Brief Definitive Report

INCREASED SIALYLATION OF SURFACE GLYCOPEPTIDES OF HUMAN TROPHOBLAST COMPARED WITH FETAL CELLS FROM THE SAME CONCEPTUS

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It is a basic thesis of current research concerning the nature of neoplasia, that alteration of cell surface components is an essential feature of transformation (1). Surface alteration can manifest itself in several ways, but perhaps the most universal modification so far described for both malignant and virally transformed cells is an alteration in the trypsin-sensitive cell surface glycopeptides (2). Because the human trophoblast shows some of the features commonly associated with neoplasia, including cellular proliferation and localized invasion (3, 4), we decided to investigate the nature of its surface glycosylation in comparison with other fetal cells derived from the same conceptus.

Materials and Methods

Human trophoblasts and fetal tissues from the same embryos were obtained from the Royal Marsden Hospital, London. These were derived from therapeutic terminations performed in the 8- to 12-wk period of gestation. Trophoblast was cultured in medium containing 20% fetal calf serum (FCS) as previously described (5). Fetal cells were treated similarly except that 10% FCS was used. 4 days before the addition of radiolabel the trophoblast medium was exchanged for one containing 10% FCS. This control was also performed in reverse; i.e., the fetal medium was replaced by one containing 20% FCS. All cells were used at the primary stage or at first subculture, and all the cultures were used during the logarithmic phase of cellular growth. To radiolabel the cells 20 μCi/ml of L-[1-^{3}H]-fucose (5.3 Ci/mmol) or 10 μCi/ml D-[6-^{3}H]-glucosamine hydrochloride (19 Ci/mmol) were added to the trophoblastic cell media, and 5 μCi/ml of L-[1-^{14}C]-fucose (61 mCi/mmol) or D-[1-^{14}C]-glucosamine hydrochloride (52 mCi/mmol) were added to the fetal cell media. A total of six experiments was performed with the radiolabels reversed. All isotopically labeled compounds were obtained from the Radiochemical Centre, Amersham, England. After 48 h of incubation with the radiolabel, the medium was decanted and its sterility was checked by using dextrose broth cultures. Trypsinates and pronase-digested trypsinates were prepared and chromatographed on Sephadex G-200 and G-50 Fine as described elsewhere (6). Cell viability after trypsinization varied from 85 to 95% as assessed by trypan blue exclusion. Neuraminidase digestion was performed using the method of Beek et al. (7). The samples were counted for radioactivity and plotted by computer as previously described (8).

Results

The results obtained were reproducible when both 10 and 20% FCS were used in the media, and consequently the lower value was routinely adopted. The results were also stable when the cells were maintained in either tritium-labeled or carbon-14-labeled medium.

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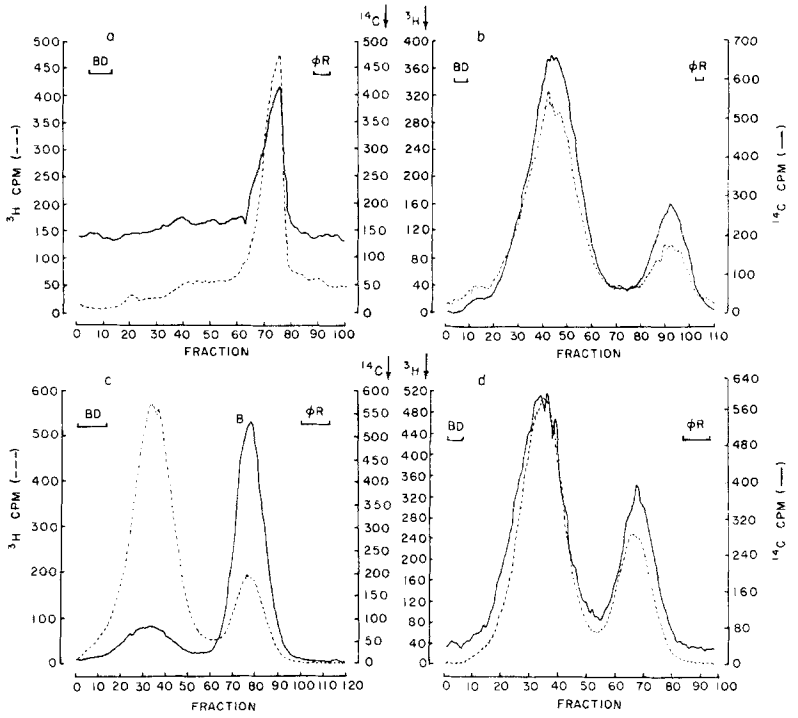

