GLYCOPROTEIN DIFFERENCES BETWEEN THE ASCITES AND CULTURED FORMS OF THE SARCOMA 180 TUMOR

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Summary: The cell surface glycoproteins of Sarcoma 180 tumor cells grown *in vitro* in cell culture or *in vivo* as an ascites form in mice have been examined by periodate-Schiff staining of dodecyl sulfate acrylamide gels and by lactoperoxidase labeling. The cells grown in culture show the presence of a set of surface membrane glycoproteins which are absent or markedly decreased in the ascites form. Conversely, the ascites form has a glycoprotein which is not evident in the cells in culture. The other surface membrane polypeptides and enzyme activities are essentially the same for the two cell types. The glycoprotein differences may be related to the ability of the cells to interact with each other or a substratum.

Cell surface macromolecules, particularly glycoproteins, are generally believed to play an important role in phenomena such as cell-cell interactions, cellular growth and transformation and cellular differentiation (1, 2). In the case of cell transformation cell surface glycoprotein differences between normal and transformed cells have been demonstrated by lectin agglutination (3), glycopeptide analyses (4), gel electrophoresis (5), and enzymatic cell surface labeling (6-8). The last technique has been used to show the presence of a high molecular weight protein in normal cultured fibroblasts which is not labeled in their virus-transformed counterparts (7). The labeling of this protein is also sensitive to the cellular growth state and stage in the cell cycle (2).

Recent studies from this laboratory have demonstrated the presence on the surface of the mouse Sarcoma 180 ascites tumor cell of two glycoproteins which can be labeled with lactoperoxidase (9). Since the glycoprotein with the higher apparent molecular weight showed variable labeling behavior, we suspected that variations in growth conditions might affect the expression of this molecule at the cell surface. Therefore we have compared proteins and

glycoproteins of membranes isolated from the Sarcoma 180 ascites form and from an established line of this tumor adapted to grow in cell culture. The line in cell culture consistently shows greater amounts of a set of glycoproteins with high apparent molecular weight than the ascites form.

Methods. Sarcoma 180 (CCRF-S180 II), purchased from the American Type Culture Collection Cell Repository, was grown in milk dilution bottles on McCoy's 5a modified medium (10). At confluency cells were removed by scraping with a rubber policeman and washed three times with Hepes balanced salts (11) prior to membrane isolation. Sarcoma 180 ascites cells were grown in the peritoneal cavity of mice and isolated as previously described (9). Surface membranes were prepared by a modification (12) of previously published procedures (9, 13). Lactoperoxidase labeling, sodium dodecyl sulfate (SDS) acrylamide gel electrophoresis and other analytical procedures were performed as previously described (9).

Results. Plasma membrane-enriched fractions were isolated from both cell types by homogenization in zinc solutions (13) followed by sucrose gradient centrifugation (12). 5'-Nucleotidase was assayed as a plasma membrane marker (12) and was found to be 19- and 18-fold purified compared to homogenates in the membranes isolated from ascites and cultured cells, respectively. Membranes from both cell types appeared by phase-contrast microscopy as ruptured envelopes or large fragments. In order to compare constituents accessible to the cell surface in the two cell lines, cells were labeled with ^{125}I and lactoperoxidase (9). The labeling pattern obtained after SDS electrophoresis of the ascites cell membranes is essentially the same as that reported previously (9), with a major peak at an apparent molecular weight of about 125,000 and a lesser peak near the top of the gel (Fig. 1). Membranes from the cells labeled in culture show a distinctly different pattern, with a single major peak near the top of the gel and essentially no label in the 125,000 molecular weight region (Fig. 1).

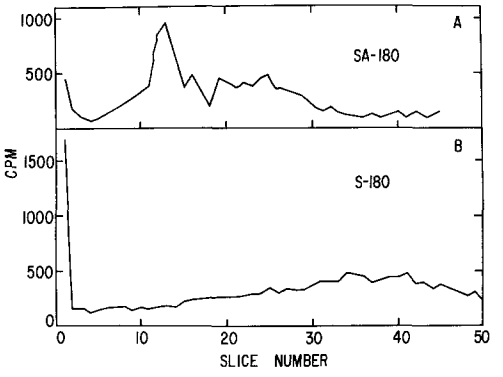

Fig. 1. - Lactoperoxidase labeling profiles of membranes isolated from ascites (A) and cultured (B) cell lines labeled with lactoperoxidase, H_2O_2 and ^{125}I (9). Electrophoresis was performed on 5% acrylamide gels using 0.1% SDS for 5 hr.

