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GANGLIOSIDE COMPOSITION OF MALIGNANT AND ACTINOMYCIN D-RESISTANT NONMALIGNANT CHINESE HAMSTER CELLS

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Abstract—The ganglioside composition of three Chinese hamster cell lines was investigated. The parental spontaneously transformed DC-3F cell line has a full complement of gangliosides from hematosides to disialogangliosides. An actinomycin D-resistant subline, DC-3F/AD X, which lost its tumorigenicity and regained a normal morphology and growth pattern in cell culture, exhibits only hematosides. A third cell line, derived from the latter and grown in the absence of actinomycin D for 3 years, regained tumorigenicity and displays a ganglioside profile similar to that of the malignant parental cell line. In addition, tumors formed from DC-3F cells grown in athymic nude mice have a ganglioside profile similar to that exhibited by the malignant cells in culture. The results suggest that the ganglioside composition of these spontaneously transformed, tumorigenic Chinese hamster cells differs from that of the majority of transformed cell lines reported in the literature in that our malignant cells are not blocked in higher ganglioside biosynthesis. We also find that the experimentally derived, nontumorigenic cells with a high degree of acquired resistance to actinomycin D are blocked at the level of hematosides.

Alteration of ganglioside [1] patterns as a concomitant of malignant transformation has been well documented [2–4]. Ganglioside alteration is exemplified by a marked reduction in the higher, more complex glycosphingolipids [5]. In one laboratory alone, 24 out of 26 cell lines tested displayed this simplified ganglioside pattern [2]. Another reliable indicator of malignant transformation is a reduction in the level of hematosides irrespective of the level of higher gangliosides, as observed by several investigators [6–10]. In addition to the *in vitro* studies, Siddiqui and Hakomori [11] observed an absence of trisialogangliosides and a high level of disialogangliosides with a concomitant increase in precursor glycolipids in Morris hepatomas 5123 and 7800. Dnistrian *et al.* [12] observed that Morris hepatoma cells have no trisialogangliosides, while hematosides, monosialogangliosides, and disialogangliosides were increased up to 9-fold when compared to normal liver. Skipski *et al.* [13] found that serum composition reflected the differences shown in the tumor cells *in situ*. We have examined the ganglioside content of a series of Chinese hamster cell lines in our laboratory: the spontaneously transformed, tumorigenic DC-3F line, the phenotypically normal (nontumorigenic) DC-3F/AD X derivative with acquired resistance to actinomycin D, and a revertant derived from the latter cell line [14]. In this paper we report the finding that the malignant and revertant cell lines exhibit a complete set of gangliosides from G_{M3} to G_{D1a}, whereas the drug resistant, nontumorigenic DC-3F/AD X cells are blocked at the level of hematosides.

MATERIALS AND METHODS

Cells and methods of culture. Details of the origins of the Chinese hamster cell lines and their resistant sublines were given earlier [14, 15]. Substrate-attached cultures were maintained in Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml). Resistant sublines were grown in drug-free medium for 15 days prior to experimental analysis. Exponentially growing cells were used throughout this study. Cells were grown in 75 cm^2 flasks (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, CA), in a standardized atmosphere of CO_2 and air. Routinely, cultures were grown in the presence of [U-^{14}C]glucosamine (238 mCi/m-mole), 20 μCi/flask for 72 hr. After the labeled medium was aspirated, the cultures were washed with Dulbecco's phosphate-buffered saline (PBS) without calcium and magnesium, detached from the polystyrene flasks with 0.5 mM potassium ethylenediamine tetra-acetic acid (EDTA), and collected by centrifugation.

Gangliosides were extracted essentially by the method of Suzuki [16] with the following modifications. After partitioning with water, the Folch [17] upper phase was adjusted to 1.0 mg/ml of cholesterol [18] to minimize loss of gangliosides during dialysis. After dialysis against ice-cold distilled water for 2 days, the cholesterol was extracted from the gangliosides three times with 3 ml portions of hexane and taken to dryness *in vacuo*. The gangliosides were separated by thin-layer chromatography on silica gel G with chloroform–methanol–0.25% $CaCl_2$ (60:35:8, v/v) [19]. Thin-layer chromatograms were scanned on a Packard Radioscan (Packard Instruments Co., Inc., Downers Grove, IL). Radioautography was performed on Kodak No Screen X-Ray film. The individual gangliosides were identified and quantitated from the area under the peaks of the radioscans. Identification of the gangliosides was made with reference standards (Supelco, Inc., Bellefonte, PA) by detection with resorcinol reagent [20].

RESULTS AND DISCUSSION

Specific cell surface changes associated with malignant transformation, in comparisons of transformed cells and their "normal" progenitors, are widely documented phenomena. One of the salient events of this transition is ganglioside simplification. In the course of our studies dealing with experimentally induced loss of oncogenic potential and associated biochemical alterations [14, 15], we undertook an investigation of the ganglioside composition of the spontaneously transformed tumorigenic DC-3F cells and the experimentally derived drug resistant subline, DC-3F/AD X (2450-fold increase in resistance to actinomycin D), that lost its tumor-forming capacity.

