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Lactoperoxidase-catalyzed Iodination of Membrane Proteins in Normal and Neoplastic Epidermal Cells

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Cell surface proteins of normal human, mouse, and rat cells in primary culture, of human basal cell carcinoma, and of carcinogen-transformed cell lines were examined by lactoperoxidase-catalyzed iodination. Autoradiography was used to record the distribution of label in the polypeptide subunits separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis. There was no significant difference in the results for normal cells of human, mouse, and rat. On the other hand, carcinogen-transformed mouse cells had many more labeled polypeptide bands of widely distributed molecular weights. The iodination profiles from human basal cell carcinoma cells were much more akin to those from normal cells than to those from carcinogen-transformed cells. Treatment of iodinated cells with proteolytic enzymes visibly altered the polypeptide bands.

Cell membranes are associated with many cell functions, such as cell recognition, adhesion, and antigenicity, which often become altered in neoplastic cells. Lactoperoxidase-catalyzed iodination has been a major tool for studying cell surface proteins [1,2]. This technique selectively labels with ^{125}I the exposed tyrosine residues of membrane proteins. The high molecular weight of lactoperoxidase prevents its penetration through the cell membrane, so that only the external surface of the cell is labeled.

Iodination has not heretofore been applied to epidermal cells. Surface changes in the epidermis have been studied by specific lectin binding [3,4] and incorporation of labeled sugars [5,6]; these methods, however, label cell constitutents different from those affected by iodination. This paper describes iodination experiments with normal human, mouse, and rat epidermal cells, with human basal cell carcinomas, and with carcinogen-transformed mouse epidermal cells [7,8].

MATERIALS AND METHODS

Culture Procedures

Tissue culture media, trypsin (1:250), penicillin (100 IU/ml), streptomycin (100 μg/ml), and salts were purchased from GIBCO (Grand Island, N.Y.). Fetal bovine serum (FBS) was obtained from Flow Laboratories (Rockville, Md.). Newborn animals used for the preparation of primary rodent cultures were Sprague-Dawley rats and Swiss-Webster mice. Normal adult human skin was excised from surgical specimens with a dermatome. Epidermis was separated from dermis by the trypsin flotation procedure [9] which consists of overnight incubation with 0.25% trypsin at 4°C. Separated epidermal fragments were finely minced, then dissociated by stirring for 1 hr in Waymouth tissue culture medium containing 10% FBS. Dissociated cells were separated from stratum corneum by filtration through a 125 μm nylon mesh, plated on collagen-coated plates [10] and grown in a Waymouth medium as modified by Marchok [11] with antibiotics added. Cells were maintained at 37° in a humidified 4% CO_2 atmosphere.

Biopsy samples of human basal cell carcinomas were exposed to 0.25% trypsin for 16 hr at 4°, minced, then grown in the same fashion as normal epidermal cells on collagen-coated plates.

Carcinogen-transformed cell lines of mouse epidermal cells (JB-8, D-11a and T-6272), generously supplied by Dr. Nancy H. Colburn of NIH, were maintained on Corning plastic tissue culture vessels in Minimal Essential Medium containing 10% FBS, nonessential amino acids, and antibiotics.

Lactoperoxidase Labeling

Cells growing in tissue culture vessels, at 75–100% confluency, were washed 3 times with Dulbecco's phosphate buffered saline (DPBS), pH 7.2, then iodinated in a 1 ml mixture of DPBS containing 60 μg lactoperoxidase (Worthington, Millipore Corp., Freehold, N.J.), 55 mU glucose oxidase (Worthington), 5 mM glucose, and 150 μCi carrier-free $Na^{125}I$ (New England Nuclear, Boston, Mass.). The reaction vessels were gently swirled for 10 min at room temperature, after which the cells were washed 3 times with DPBS containing 5 mM NaI. Then the cells were scraped into 0.1 ml DPBS containing 2 mM phenylmethylsulfonylfluoride (PMSF) at 4°, sonicated for 10 sec, and mixed with 0.1 ml sodium dodecyl sulfate (SDS) sample buffer (0.125 M Tris HCl, pH 6.8; 20% glycerol; 4% SDS; 10% β-mercaptoethanol; 2 mM PMSF; 0.004% bromphenol blue).

