Loss of Cell-Population-Density-Dependent Incorporation of Fucose into the Lipid Fraction of Cultured Human Tumour Cells

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The incorporation of radioactively labelled fucose into the lipid fraction of cultured normal human cells and several human tumour-cell lines was examined as a function of the cell population density. Normal cells exhibited a density-dependent pattern of incorporation, whereas in tumour cells the radioactivity incorporated was independent of the cell population density. An exception was found among the tumour cells, which suggests a possible correlation between the loss of this marker and the ability to produce tumours.

One of the major characteristics that accompanies oncogenic transformation is the lack of cellular growth control in response to increased cell population density. Studies have suggested that the carbohydrate portion of surface glycoconjugates is involved in the regulation of cellular growth (Roseman, 1970; Hynes & Bye, 1974; Hakomori, 1975; Richardson *et al.*, 1975). It has been shown for a variety of cultured normal rat, mouse and hamster cells that the synthesis of certain gangliosides (Yogeeswaran & Hakomori, 1975) and neutral glycolipids (Hakomori, 1970; Robbins & MacPherson, 1971; Critchley & MacPherson, 1973; Sakiyama & Robbins, 1973; Sakiyama & Terasima, 1975) is cell-population-density-dependent and increases at confluency. In contrast, a common feature of spontaneously and virus-transformed cells appears to be a lack of cell-density-dependent synthesis of these components.

Previously an altered pattern of fucose incorporation into the lipid fraction of cultured human tumour cells as compared with normal cells has been reported (Steiner & Melnick, 1974). Transformed cells characteristically exhibit a decreased incorporation of radioactively labelled fucose into a lipophilic component of low chromatographic mobility designated as fucolipid FLIV, with a concomitant increase in a more mobile component. The nature of these fucosylated organic-extractable compounds has recently been established (Larriba *et al.*, 1977). In the present study the above observations have been extended to examine the incorporation of radioisotopically labelled fucose into the lipid fraction of normal and cultured human tumour cells as a function of the cell population density. The results show that in normal cells the synthesis of the fucosylated compounds is markedly dependent on the cell culture density, whereas in tumour cells

synthesis is not dependent on the cell population density.

Experimental

The cell lines used in the present study include normal human embryo lung (HEL) cells and isolates from different human tumours, namely rhabdomyosarcoma (A204), malignant melanoma (A375), lung carcinoma (A549) and cells originally derived from a human fibrosarcoma (8387). The properties of these tumour-derived cell lines have been described elsewhere (Giard *et al.*, 1973; Arnstein *et al.*, 1974). Seed cultures were provided by Dr. S. Steiner, Baylor College of Medicine, Houston, TX, U.S.A. The cells were cultured in Eagle's minimal essential medium supplemented with 10% (v/v) foetal calf serum and routinely examined for the presence of mycoplasma. In labelling experiments the above medium was supplemented with 5 μ Ci of L-[6-³H]fucose (24 Ci/mmol; Amersham/Searle, Des Plaines, IL, U.S.A.) per ml of medium. In pulse and chase experiments, the chase medium was Eagle's minimal essential medium supplemented with 20 μ M-L-[¹⁴C]fucose. Unless otherwise indicated, high- and low-population-density cultures were seeded at 1.5×10^6 and 6×10^4 cells respectively per 475 ml (16 oz) glass bottle (approximate area 72 cm²). The cells were allowed to attach overnight and pulse-labelled for 16 h with [³H]fucose-supplemented medium.

Lipid extraction was performed as previously described (Gacto & Steiner, 1976). The lipid extract was partitioned as described by Folch *et al.* (1957) and the upper phase analysed by t.l.c. on silica-gel G plates (E. Merck, Darmstadt, Germany) by sequential one-dimensional chromatography, first in chloroform/methanol/water (60:35:8, by vol.) and then in propan-2-ol/conc.NH₃/water (7:2:1, by vol.). The

plates were scraped in 1 cm bands from origin to solvent front (18 cm) and the radioactivity was measured by liquid-scintillation spectrometry. The two major fucose-labelled components, i.e. FLIII and FLIV, are quantitatively included in fractions 4–10. Analysis of radioactively labelled water-soluble precursors in the cytosol fraction of the cells was performed as previously reported (Gacto & Steiner, 1976). Determination of trichloroacetic acid-insoluble radioactivity was performed by a filter-paper-disc method as described by Mans & Novelli (1961). The amount of total cell protein was determined by the method of Lowry *et al.* (1951).