Results of analysis of gangliosides separated by thin-layer chromatography are shown in Fig. 1. Malignant DC-3F cells display a full complement of gangliosides from G_{M3} to G_{D1a}, whereas phenotypically normal DC-3F/AD X cells have only ganglioside G_{M3}. This is surprising in view of the observation that spontaneously transformed cell lines are frequently blocked in the biosynthesis of higher gangliosides [2, 3]. The transformed DC-3F cells appear to have a ganglioside pattern similar to that reported by others for normal mouse cells grown in culture [2, 5] and show a reduction in hematosides [7, 8]. These results are compatible with the observations reported by Hakomori *et al.* [8] indicating that there may also be a change in the organizational architecture of transformed cells. They found that although the total amount of hematosides in their virally transformed cells was substantially lower, the reactivity to antihematoside serum was much higher for the transformed cells than for normal cells. In an experimental system analogous to ours, Nigam *et al.* [21] observed higher levels of G_{M2} in phenotypically normal actinomycin D-resistant hamster cells as compared to drug-sensitive, SV40-transformed parental cells and revertant cells grown in the absence of actinomycin D. These authors attribute this increment to higher levels of G_{M3}:UDP-*N*-acetylgalactosaminyltransferase activity in the antibiotic resistant cells. Although we have not as yet measured this enzyme activity, clearly our actinomycin D-resistant DC-3F/AD X cells do not synthesize any ganglioside higher than G_{M3} (Figs. 1a and b).

The cell line derived from the drug resistant DC-3F/AD X subline and grown in the absence of actinomycin D for approximately 3 years was also examined. The revertant line, designated DC-3F/AD X-U, is characterized by a level of drug resistance (50-fold) and tumorigenic capacity close to that of control DC-3F cells [14]. Also, revertant cells exhibit a ganglioside composition that is similar to that of the malignant control cells; the full complement of gangliosides is expressed (Fig. 2, Table 1). To investigate whether the ganglioside patterns displayed by transformed DC-3F cells might be related primarily to long-term growth in a cell culture environment, we examined tumors produced by DC-3F in athymic nude mice. Gangliosides were extracted from tumor cells labeled *in vitro* with radioactive glucosamine. The ganglioside composition of these cells is similar to that of parental and revertant lines maintained *in vitro* (Fig. 2b, Table 1). No trisialogangliosides were detectable in any of our Chinese hamster cells.

Several exceptions to the general finding that malignant transformation is accompanied by a block in synthesis of higher gangliosides have been reported. For example, Yogeeswaren *et al.* [22] described two clonal isolates of SV40-transformed 3T3 cells that exhibited a complex ganglioside pattern qualitatively similar to that of "normal" control cells but with an increase in the amount of G_{D1a}. However, only one of these lines was tumorigenic. Another SV40-transformed line and a polyoma-transformed isolate showed a marked simplification in ganglioside composition. Mora [23] found that two spontaneously transformed, tumorigenic cell lines, T AL/N and BALB 3T12, appeared to have the ganglioside pattern of normal cells.

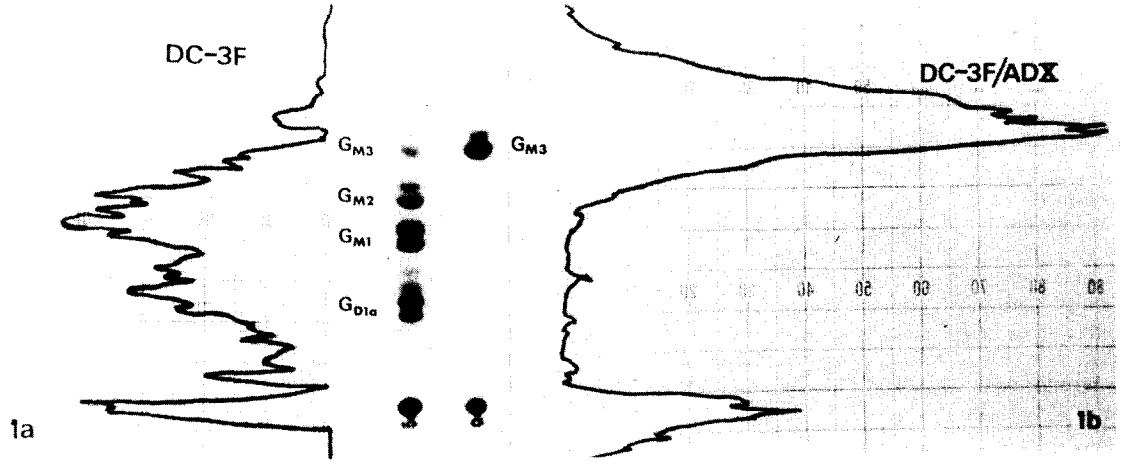

Fig. 1. Scan of radioactivity and radioautogram of gangliosides from thin-layer chromatograms. The spot just above the origin did not stain with resorcinol and is presumably a glycopeptide. Panel a: DC-3F; panel b:DC-3F/AD X. The approximate cpm applied to the chromatograms were: DC-3F: 27,000; and DC-3F/AD X: 30,000.