The membrane components from the two cell lines were further characterized by the polypeptide and carbohydrate distributions on SDS acrylamide gels. Scanning profiles for the Coomassie blue stained species are shown in Fig. 2. The polypeptide distributions from the two cell lines are essentially the same, although there are some differences in band intensities. By comparison the periodate-Schiff staining patterns show striking differences (Fig. 3). The ascites form has a major band near the center of the gel with an apparent molecular weight of 125,000 and lesser bands near the bottom of the gel. The membranes from the line grown in culture show two intense, sharp bands (1 and 2) which have barely migrated into the gel plus small amounts of staining material near the center and bottom of the gel. Electrophoresis of samples of calf serum did not show glycoprotein bands in the areas of those found in the membranes of two cell types.

Discussion. These results show the presence on the surface membranes of Sarcoma 180 tumor cells grown in culture of glycoproteins which appear to be absent or drastically reduced in the ascites form of the tumor. These well-defined glycoprotein differences contrast sharply with the comparisons of membrane polypeptides, which are essentially the same for the two cell lines.

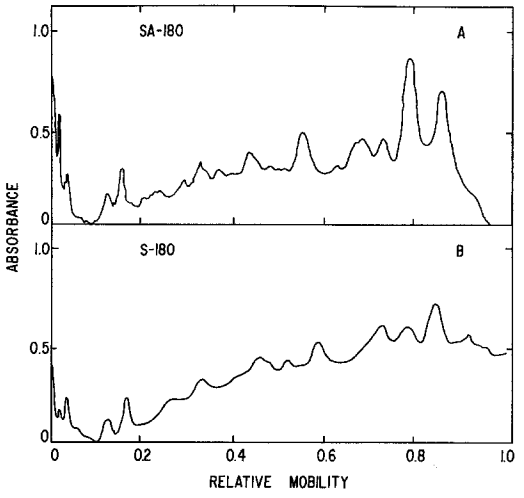

Fig. 2. - Polypeptide patterns for ascites (A) and cultured (B) Sarcoma 180 cell lines. Electrophoresis was performed as in Fig. 1. The two profiles are slightly offset due to different electrophoresis conditions.

A number of lines of evidence indicate that these are surface membrane-associated glycoproteins. They are present predominantly on the surface membrane fraction, which is distinguished morphologically and by enzyme markers from other cell membrane fractions. They are not readily removed from the membrane by extraction with 1 M salt or dilute EDTA solutions, although other proteins can be removed from these membranes by these methods (14). The glycoproteins are cleaved by trypsin, which causes the release of glycopeptides and disappearance of the glycoproteins from the gel patterns (15).

The basis for the glycoprotein differences between the cell lines is not clear. Possible explanations include alterations in the biosynthesis, insertion into the membrane or degradation of the glycoproteins. The last might occur by proteolysis (2) in the ascites cells. It seems less likely that these differences are due to crypticity or lowered sialic acid content, since the differences were demonstrated by two very different techniques. The possibility of selective glycoprotein aggregation in one membrane type cannot be ruled out at present, although heating at 100° in the dodecyl sulfate before

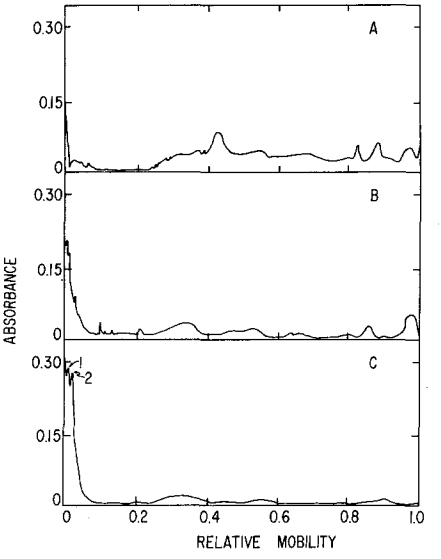

Fig. 3. - Periodate-Schiff staining profiles for ascites (A) and cultured (B and C) Sarcoma 180 cell lines. Electrophoreses were run for 5 hr for samples A and B and for 10 hr for sample C.

electrophoresis does not alter the periodate-Schiff staining patterns (16).

The function of these glycoproteins is unknown. Similar glycoproteins with high apparent molecular weights have been demonstrated for other cells grown in culture (6, 7, 17), and the absence of a high molecular weight glycoprotein has been suggested to be a characteristic of transformed cells (2). The presence of the glycoproteins on the Sarcoma 180 cells in culture is dependent on the cell density (J. W. Huggins, unpublished). This dependence is similar to the behavior exhibited by the transformation-sensitive glycoprotein(s). However, our results do not appear to indicate a correlation of the absence of the glycoproteins with malignancy, since both cell types will form tumors. The preferential association of these glycoproteins with cells grown in culture suggests that the glycoproteins may be involved in the association of the cells with the substratum on which they grow. The fact that cells are released from the substratum by trypsinization, which cleaves the glycoproteins, lends some credence to this idea. Attempts to grow the as-

cites form in culture have not been successful to date, possibly because of this inability to attach to a substratum. Further studies on conditions which promote attachment or release of the cells are needed in order to evaluate the role of the glycoproteins in these phenomena.

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