Results and Discussion

Previous studies have shown that the incorporation of radioisotopically labelled fucose into the lipid fraction of normal rat cells varies as a function of the cell population density (Gacto & Steiner, 1976). The results shown in Fig. 1(a) illustrate the incorporation of [^3H]fucose into the lipid fraction of normal human (HEL) cells as a function of the surface area on which the cells were plated. The number of cells seeded per culture and the volume of medium was kept constant while the area of cell distribution was varied. It can be observed that in normal human

cells there is an inverse relationship between the fucose radioactivity incorporated into the lipid fraction and the surface area occupied by the cells. The examination of the patterns of incorporation (Fig. 1a') revealed that there is little of components FLIV and FLIII being synthesized in sparsely distributed cells and that the increase in cell population density that results from diminishing the area of cell distribution is accompanied by an increase in the synthesis of both components. Similar experiments performed with cultured human tumour cells (A204) revealed little or no difference in the amount of incorporation or in the distribution of the label at high and low cell-population densities (Figs. 1b and 1b'). These observations are consistent with the interpretation that the synthesis of components FLIV and FLIII in normal human cells is the result of cell-to-cell contact. The concept of cell-to-cell regulation of glyco-conjugate synthesis has been suggested previously (Yogeeswaran *et al.*, 1974). On the other hand, the data on A204 cells suggest a lack of cell-density-dependent regulation in the synthesis of these components in tumour cells.

The results obtained with sparse normal cells might be alternatively interpreted to indicate a marked decrease in the specific activity of the water-soluble precursor pools (i.e., fucose, fucose 1-phosphate

Fig. 1. Incorporation of [^3H]fucose into the lipid fraction of HEL and A204 cells plated at various cell population densities. In the lower portion of the Figure each point represents ten cultures of 2×10^4 normal (a) or tumour (b) cells plated at the cell densities shown on the abscissa. To minimize cellular migration all but the most sparse cultures were seeded on glass coverslips (Corning no. 1; 1.77 cm^2), which were placed into plastic Falcon plates. After attachment, the medium was replaced with 20 ml of minimal essential medium supplemented with [^3H]fucose ($5 \mu\text{Ci/ml}$; 24 Ci/mmol) and the cells labelled under CO_2/air (7:93) at 37°C for 16 h. The upper portion of the Figure (a', b') shows the profiles of the distribution of radioactivity into components FLIII and FLIV. Each profile represents the corresponding point on the graph below from left to right. Cell saturation densities of HEL and A204 cells are about 8×10^4 cells/ cm^2 and 2×10^5 cells/ cm^2 respectively.

Table 1. Incorporation of [^3H]fucose into trichloroacetic acid-insoluble material and lipid fraction of normal and tumour human cells at high and low cell population density

Cells were labelled for 16h in radioactive medium at high [1.5×10^6 cells/475 ml (16 oz) culture bottle] and low (6×10^4 cells/culture bottle) cell population density. The incorporation of radioactivity was examined as described in the Experimental section. Longer-term incorporation (60h) at high and low cell population densities resulted in a net increase in the radioactivity incorporated into the protein and lipid fractions, but the relative distribution of the label into the lipid fraction at high as against low population density yielded identical observations.

Type of cell	Population density	$10^{-3} \times$ Radioactivity (d.p.m./mg of protein)		
		Acid-insoluble material	Lipid fraction	
			Component FLIV	Component FLIII
HEL	High	150.7	8.1	2.1
	Low	166.6	0.3	0.1
A204	High	58.9	0.2	1.8
	Low	51.1	0.2	1.6
8387	High	64.2	16.5	2.4
	Low	74.5	1.3	0.8
A549	High	86.7	1.0	2.0
	Low	75.5	1.1	2.0
A375	High	90.2	2.0	2.4
	Low	103.7	1.9	2.5

and GDP-fucose) due to enhanced fucose synthesis *de novo* or decreased uptake of the exogenous radioactively labelled sugar. However, if the decreased incorporation into lipid at low cell density were due to this, one might predict a similarly decreased incorporation of fucose radioactivity into acid-insoluble material on the assumption that the water-soluble fucose precursors to both lipid and protein fractions are from a common pool. The analysis of the radioactivity incorporated into the trichloroacetic acid-precipitable fraction of HEL cells at low as against high cell population density (Table 1) did not reveal a significant difference as compared with the changes in the lipid fraction. As with HEL cells, there was little difference in the amount of fucose radioactivity incorporated into acid-insoluble material of A204 cells at high as against low cell population density (Table 1).