FIG. 1. (a) Trypsinates from trophoblastic and skin fibroblastic cells labeled with L-fucose and analyzed on Sephadex G-200. Trophoblast glycopeptides (^{3}H - - -) and skin glycopeptides (^{14}C —) coeluted. BD is the blue dextran marker (high molecular weight; totally excluded material) and ϕR is the phenol red marker (low molecular weight; totally included material). (b) The trypsinates shown in (a) after exhaustive digestion with pronase and chromatography on Sephadex G-50 Fine. Two peaks are resolved, but again both coelute. (c) L-fucose-labeled pronase-digested glycopeptides from trophoblastic cells (^{3}H - - -) analyzed in comparison with fetal heart cells (^{14}C —) on Sephadex G-50F. Again the two peaks comigrate, though the heart cells showed considerable enrichment in the glycopeptides which comprise the second peak (B). (d) Trophoblastic glycopeptides compared with those of fetal intestine and labeled with L-fucose as in (c).

Fig. 1 a shows fucose-labeled trypsinates from trophoblast analysed in comparison with fetal skin fibroblasts. Only one low molecular weight peak is present in each case, and it coelutes on Sephadex G-200. Pronase digestion of these trypsinates resulted in the production of two fractions after Sephadex G-50 chromatography (Fig. 1 b). Again both peaks comigrate. No faster or slower eluting fractions were observed when the pronase-digested glycopeptides were compared with fetal heart cells (Fig. 1 c) or fetal intestine cells (Fig. 1 d) from the same embryos. It would appear, therefore, that there are no qualitative differences resolvable by gel filtration on Sephadex in the fucose-containing surface-derived glycopeptides of trophoblast when compared with fetal cells from the same embryos. However, the different fetal cells appear to possess different amounts of glycopeptides, e.g. heart cells appeared to be considerably enriched in glycopeptides comprising the slower eluting peak (Fig. 1 c). The fucose-containing glycopeptides of trophoblast contain slightly less neuraminidase-sensitive sialic acid than do those of fibroblasts because neuraminidase treatment caused slower elution (lowered molecular weight) of the fibroblast-derived glycopeptides compared with the trophoblastic glycopeptides (Fig. 2 a). It may be, therefore, that both trophoblastic and fetal skin cells contain the same number of sialic acid residues in

