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Heterogeneity of the Surface Material in Isolated Cells of Transplantable Hamster Melanomas[1]

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ABSTRACT

The heterogeneity of the surface material released by trypsin in isolated cells of melanotic and amelanotic melanomas was studied by the method of separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

The electrophoretic patterns of the surface material derived from two kinds of melanoma showed some differences. The differences in the surface proteins and glycoproteins seem to be related to the biological properties of both melanomas.

INTRODUCTION

Changes in the qualitative and quantitative contents of the surface glycoproteins accompanying oncogenic transformation have been studied by many authors (4-7, 16, 17).

However, as emphasized in different reports, at present the most important problem is to determine which of the observed differences can be directly connected with the malignant transformation of cells (2, 3, 19, 20).

In our previous investigation of the differentiation of the surface glycoproteins of cells from 2 kinds of transplantable hamster melanoma, we have found that a spontaneous change of a melanotic melanoma to an amelanotic form is associated with an altered content of surface glycoproteins, antigenicity and immunogenicity, and binding of concanavalin A (10, 11).

In continuation of these studies, we attempted to see if the alteration of surface glycoproteins is accompanied by changes in the heterogeneity of the surface proteins and glycoproteins. We compared the heterogeneity of the surface material released by trypsin from isolated melanoma cells and examined it by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Animals and Tumors. Male Syrian (golden) hamsters, 2 to 3 months old, were used. Tumors were transplantable melanotic and amelanotic melanomas. The latter originated from the former by a spontaneous transformation in which a loss of pigment was accompanied by an acceleration of growth, a greater anaplasia, and changes in surface glycoproteins (Table 1).

Isolation of Melanocytes. Melanoma cells were isolated from solid tumors by a nonenzymatic method, described earlier (1), to obtain single-cell suspensions without loss of surface polysaccharides (12). The suspension contained 78 to 90% viable cells.

Digestion of Surface Material. After isolation, the cells of 2 kinds of melanocytes were incubated for 1 hr at 37° with trypsin (200 μg/ml; Sigma Chemical Co., St. Louis, Mo.) and DNase I (50 μg/50 μl/10^8 cells; Koch-Light, Colnbrook, England). After incubation, the cells were centrifuged, and the resulting supernatant was used for protein estimation and for polyacrylamide gel fractionation.

Protein Estimation. Total protein was determined by the method of Lowry *et al.* (15), using bovine albumin Fraction V (Sigma) as a standard.

Polyacrylamide Gel Electrophoresis Method. Proteins were fractionated electrophoretically on polyacrylamide gel columns containing sodium dodecyl sulfate according to the method of Fairbanks *et al.* (8).

Aliquots (25 or 50 μl) of the sample solution containing 250 or 500 μg proteins (in 10 to 20 μl H_2O, 5 to 10 μl 40% saccharose, 2.5 μl 10% sodium dodecyl sulfate, 2.5 μl 0.01 M EDTA, 2.5 μl pyronin, and 2.5 to 5 μl Tris-HCl buffer, pH 8) were deposited on top of the gel.

Electrophoresis was carried out for 4 hr at 5 ma/gel at room temperature. Afterwards, the gels were immediately removed from the glass tubes and stained with 0.025% Coomassie Brilliant Blue R solution in 25% isopropyl alcohol and 10% acetic acid. Destaining was carried out by soaking the gels in 7% acetic acid. Some gels were stained by the periodic acid-Schiff reaction to detect glycoproteins (8).

Molecular weights were determined by coelectrophoresing marker proteins (18). The molecular weight markers were: cytochrome *c*, M_r 12,400; chymotrypsinogen A, M_r 25,000; bovine albumin, M_r 67,000; aldolase, M_r 147,000; catalase, M_r 240,000 (Serva, Heidelberg, Germany).

Destained gels and developed films were scanned on a Zeiss microphotometer (d = 0.05 mm) fitted with a linear gel transport device.

RESULTS

An analysis of the surface material released by trypsin from isolated cells of both kinds of melanomas by polyacrylamide gel electrophoresis revealed a high heterogeneity.