For an investigation of the impact of proteolytic enzymes, cells were iodinated, swirled and washed as above. At this point, the cells were reacted with dispase, 100 μg/ml (Sigma Chemical Co., St. Louis, Mo.), or trypsin, 10 μg/ml (Sigma), for 10 min at room temperature, then washed 3 times with DPBS containing 2 mM PMSF. Thereafter, the above procedure was resumed, starting with scraping the cells off the reaction vessels.

Each iodination experiment was carried out three or more times, except for the basal cell carcinoma which was done twice (with different tumor samples).

Electrophoresis and Autoradiography

Samples in SDS buffer were heated at 100° for 3 min. Proteins were separated by the method of Laemmli [12] on 1.5 mm vertical slabs of 7.5% polyacrylamide below a 3% stacking gel. Electrophoresis was for 3 hr at 30 mA per slab (at constant current). Gels were stained for 16 hr in 0.1% Coomassie Blue in 50% methanol, 10% acetic acid, then destained in the same solvents without the dye and dried on Whatman 3 MM paper in a Biorad gel drier. Sheets of Kodak SB-5 film were exposed to the gels for 3 days at −70°. Molecular weight calibration was achieved with the following standards: myosin (200,000 daltons), phosphorylase B (92,500), bovine serum albumin (68,000), ovalbumin (46,000), and carbonic anhydrase (30,000).

RESULTS

Normal Epidermal Cells

Collagen-coated plates were required for the growth of human epidermal cells and were often also used for rodent cells to improve cell attachment during initial plating. Since collagen is composed of protein, it can be expected to become labeled during iodination. To separate out the contribution of the collagen, controls were run with collagen-coated plates devoid

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Abbreviations:
DPBS: Dulbecco's phosphate buffered saline
FBS: fetal bovine serum
PMSF: phenylmethylsulfonylfluoride
SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis

of cells. After several days in culture, the collagen is more loosely bound to the plates, while the cells become attached directly to the tissue culture vessels; one can then flush loose the collagen with a jet of culture medium without detaching the cells. Figure 1 is an autoradiogram of the labeled polypeptides obtained for collagen alone, for human cell cultures on collagen, and for human cell cultures from which collagen was mechanically removed. The collagen produced bands at about 200,000 and 135,000 daltons. The cell samples without collagen did not have these bands. In all the results for normal cells, no labeled bands appeared above 100,000 daltons other than those due to collagen.

Figure 2 presents the autoradiograms for normal rat, mouse, and human cells. The top of the gel (above 100,000 daltons) is not shown, in order to omit the collagen contribution (where present). The results for all 3 species look alike, with the labeled polypeptides appearing at the same molecular weights. The relative intensities of the bands are more similar between the rat and mouse cells than they are between human and rodent cells. The most intense radioactivity is found at 56,000 and 65,000 daltons, with weaker bands appearing in the ranges 45,000–55,000 and 25,000–35,000 daltons.

Neoplastic Cells

The autoradiograms obtained from cultures of 3 carcinogen-transformed mouse cell lines (grown without collagen) are displayed in Figure 3. The labeling patterns of the 3 lines are very similar to each other and dramatically differ from the results for normal cells (Fig 2). There are a large number of radioactive polypeptides, extending over the entire range of molecular weights.

Figure 4 compares the iodination pattern for human basal cell carcinoma with those of normal human cells and of one of the carcinogen-transformed lines. The autoradiogram of the basal cell carcinoma (grown on collagen) resembles that of the normal cells in not showing the profusion of bands at widely distributed molecular weights that is characteristic of the transformed cells. The main differences are that very intense radioactivity now occurs at 79,000 daltons where there is no band for