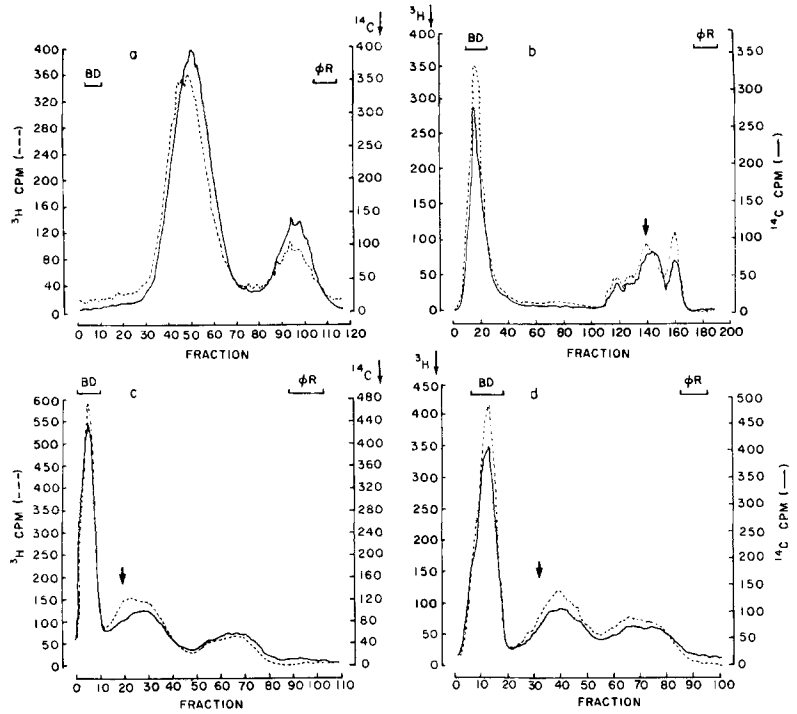

FIG. 2. (a) L-fucose-labeled glycopeptides from trophoblast compared with those derived from fetal skin fibroblasts. The pronase-digested trypsinates were further treated with neuraminidase and chromatographed on Sephadex G-50F. (b) Glycopeptides from trophoblastic cells (^{3}H – –) chromatographed with those from skin fibroblasts (^{14}C —). A difference in the elution patterns of the trypsin-sensitive glycopeptides of the trophoblast is indicated (arrow). D-glucosamine was used as precursor and Sephadex G-200 for chromatography. (c) Chromatography on G-50F of the glycopeptides shown in (b) after digestion with pronase. A faster eluting fraction was again present in the trophoblast spectrum. (d) As (c) after treatment with neuraminidase. The two profiles cochromatograph, indicating that the faster eluting fraction of (c) contains sialic acid.

their fucose-containing glycopeptides, but that in trophoblast fewer of these residues are sensitive to the action of neuraminidase.

When D-glucosamine was used as sugar precursor, however, a more interesting pattern emerged. Fig. 2b illustrates the results of analysis of trypsinates from trophoblast and fetal skin cells. A faster eluting fraction is present in the trophoblast spectrum (arrow). This faster eluting fraction was again seen when the trypsinates were digested with pronase (Fig. 2c). Neuraminidase treatment resulted in the disappearance of the faster eluting fraction, and in the comigration of the two spectra (Fig. 2d). This suggested that neuraminidase-sensitive sialic acid was at least partly responsible for the faster elution of the trophoblastic glycopeptides. This faster eluting fraction was not affected by the isotope used for labeling. The faster eluting glucosamine-derived glycopeptides were also observed when trophoblast was analyzed in comparison with fetal heart cells (Fig. 3a). Again the heart cells showed considerable enrichment in the low molecular weight glycopeptides which comprised the second peak. Fetal intestinal cells did not appear to have measurable quantities of the first peak (Fig. 3b), although they too showed slight enrichment in the glycopeptides of the second peak.

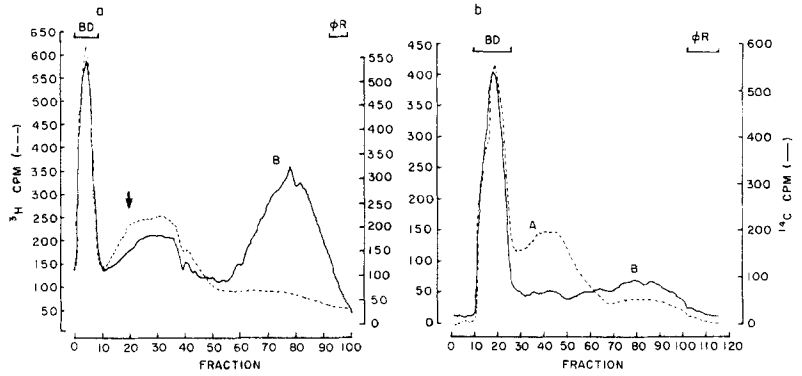

FIG. 3. (a) Glucosamine-labeled pronase-digested trypsinates of trophoblast (^{3}H - -) compared with fetal heart (^{14}C —) on Sephadex G-50F. A faster eluting fraction was again observed (arrow). Note the enrichment of the heart cells in the lower molecular weight glycopeptides of peak B. (b) As (a) except that the trophoblast was compared with intestinal cells. Peak A material was virtually absent in the intestinal cells.

