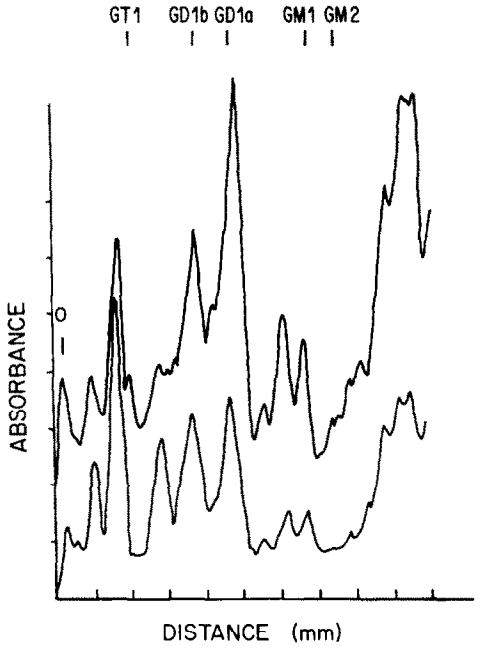

Fig.1. Densitometric scan of autoradiogram of [^{14}C]galactose-labeled BeWo gangliosides. Cultures were labeled with D-[1-^{14}C]galactose from 27–70 (lower trace) or 70–117 (upper trace) hours after plating. Gangliosides were extracted as described in section 2 and chromatographed on silica gel 60 thin layer plates in solvent system A. Autoradiograms were scanned at zero wavelength in the absorbance mode. Peak areas are not linearly proportional to radioactivity on the scan shown here. Peaks were identified by comparison with co-chromatographed standard gangliosides stained with resorcinol.

tent than all of the other species. By using solvent system C, which is capable of resolving highly sialylated gangliosides from fish and chick brain [23], it was possible to separate the low mobility fraction into 7 discrete peaks of radioactivity (fig.2A). Most of the label was removed from the polysialoganglioside region of the chromatogram following treatment of intact cells with *Cl. perfringens* neuraminidase suggesting that these gangliosides are primarily cell surface-localized (fig.2B).

Although BeWo cells synthesize multiple species of less complex gangliosides from radioactive precursors (galactose, D-glucosamine), 50–75% of ganglioside-bound sialic acid is contained in the polysialoganglioside fraction, as determined by quantitative scanning densitometry of resorcinol-

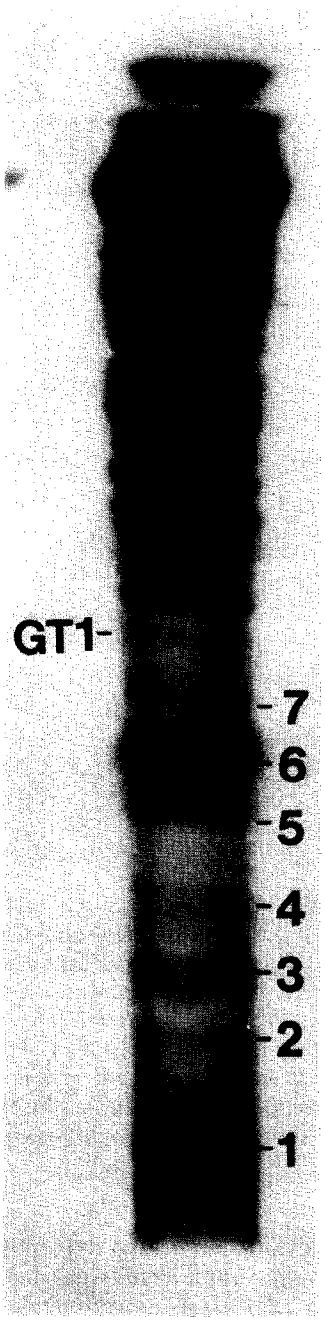

Fig.2. (A) Autoradiogram of biosynthetically-labeled BeWo polysialogangliosides. Gangliosides were extracted from cultures which were grown to confluency in [^{14}C]galactose. Following chromatography on silica gel 60 hptlc plates in solvent system C, the thin layer was autoradiographed on Kodak Blue Brand X-ray film at −80°C. Numbered bands correspond to those referred to in the text.