The electrophoretic pattern of the surface material from both kinds of melanomas shows 9 bands staining with Coomassie blue (Charts 1 and 2). It should be stressed, however, that these fractions are not identical in both kinds of tumor, because of differences in the molecular weight and in the presence of carbohydrates, as shown by Schiff reactions (Charts 3 and 4).

The separation of the surface material from melanotic melanoma cells revealed 3 fractions staining with Coomassie blue only (Chart 1, *band* and *Peaks* 6, 7, and 8) with molecular weights between 60,000 and 20,000, and 6 fractions detected by the periodic acid-Schiff method (Chart 3; Fractions 1, 3, 4, 5, 9, and 10); these glycoprotein fractions are characterized by a diversified molecular weight (above 155,000 and below 12,000).

A component, designated as 10, with a high electrophoretic mobility, does not separate very well (R_f range, 0.85 to 0.95). It seems to be heterogeneous; besides, it is rich in carbohydrate components, as indicated by the strong staining in the periodic acid-Schiff reaction.

Separation of the surface material from the amelanotic melanoma cells showed the same number of bands stained with Coomassie blue as in its melanotic counterpart, but in this kind of tumor only one fraction (Fraction 10) does not contain the

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Table 1
Pathobiological data on transplantable melanotic and amelanotic melanoma

	Manner of appearance	Yr of appearance	Amount of tissue causing 100% transplantability[a] (mg)	Mean transplantation interval[a] (days)	Survival time of implanted hamsters[a] (days)	Most frequent location of metastases[a]	Histological structure and ultrastructure[a]	Content of carbohydrate components in surface material[c] (nmol/mg protein)	Antigenicity[d, e]	Immunogenicity[f]
Melanotic melanoma subline Ma	Developed spontaneously from a spontaneous malignant melanoma of the skin of golden hamster[g]	1959[g]	200	31 ± 3[h]	81 ± 5.8	Lungs and regional lymph nodes.	Cells characterized by numerous cytoplasmic processes, melanosomes, and premelanosomes. Golgi area is more extensive. Ribosomes are more abundant. Cells adhere more closely to each other.	1702		
Amelanotic melanoma subline Ab	Formed by a spontaneous alteration of melanotic melanoma[i]	1963[i]	50	16 ± 0.8	27 ± 1.5	Kidneys and liver.	Polygonal cells with few thin processes, without melanin and melanosomes. Loosened intercellular connections.	631	Altered in comparison to melanotic melanoma (% of inhibition of macrophage migration)	Stronger than in melanotic melanoma

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[g] Bomirski, A., Dominiczak, T., and Nowińska, L. Acta Unio Intern. Contra Cancrum, *18:* 178–180, 1962.
[h] Mean ± S.D.
[i] Bomirski, A., Nowińska, L., and Pautsch, F. International Pigment Cell Conference, pp. 252–258. Berlin: Springer-Verlag, 1966.

carbohydrate moiety whereas the remaining 8 bands are of a glycoproteinic nature (Chart 4, Bands *1, 2, 4, 5, 6, 7, 8,* and *9*).

Thus, the comparison of the electrophoretic pattern of the surface material from melanotic and amelanotic melanomas shows that, as regards their electrophoretic mobility and molecular weight, only 3 fractions (Fractions 1, 4, and 9) are common to both kinds of transplantable melanomas. Apart from the above-mentioned differences concerning Fraction 10, it should be noted that Fraction 2 (molecular weight, about 100,000) appears only in the amelanotic form of tumor (Chart 2) and cannot be observed in the original form (Chart 1), while the glycoprotein Fraction 3 (molecular weight, about 83,000) is present only in its native melanotic form (Charts 1 and 3).

DISCUSSION

The results show that a spontaneous transformation of the melanotic melanoma to an amelanotic one is associated with changes in the quality and quantity of proteins and glycoproteins in the surface material released by trypsin.

In comparison with the native melanotic form, the amelanotic melanoma showed an increased number of glycoprotein fractions. Carbohydrate components were not detectable in only one fraction.