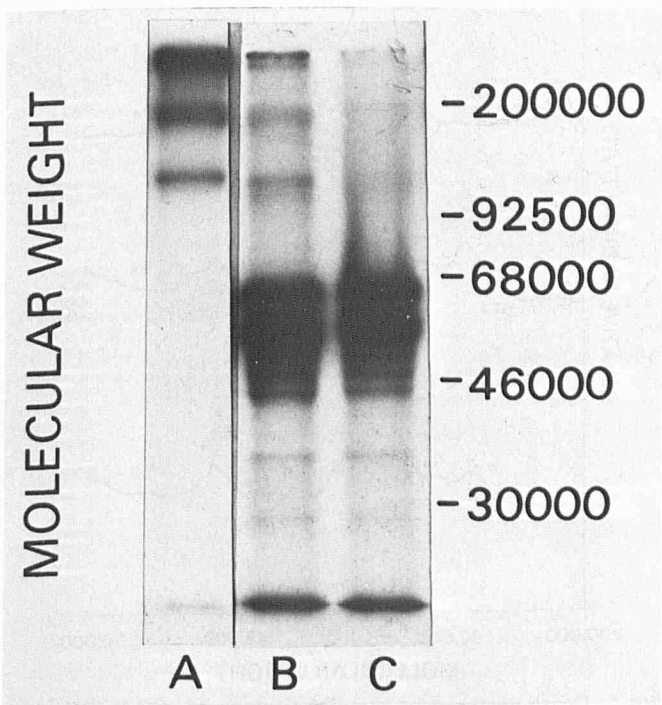

FIG 1. Autoradiograms of SDS-PAGE of (*A*) collagen alone, (*B*) normal human epidermal cells grown on collagen, and (*C*) same as (*B*) except collagen mechanically removed.

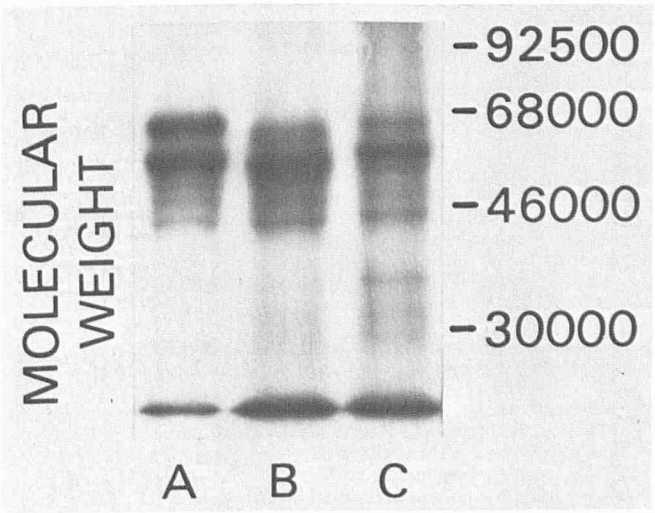

FIG 2. Autoradiograms of SDS-PAGE of normal epidermal cells of (*A*) human, (*B*) mouse, and (*C*) rat. Section above 100,000 daltons not shown, as it contains only collagen contribution (see Fig 1).

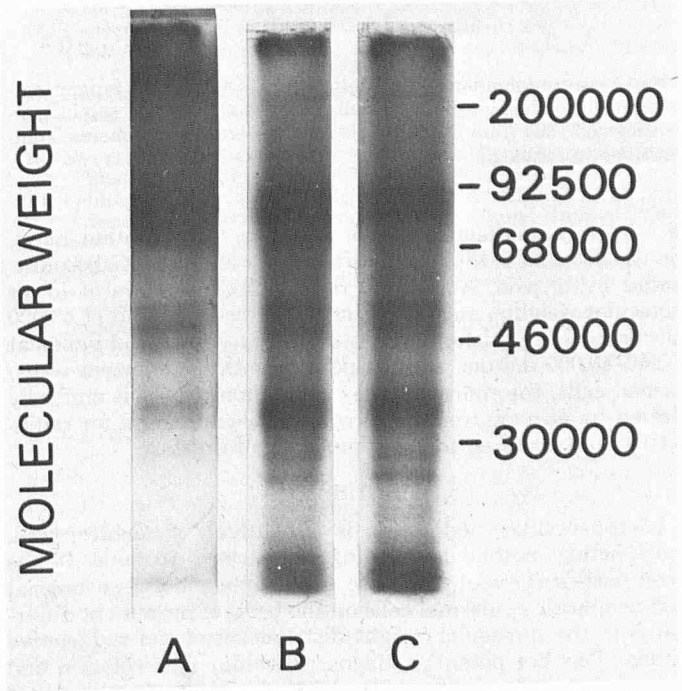

FIG 3. Autoradiograms of SDS-PAGE of carcinogen-transformed mouse epidermal cell lines (*A*) T-6272, (*B*) JB-8, and (*C*) D-11a.

the normal cells and that, conversely, the 56,000 dalton band is no longer seen.

