THE MAJOR SURFACE PROTEIN OF EPITHELIAL CELLS FROM NEWBORN AND ADULT RAT LIVERS IN PRIMARY CULTURES

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SUMMARY: Epithelial cells from newborn and adult rat livers were grown as large colonies in primary cultures. Fibroblasts were also isolated from the same organ and grown at confluency. The cell surface proteins of each population were radioiodinated via the lactoperoxidase-catalysed reaction and compared in terms of radioactivity profiles obtained after electrophoresis on sodium dodecyl sulphate polyacrylamide gel. A protein of 80,000 daltons is predominantly exposed on epithelial cells from newborn and adult liver comparatively to a protein of 225,000 daltons on fibroblasts from both origins. The finding of a distinct major protein expressed on epithelial cells is most appealing in terms of its possible role in cell-cell specific adhesion, cell proliferation, differentiation and antigenecity, and malignant transformation.

A protein of apparent molecular weight 225,000 daltons is predominantly labeled on the surface of "normal" fibroblasts in culture by lactoperoxidase-catalysed iodination (LPO). The protein, which has been detected on cells from a number of established fibroblastic cell lines (1-4), is most likely involved in cell adhesion, antigenecity and proliferation, and virus transformation (5-11). To our knowledge, no comparable work has been reported for normal epithelial cells, most probably because of difficulties in obtaining primary cultures of this type of cells in vitro. Thus, our first effort has been aimed to develop methods for culturing rat liver epithelial cells as opposed to the fibroblasts that can be readily obtained from the same organ with standard culture procedures. We report here on the use of the LPO-probe to detect the surface membrane proteins of epithelial and fibroblastic cells from adult and newborn rat livers in primary cultures. The results demonstrated that a major

Abbreviations: LPO: Lactoperoxidase; PBS: Phosphate buffered saline; SDS: Sodium dodecyl sulphate; BSS: Balanced salt solution; EGTA: Ethyleneglycol-bis-(β-amino-ethyl ether) N,N'-tetra-acetic acid.

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protein is expressed on cells of epithelial morphology, which is different in size however from the one exposed on cells of typical fibroblastic appearance.

MATERIAL AND METHODS:

Animals: Cells were isolated from the livers of adult (about 175 gm) and newborn (6 day-old) rats. The adult animals had been fed ad libitum up to 18 hours before sacrifice. The newborn rats were taken from their mother one hour before sacrifice.

Cell isolation and cultures:

Adult rats - The method for the isolation of liver cells is a modified version of the double-perfusion technique of Seglen (12) using only collagenase (13). Our approach has been to determine the best combination of cell isolation and culture conditions which would yield a particular cell type. Details of the procedures will be given in a subsequent publication (14).

Briefly, the animal was anesthetized under ether and the abdominal cavity was opened to canulate the portal vein and to section the vena cava. To obtain epithelial cell cultures, the liver was first washed with 150 ml of Ca^{++}-Mg^{++} free BSS (Earle's salt) containing 0.5 mM EGTA at a rate of 50 ml/min. The organ was then perfused with 200 ml of William E medium (Flow, Maryland) containing 0.025% collagenase and supplemented with $CaCl_2$ at a final concentration of 5 mM. After liver decapsulation, the cells were detached by manual agitation in normal E medium at room temperature. The cells were washed by centrifugation and plated at a concentration of 1×10^6 cells/ml in 3 ml of pre-warmed medium (pH 7.3) containing 10% fetal calf serum, and finally incubated in 24 cm^2 T-Flasks (Falcon Plastic, #3013) at 37°C in a 5% CO_2-95% air atmosphere. The medium was removed after one hour and 20 ml of fresh milieu at pH 7.6 was added. The medium was changed again at 24 hours post-inoculation. Within 7 days, numerous large colonies of epithelial cells were seen under phase contrast microscopy. To obtain fibroblast cultures, the liver cells were isolated by Seglen's method (12, 13) and then plated at the same concentration as above but in cold medium. The cultures were maintained in 3 ml medium (pH 7.3) at 37°C and medium was changed twice a week. By day 7, the flasks contained cells of all typical fibroblastic morphology and the cultures attained near confluency. Surface labeling was performed at day 10 after plating.