The possibility that the decreased radioactively labelled fucose incorporated into the lipid fraction of sparse HEL cells might be due to increased catabolism or shedding of these components to the medium was also considered. HEL cells were labelled with [^3H]-

fucose for 24h at high cell population density and collected by trypsinization. The pattern of incorporation in one portion of these cells was determined and the remaining portion was seeded in chase medium at low cell density and collected after 16h. After the shift to low cell population density there was little loss of label from either component FLIII or component FLIV, and the decrease (less than 10%) was not significant enough to account for the marked decrease observed in the lipid fraction of fucose-labelled sparse cells. Under these conditions radioisotopically labelled fucose, fucose 1-phosphate and GDP-fucose are not detectable in the cytosol fraction of the cells after 6h in the non-radioactive medium, indicating an effective chase of the precursor pools (Gacto & Steiner, 1976). Hence these results are consistent with the interpretation that the decreased incorporation of label in the lipid fraction of HEL cells at low cell population density is due to inhibited synthesis rather than to loss of these components.

The incorporation of [^3H]fucose into the lipid and protein fractions of other tumour-derived human cell lines was also examined as a function of the cell population density (Table 1). The A549 and A375 cell lines manifested a relatively marked decrease in the ratio of components FLIV/FLIII as compared with HEL cells, whereas in 8387 cells the relative distribution of label into the lipid fraction was comparable with that of normal cells. Moreover, in 8387 cells the incorporation of labelled fucose into lipid was also cell-population-density-dependent, whereas in A549 and A375 cells it was practically unaffected by changes in the cell population density. None of the cell lines showed significant differences in [^3H]fucose incorporation into acid-insoluble material at high as against low cell population density (Table 1). The common characteristic of the transformed human cell lines examined is that they were derived from tumours (Giard *et al.*, 1973; Arnstein *et al.*, 1974), although only the A204, A549 and A375 cell lines were reported capable of producing tumours in anti-thymocyte-serum-treated mice. The one tumour-derived cell line that was reported unable to produce tumours, i.e. 8387, manifests changes in the incorporation of [^3H]fucose into the lipid fraction in response to changes in the cell population density and exhibits a pattern of incorporation resembling that of normal cells. The usefulness of the loss of cell-population-density-dependent synthesis of these components as a marker *in vitro* for oncogenicity in human cancer cells awaits broader testing. However, it is already apparent that some cultured human tumour cells manifest a loss of density-dependent regulation of incorporation of labelled fucose into lipid that closely parallels observations made in studies with virus-transformed cell lines (Gacto & Steiner, 1976).

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References

- Arnstein, P., Taylor, D. O. N., Nelson-Rees, W. A., Huebner, R. J. & Lenette, E. H. (1974) *J. Natl. Cancer Inst.* **52**, 71–84
- Critchley, D. R. & MacPherson, I. (1973) *Biochim. Biophys. Acta* **296**, 145–159
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Gacto, M. & Steiner, S. (1976) *Biochim. Biophys. Acta* **444**, 11–22
- Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H. & Parks, W. P. (1973) *J. Natl. Cancer Inst.* **51**, 1417–1423
- Hakomori, S. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **67**, 1741–1747
- Hakomori, S. (1975) *Biochim. Biophys. Acta* **417**, 55–89
- Hynes, R. O. & Bye, J. M. (1974) *Cell* **3**, 113–120
- Larriba, G., Klinger, M., Srameck, S. & Steiner, S. (1977) *Biochem. Biophys. Res. Commun.* **77**, 79–85
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53
- Richardson, C. L., Baker, S. R., Morre, D. J. & Keenan, T. W. (1975) *Biochim. Biophys. Acta* **417**, 175–186
- Robbins, P. W. & MacPherson, I. (1971) *Nature (London)* **229**, 569–570
- Roseman, S. (1970) *Chem. Phys. Lipids* **5**, 270–297
- Sakiyama, H. & Robbins, P. W. (1973) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **32**, 86–90
- Sakiyama, H. & Terasima, T. (1975) *Cancer Res.* **35**, 1722–1726
- Steiner, S. & Melnick, J. L. (1974) *Nature (London)* **251**, 717–718
- Yogeewaran, G. & Hakomori, S. (1975) *Biochemistry* **14**, 2151–2156
- Yogeewaran, G., Laine, R. A. & Hakomori, S. (1974) *Biochem. Biophys. Res. Commun.* **59**, 591–599