Enzyme-treated Cells

Proteolytic enzymes are often used for tissue dissociation and cell dispersal. Trypsin has been the most common choice; more recently, dispase has come into favor because it is gentler on the cells and is not inhibited by serum. Iodinated normal and carcinogen-transformed cells were treated with these two enzymes before electrophoresis. Autoradiograms for cells that were not exposed to enzymes, for those treated with dispase, and for those treated with trypsin are compared in Fig 5, for normal rat cells in Fig 5*a* and for transformed cells (line *JB-8*) in Fig 5*b*. The results are displayed as densitometer scans of the autoradiograms for better resolution.

For the normal cells, the 56,000 dalton polypeptide seems to

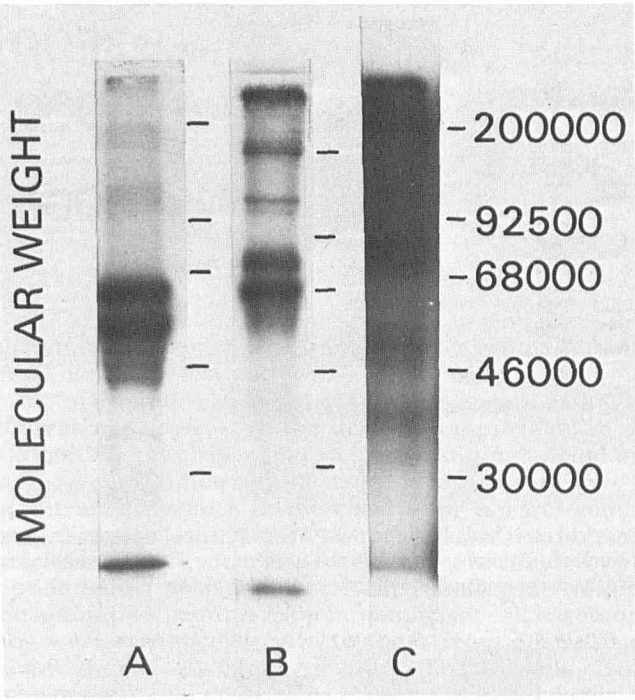

FIG 4. Autoradiograms of SDS-PAGE of (*A*) normal human epidermal cells, (*B*) human basal cell carcinoma, and (*C*) carcinogen-transformed cells (line D-11a). Gels from different experiments; standards shown for each.

be relatively unaffected by the enzymes. On the other hand, the 65,000 band is attenuated by dispase and essentially obliterated by trypsin. Additional radioactivity appears at lower molecular weights, most prominently a distinct band at 43,000 daltons with trypsin as against three partly resolved bands at 40,000–46,000 daltons with dispase. For the carcinogen-transformed cells, the more complex iodination profile is similarly altered by enzyme treatment, with an overall trend for radioactivity to be shifted to lower molecular weights.

DISCUSSION

Lactoperoxidase iodination is a relatively straightforward, quick-acting method of labeling cell-surface proteins. It has been used successfully here to discriminate between normal and neoplastic epidermal cells on the basis of prominent differences in the molecular-weight distribution of the radioactive bands. This has potential diagnostic value. The isolation and chemical characterization of the labeled macromolecules would obviously contribute significantly to an understanding of the neoplastic process. A drawback of iodination is that it requires intact viable cells, since the penetration of lactoperoxidase into damaged cells could label internal components as well as the cell surface. Another limitation is that the cells must be dispersed so that their surfaces can be exposed to the action of lactoperoxidase, but care must be taken that the cells have recovered in tissue culture from the damage caused by the proteolytic enzymes used in their dispersal. These restrictions prevent the application of iodination *in vivo*.

The similarity in the labeling patterns of the normal human and rodent cells is reassuring as to the use of rodents as a model system for the study of human epidermal membranes. That the rat and mouse cells resemble each other more closely than they do the human cells may be due either to their being related species or to the fact that these are cells from newborn animals whereas the human cells come from adult tissues.