Our previous investigations concerned with the content of glycoprotein components in the surface materials obtained after treatment with trypsin from both kinds of melanomas showed that the surface material from amelanotic melanoma is poorer in glycoprotein components than is its melanotic counterpart (10). The observations connected with the present results seem to suggest that the transformation of a melanotic form to an amelanotic one is accompanied by a lowering of the rate of glycosylation of the particular surface glycoproteins without a decrease in their heterogeneity. The studies of Finne *et al.* (9) showed some correlation between the differentiation of the rate of glycosylation of the particular surface glycoproteins and the changed biological features of different clones of melanoma.

Our present study showed also that, although the number of bands in the electrophoretic patterns was the same, only 3 fractions were common to both kinds of melanomas.

These observations show that a spontaneous transformation of a melanotic melanoma to an amelanotic form, characterized

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by an acceleration of growth, is associated with a distinct reorganization of surface membrane proteins and glycoproteins.

Perhaps the appearance of a new glycoprotein with a high molecular weight has some correlation with the changed biological features of the amelanotic melanoma mentioned above. This seems probable because, as indicated by the reports of Bramwell *et al.* (3), the surface macromolecules with molecular weights of about 100,000, visualized in different tumors including melanoma, could have some connection with the regulation of the growth of neoplastic cells.

Our results, showing a high heterogeneity of the surface glycoproteins from both kinds of melanoma, agree with the reports of other authors (14, 21).

Lloyd *et al.* (14) demonstrated the presence of 5 common glycoproteins in the surface material separated in polyacryl-

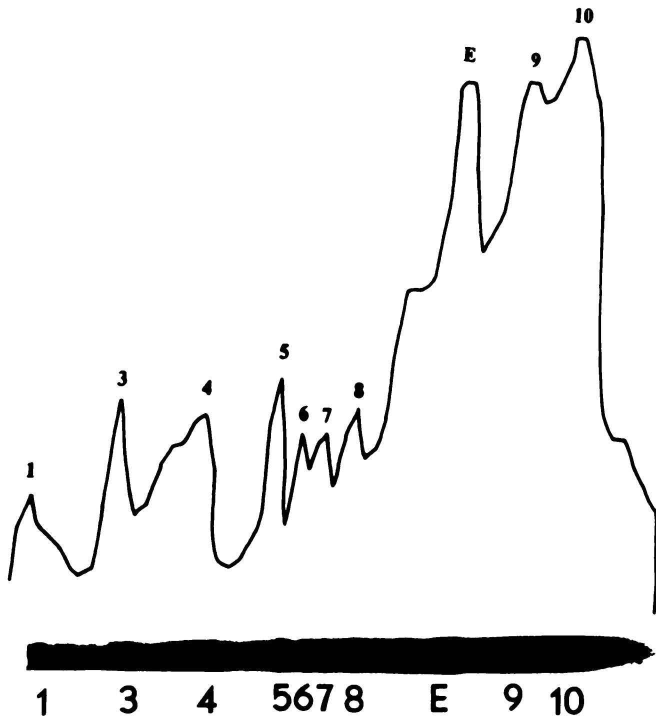

Chart 1. Densitogram and polyacrylamide gel electrophoresis of proteins from the surface material of melanotic melanoma, stained with Coomassie blue. Aliquots (50 µl) of the sample containing 500 µg protein were placed on top of each gel. The electrophoretic pattern and densitometer chart show 9 fractions with molecular weights between 60,000 and 20,000. *E*, enzymes; *abscissa*, relative mobility; *ordinate*, relative absorbance.