Newborn rats - Liver cells were isolated by an adapted version of Takaoka et al's method using Dispase I (15). The animal was decapitated and the liver was minced in 5 ml of E medium (pH 7.3) containing 10% fetal calf serum added with 10^3 units of Dispase I (Godo Shusei Co., Tokyo). The suspension was incubated in a shaking water-bath for 1 hour at 37°C. Large tissue aggregates were removed by 1 x g sedimentation and the cells were washed by a centrifugation at 2000 g-min. To obtain epithelial cells, the centrifugation was done at room temperature and the cells were plated at 3×10^7 nucleated cells per flask (#3013) in 10 ml of pre-warmed medium (pH 7.6). Medium was changed 24 hours later. Numerous epithelial colonies were seen at day 5 post-inoculation. Fibroblastic cells were obtained by a plating of cells at the same concentration in cold medium (pH 7.3) and a subsequent incubation of 37°C in 5 ml medium. Surface labeling was done at day 7 after plating.

Surface labeling: Surface radioiodination of the cells was achieved by adding successively to a flask containing 1 ml PBS 100 mU of lactoperoxidase (Sigma), 100 mU of glucose oxidase (Worthington), 100 µCi of [^{125}I] sodium iodide (New

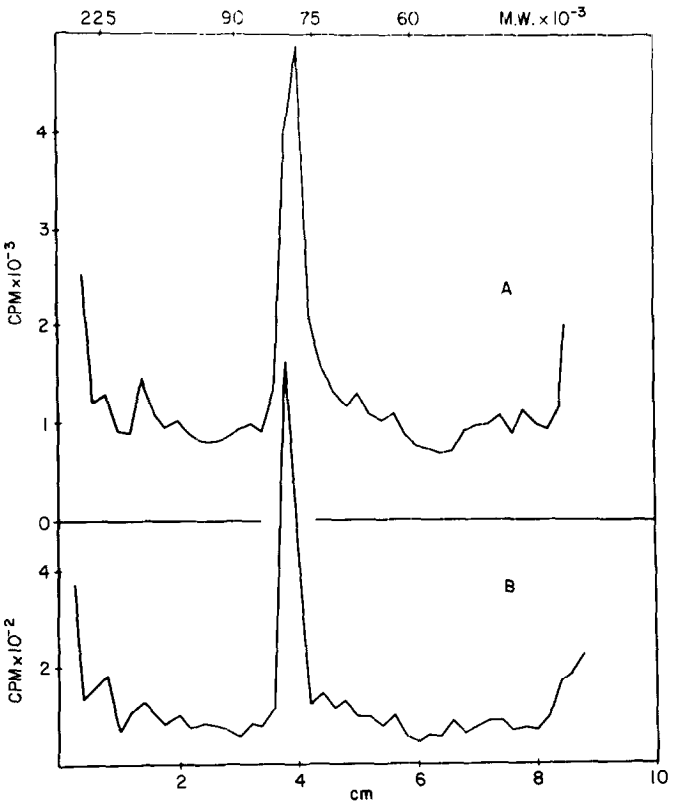

Figure 1. Radioactive profiles of SDS-polyacrylamide gels of stromal proteins. Cell stroma of ^{125}I surface-labeled epithelial cells from adult (curve A) and newborn (curve B) rat livers in primary cultures were solubilized in SDS and subjected to electrophoresis in 7% polyacrylamide gels. The gels were cut into 2 mm slices and counted for radioactive iodide.

England Nuclear) and 900 µg glucose as reported before (16, 17). The reaction was allowed to proceed for 10 min at room temperature and free iodide was removed by three successive washings with PBS.

Protein fractionation: The labeled cells were detached in 2 ml PBS with a rubber policeman and the flask was rinced once with 3 ml PBS. The two suspensions were combined and the cells were spun at 500 x g for 10 min. The pellet was resuspended in 125 µl distilled water before addition of SDS, sodium carbonate and β-mercaptoethanol (16). Electrophoresis on SDS-polyacrylamide gel was carried out as before (18, 19). At the end of the run the gels were cut in 2 mm slices and counted in a gamma well counter (Nuclear Chicago). The results are presented as ^{125}I-radioactivity profiles along a gel of 10 cm.