Discussion

Alteration of cell membrane glycopeptides associated with neoplastic transformation is now well established (2). These modifications have been detected mainly by use of radiolabeled fucose, although glucosamine is also a suitable precursor (6). It would seem that in the case of the trophoblast the faster eluting glycopeptides do not contain fucose, but do contain glucosamine-derived products, including sialic acid. In view of the observation that galactose and *N*-acetylglucosamine, in addition to sialic acid, may be involved in the increased glycosylation of neoplastic cells (9), these two sugars may also be involved in the case of the trophoblast. The recently described wheat-germ lectin receptor of the trophoblast (10), which contains *N*-acetylglucosamine, may possess the glycopeptide(s) responsible for the faster eluting peak observed after gel filtration (Figs. 2 and 3). Alternatively, it may be that human chorionic gonadotrophin (HCG) the major saccharide of which is acetylglucosamine (11), and which may be associated with the trophoblastic plasma membrane, contains these unique glycopeptides. It is of interest that HCG has been demonstrated on the surfaces of various malignant cells (12).

The observations that increased sialylation of fucose-labeled glycopeptides was not associated with cellular growth per se nor with the expression of an embryonic phenotype (2), and also does not appear to be a function of tissue regeneration (13), led to the suggestion that such increased sialylation was associated with a neoplastic phenotype (2). Because radioactive glucosamine also labels an altered glycopeptide species in neoplastic cells (6), it may be that two types of glycopeptide populations are involved in atypical cellular behavior. The first contains glucosamine-derived sugars including sialic acid but excluding fucose. Because such a glycopeptide has been described here for normal trophoblast, and also appears in preimplantation mouse blastocysts when compared with cleavage-stage embryos (14), it may be associated with the phenomenon of localized cellular invasion. The second type of glycopeptide also contains sialic acid and perhaps other sugars (9), but in addition contains fucose. This latter glycopeptide may be associated with a neoplastic phenotype (2). The faster eluting peak observed here for 8- to 12-wk trophoblasts is not as marked as that seen

in neoplastic cells (2). This is perhaps due to the fact that trophoblast is not normally as invasive as neoplastic cells and at the 8-wk stage it is not as invasive as it is at earlier stages of gestation. It would be of interest to determine if the invasive, but nonneoplastic, placenta creta (4) manifest increased amounts of the faster eluting glucosamine-derived glycopeptides. Further support for this hypothesis may also come from a comparison of normal trophoblast with its benign (hydatidiform mole) and malignant (choriocarcinoma) neoplastic counterparts to determine if there is a progressive increase in the fucose-labeled glycopeptides associated with increasing neoplastic potential.

Summary

The surface glycopeptides of human trophoblastic cells have been compared with those of fetal cells from the same embryos using double-labeling methods with isotopes of L-fucose and D-glucosamine. A faster eluting, neuraminidase-sensitive, fraction was observed on Sephadex chromatography of the trophoblast spectra when D-glucosamine was used as precursor. Labeling with fucose did not appear to result in any differences, thus suggesting that the glycopeptides characteristic of trophoblast contained glucosamine-derived metabolic products, including sialic acid, but excluding fucose. This increased sialylation is similar to, but not identical with, modifications observed in neoplastic cells, and on this basis it is postulated that two species of glycopeptides may be involved in atypical cellular behavior. The first contains sialic acid and other sugars excluding fucose, and is associated with localized cellular growth and invasion. The second contains both sialic acid and fucose and is characteristic of neoplastic cells.

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