The basal cell carcinoma, like the normal cells, has an iodination profile with labeled bands in the range 40,000–80,000 daltons, whereas the labeling pattern of the carcinogen-transformed cells shows a greater profusion of bands extending over a much wider range of molecular weights. This parallels observations that the histological and ultrastructural appearance of basal cell carcinoma is closer to normal than to transformed cells. Cells of basal cell carcinoma do not display cytoplasmic

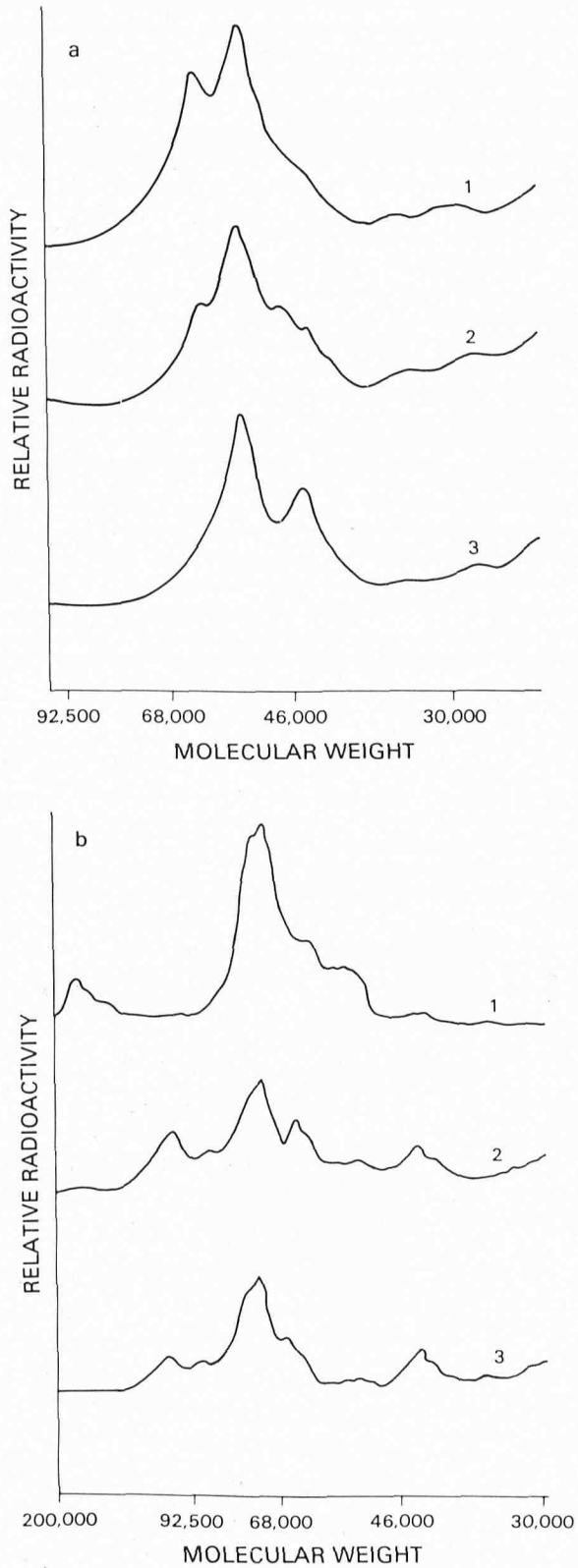

FIG 5. Densitometer scans of autoradiograms of SDS-PAGE of (*a*) normal rat epidermal cells and (*b*) carcinogen-transformed cells (line JB-8); in each section, curve (*1*) is for cells not exposed to enzyme, curve (*2*) for cells treated with dispase, and curve (*3*) for cells treated with trypsin.

differentiation and resemble normal embryonic basal cells [13–16]. While a high nuclear-to-cytoplasmic ratio is observed in histological sections, the nuclei do not exhibit the abnormal mitotic figures characteristic of tumor cells [13]. On the other hand, extensive ultrastructural changes have been noted for the transformed lines used here, such as the loss of tonofilaments and a great increase in the number of ribosomes [7]. Major changes in the cell surface composition have been observed for other types of transformed cells [17,18]. It would be interesting to also carry out iodination studies with epidermal squamous cell carcinoma. The most salient discrepancy between the labeling patterns of normal cells and of basal cell carcinoma is the intense radioactivity for the latter at 79,000 daltons which has no counterpart for normal cells. There is a labeled band for the carcinogen-transformed cells near this molecular weight. A labeled band at 80,000 daltons has also been reported for HeLa cells [19].