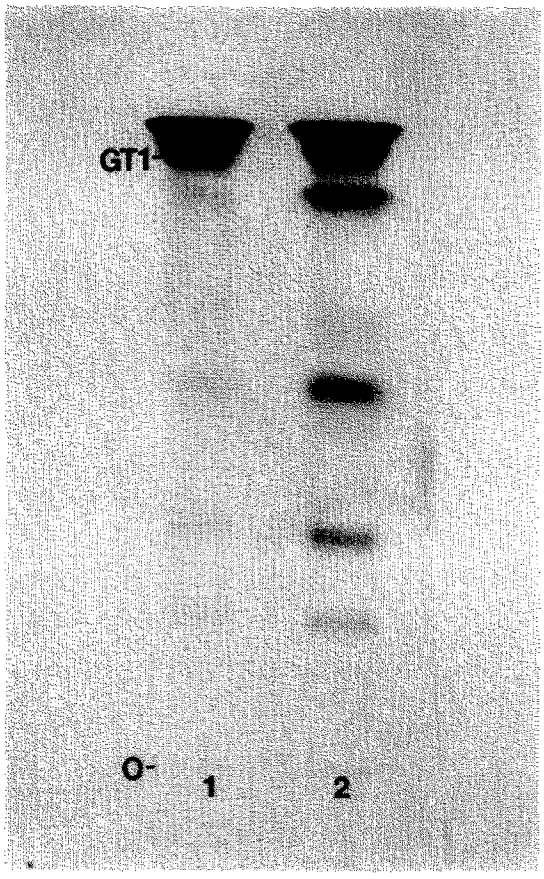

Fig.2. (B) Autoradiogram of biosynthetically-labeled BeWo polysialogangliosides before and after cell treatment with neuraminidase. Subconfluent cultures labeled for 3 days with [^{14}C]galactose were dissociated with trypsin-EDTA, replated in growth medium without radioactive isotope, and incubated for 1 h at 37°C in the presence (lane 1) or absence (lane 2) or neuraminidase as described in section 2. Gangliosides were extracted and chromatographed in solvent system C.

stained gangliosides separated in solvent system B. It is unlikely that these results can be ascribed to the presence of sialoglycoproteins or free sialic acid since similar results were obtained with exhaustively trypsinized ganglioside extracts and with gangliosides prepared by chromatography on DEAE Sephadex, Sephadex LH20, and silicic acid columns [26].

In solvent system C, the polysialoganglioside fraction separates into 4 peaks which are detected by resorcinol staining (fig.3) and correspond in R_f

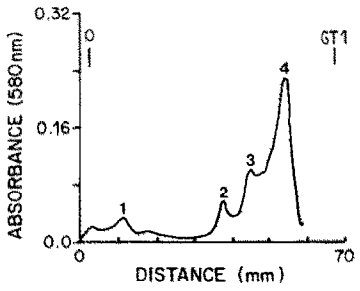

Fig.3. Densitometric scan of resorcinol-stained BeWo polysialogangliosides. About 7 μg ganglioside-NANA was spotted on silica gel hptlc plates, chromatographed in solvent system C, and resorcinol-stained. The polysialoganglioside region of the scan is shown here. Peaks are numbered to correspond with those in the text.

to peaks 1, 4, 5, and 6 of the biosynthetically labeled polysialogangliosides shown in fig.2A. A comparison of the migration rates of BeWo polysialogangliosides with those of chick embryo optic lobes with known sialic acid/sphingosine molar ratios [27] suggests that peaks 4 and 3 are tetrasialogangliosides, peak 2 is a pentasialoganglioside and peak 1 may contain more than 7 sialic acid residues/molecule.

4. DISCUSSION

BeWo, a human trophoblast cell line, is the first mammalian cell line found to contain a high proportion of polysialogangliosides. In this respect it is atypical of other established cell lines of either normal or tumorigenic origin. Thus far the two examples of polysialoganglioside abundance in higher vertebrates – embryonic chick brain and BeWo – are developing tissues. Adult mammalian tissues and avian brain have not been found to contain significant amounts of polysialogangliosides. Recent studies from our laboratory have found that polysialoganglioside abundance in 6 mammalian cell lines correlates with the ability of cells to form multicellular clusters at subconfluent densities [9]. In chick embryo optic lobes, polysialogangliosides are preferentially accumulated during a specific developmental period when neuronal migration and differentiation take place and new contacts are made between optic nerve axons [13]. We suggest that polysialogangliosides may

mediate specific types of cell contact interactions during avian and mammalian development.

The availability of mammalian cell lines such as BeWo that contain a high proportion of polysialogangliosides, and lines which totally lack these gangliosides, should prove useful for further studies on polysialoganglioside synthesis and function in mammalian tissues.

ACKNOWLEDGEMENTS

These studies were supported by grants from the Medical Research Council of Canada, the Alberta Heritage Foundation for Medical Research, and the Alberta Heritage Savings Trust Fund: Applied Research-Cancer.

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