Table 1. Ganglioside composition of Chinese hamster cells*

Cell	G_T	G_{D1a}	G_{M1}	G_{M2}	G_{M3}
DC-3F	0	29	39	23	8+
DC-3F/AD X	0	0	0	0	100+
DC-3F/AD X-U	0	25	30	30	15
DC-3F tumor	0	26	30	21	23

* Percentage of total radioactivity of individual species separated by thin-layer chromatography, calculated as described in Materials and Methods.

+ Mean values of three independent experiments.

However, prolonged cultivation *in vitro* for longer than 2 years led to loss of the G_{D1a} component. No evidence of incidental viral infection or viral transformation was observed. Another biological phenomenon which may alter the organizational state of membrane glycolipid is density-dependent inhibition of growth as shown by Hakomori [24] and Sakiyama *et al.* [25]. These authors found that certain neutral glycolipids increase at high cell densities in normal hamster cells. As noted previously [15], our spontaneously transformed and highly tumorigenic DC-3F line was derived by several clonings from the Dede cell line. This line was established in culture in 1962 by T. C. Hsu from normal adult Chinese hamster lung tissue and has been grown in culture over a total period of 4–5 years at least.

In addition to the marked differences in ganglioside composition described in this report, the actinomycin D-sensitive and -resistant Chinese hamster cells show profound differences in membrane-associated biological properties. We demonstrated earlier that the primary determinant of drug resistance of the DC-3F/AD X cells is reduced permeability to actinomycin D [26]. These highly resistant cells were shown to be nontumorigenic in a heterotransplant system and to have acquired morphological and growth characteristics of normal cells in culture [14]. In contrast, drug-sensitive DC-3F cells are tumorigenic and have morphological characteristics of malignant cells. We recently reported [27] that the phenotypically normal DC-3F/AD X cells synthesize a 150,000 dalton plasma membrane glycoprotein and apparently do not express the prominent 93,000 dalton glycopeptide of DC-3F cells. We are currently focusing our attention on these differences in ganglioside and glycoprotein composition with respect to mechanisms of actinomycin D resistance and loss of oncogenic potential.

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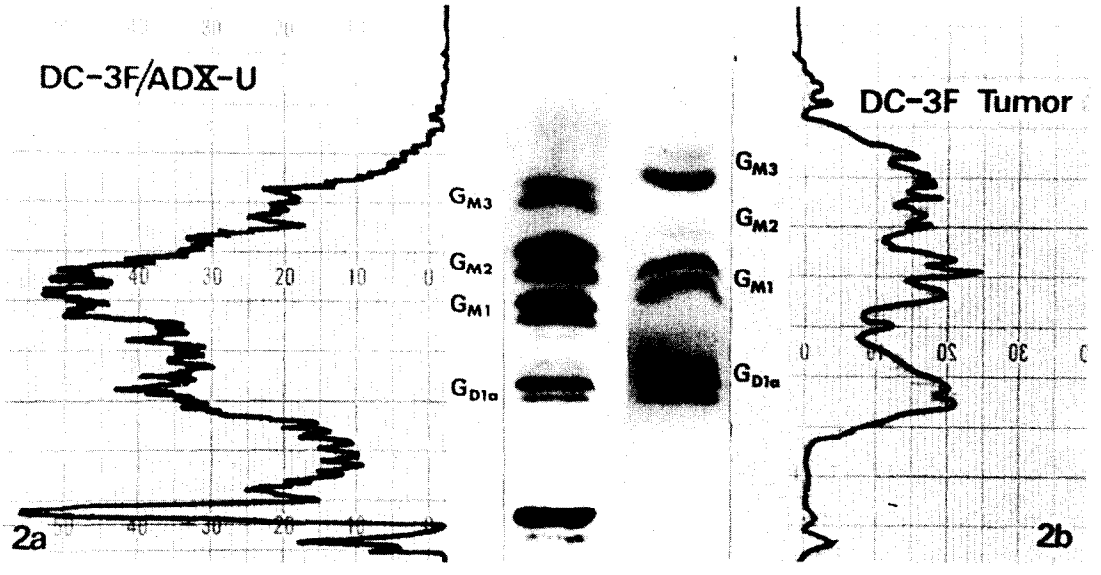

Fig. 2. Scan of radioactivity and radioautogram of gangliosides from thin-layer chromatograms. The spot just above the origin did not stain with resorcinol and is presumably a glycopeptide. Panel a: DC-3F/AD X-U; panel b: DC-3F tumor. The approximate cpm applied to the chromatograms were: DC-3F/AD X-U: 50,000; and DC-3F tumor: 52,000.

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