RESULTS AND DISCUSSION

By choosing proper culture conditions, it was possible to obtain culture flasks that contained numerous colonies of epithelial cells of more than 1 mm in diameter. Also, phase contrast microscopy of the colonies revealed that

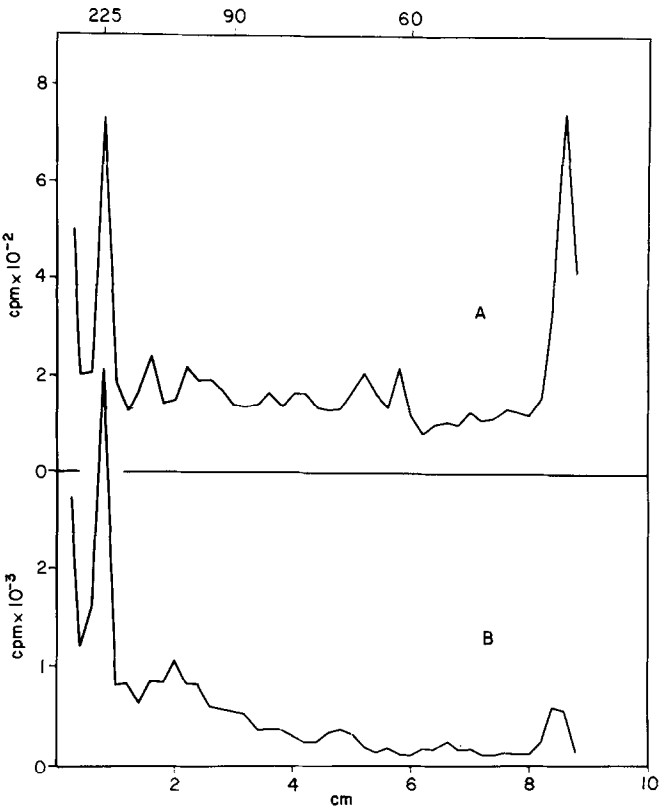

Figure 2. Radioactive profiles of SDS-polyacrylamide gels of stromal proteins. Cell stroma of ^{125}I surface-labeled fibroblasts from adult (curve A) and newborn (curve B) rat livers in primary cultures were solubilized and treated as described in Figure 1 to obtain the ^{125}I radioactivity distribution.

the epithelial cells from adult liver had essentially the same morphology as those from newborn liver, i.e. polygonal cells with granular cytoplasm (data not shown). No noticeable differences in morphology were observed between fibroblasts of both origins. Figure 1 shows the ^{125}I-radioactivity distribution over a gel after surface labeling of epithelial cells and subsequent fractionation by SDS-electrophoresis. Curve A shows the results for cells from adult liver, where most of the radioactivity is found at 3.8 cm from the origin. Curve B, which corresponds to a labeling of cells from newborn liver, shows essentially the same radioactivity distribution. A comparison between the relative mobility of this labeled protein and those of known proteins yields

an apparent molecular weight of 80,000 daltons. Thus, a major protein is expressed on surface of epithelial cells, which is different in size from that observed on fibroblasts from established cell lines (1-5). It was of interest then to determine the surface labeling of normal fibroblastic cells grown also in primary cultures, i.e. for cell cultures obtained under comparable experimental conditions. Figure 2 shows the results for fibroblasts from adult rat liver (Curve A) and newborn rat liver (Curve B). On both curves, the ^{125}I-radioactivity is found at the 0.8 cm region from the origin, which corresponds to the relative mobility of a protein of about 225,000 daltons.

These results demonstrate that the major protein found on cell surface is cell-type specific. A protein of about 225,000 molecular weight is present on surface of fibroblasts from adult and newborn rat livers, and also from established fibroblast lines of various origins. In that sense, the exposed protein on fibroblasts is cell-specific rather than being dependent on organ or animal origins. It would then be of great interest to determine if the protein of 80,000 nominal molecular weight is present on epithelial cells from other tissue or animal sources. It is worth noticing the presence of a major surface protein of a similar molecular weight on HeLa cells, a human epithelial cell line derived from a carcinoma of the cervix (15).

Moreover, the great resemblance between epithelial cells of adult and newborn rat livers suggests that the adult liver cells which give rise to a continuous line in vitro (our cells from adult liver are at the third passage) represent a special population of less differentiated cells already present in the perinatal liver and capable of sustained proliferation throughout liver maturation. This is supported by a follow-up of the cell behaviour during the first days after initial plating (12). A mass culture of adult liver cells larger in size than any newborn liver cells and exhibiting typical liver functions is obtained. Most of these adult cells are however in a stationary state and disappear after 2-3 days. Those which give rise to colonies some days later correspond to a low number of the original plating (12).

In the light of the important data reported for the major fibroblastic cell surface protein (1-11), our finding of a distinct protein on normal liver epithelial cells is most appealing in terms of the probable role of such surface protein in cell-cell specific adhesion, cell growth, differentiation and antigenecity, and neoplasic transformation.

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