When cells that have been iodinated are then treated with proteolytic enzymes, labeled polypeptides disappear at higher molecular weights and others appear at lower molecular weights. Presumably, this indicates that cell surface proteins have been broken down by the enzymes. Such an effect from trypsin has been previously reported [18,20,21]. It is also found here from dispase, although less strongly. Proteolytic enzymes are frequently used for dissociation of tissue fragments into single cells. The hazard of cell surface damage from such processing must be taken into account in experimental design and interpretation.

REFERENCES

1. Phillips DR, Morrison M: Exposed protein on the intact human erythrocyte. Biochemistry 10:1766–1771, 1971
2. Juliano RL, Behar-Bannelier M: An evaluation of techniques for labeling the surface proteins of cultured mammalian cells. Biochim Biophys Acta 373:249–267, 1975
3. Brabec RK, Peters BP, Bernstein IA, Gray RH, Goldstein IJ: Differential lectin binding to cellular membranes in the epidermis of the newborn rat. Proc Natl Acad Sci USA 77:477–479, 1980
4. Nemanic MK, Elias PM: Localization and identification of sugars in mammalian epidermis. J Cell Biol 83:46, 1979
5. King IA, Tabiowo A, Williams RH: Incorporation of L-(^{3}H) fucose and D-(^{3}H) glucosamine into cell-surface-associated glycoconjugates in epidermis of cultured pig skin slices. Biochem J 190:65–77, 1980
6. Mann PR, Williams RH, Gray GM: Distribution of glycoproteins containing fucose in normal and psoriatic keratinocytes. Br J Dermatol 102:649–657, 1960
7. Colburn NH, Vorder Bruegge WF, Bates JR, Gray RH, Rossen JD, Kelsey WH, Shimada T: Correlation of anchorage-independent growth with tumoriginicity of chemically transformed mouse epidermal cells. Cancer Res 38:624–634, 1978
8. Colburn NH: The use of tumor promoter responsive epidermal cell lines to study preneoplastic progression, Neoplastic Transformation, Differentiated Epithelial Cell Systems in Vitro. Edited by LM Franks, CB Wigley. New York, Academic Press, Inc., 1979, pp 113–133
9. Yuspa SH, Harris CC: Altered differentiation of mouse epidermal cells treated with retinyl acetate in vitro. Exp Cell Res 86:95–105, 1974
10. Karasek MA, Charlton ME: Growth of postembryonic skin epithelial cells on collagen gels. J Invest Dermatol 56:205–210, 1971
11. Marchok AC, Rhoton J, Nettesheim P: Long term culture of epithelial cells from normal rat tracheas. In Vitro 12:320, 1976
12. Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685, 1970
13. Van Scott EJ: Reaction patterns of normal and neoplastic epithelium. Advan Biol Skin 7:75–87, 1966
14. Brody I: Contribution of the histogenesis of basal cell carcinoma. J Ultrastruct Res 33:60–79, 1970
15. Flaxman BA: Growth in vitro and induction of differentiation in cells of basal cell cancer. Cancer Res 32:462–469, 1972
16. Heenen M, Achten G, Galand P: Autoradiographic analysis of cell kinetics in human normal epidermis and basal cell carcinoma. Cancer Res 33:123–127, 1973
17. Nicolson GL: Trans-membrane control of the receptors of normal and tumor cells: II. Surface changes associated with transformation and malignancy. Biochim Biophys Acta 458:1–72, 1976
18. Hynes RO: Cell-surface proteins and malignant transformation. Biochim Biophys Acta 458:73–107, 1976
19. Fehlmann M, Marceau N: Cellule HeLa: Arrangements de protein de surface en fonction de conditions de culture. Un Med Can 105: 346–350, 1976
20. Guy D, Latner AL, Turner GA: Radioiodination studies of tumor cell-surface proteins after different disaggregation procedures. Br J Cancer 36:166–172, 1977
21. Baumann H, Doyle D: Effect of trypsin on the cell surface proteins of hepatoma tissue culture cells. J Biol Chem 254:3935–3946, 1979