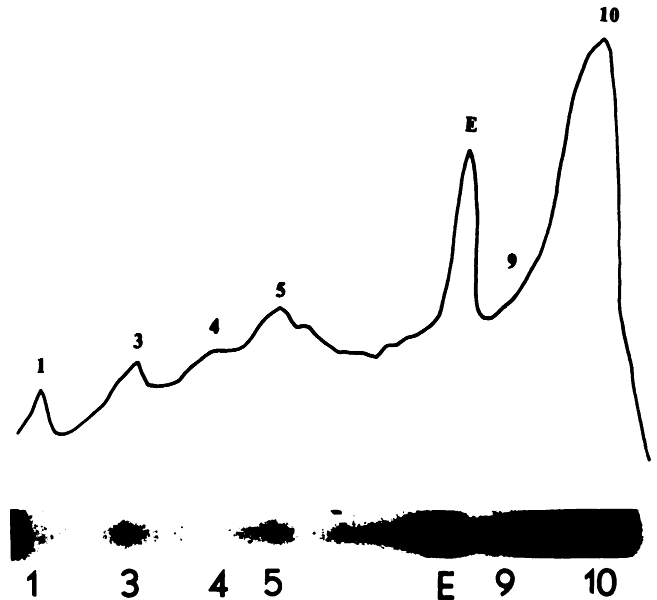

Chart 3. Densitogram and polyacrylamide gel electrophoresis of glycoproteins from the surface of melanotic melanoma, stained with Schiff reagent. The electrophoretic pattern and densitometer chart show 6 peaks and 6 bands. These glycoprotein fractions are characterized by a diversified molecular weight range (above 155,000 and below 12,000). *E*, enzymes; *abscissa*, relative mobility; *ordinate*, relative absorbance.

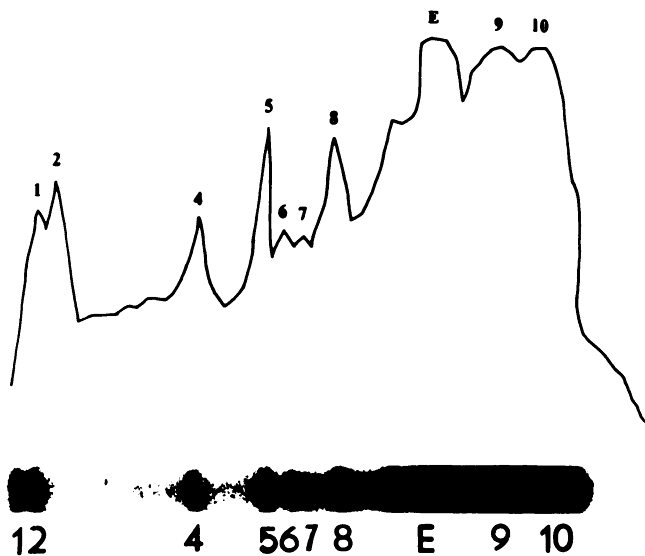

Chart 2. Densitogram and polyacrylamide gel electrophoresis of proteins from the surface of amelanotic melanoma stained with Coomassie blue. The electrophoretic pattern and densitometer chart show 9 fractions but, in comparison with the original form of the melanoma (Chart 1), the band and peak marked *3* are lost, and a new fraction (*band* and *Peak 2*) with a molecular weight of about 100,000 appears. *E*, enzymes; *abscissa*, relative mobility; *ordinate*, relative absorbance.

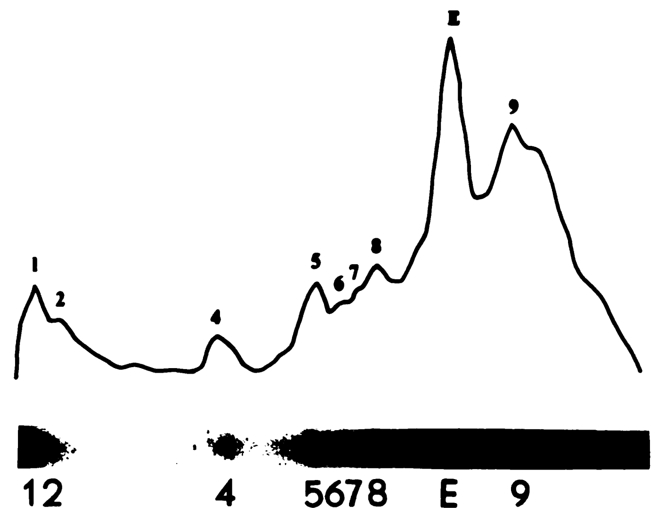

Chart 4. Densitogram and polyacrylamide gel electrophoresis of glycoproteins from the surface of amelanotic melanoma, stained with Schiff reagent. The electrophoretic pattern and densitometer chart differ from Chart 3 by the number of fractions and their molecular weight. In the amelanotic counterpart, the surface glycoproteins show a greater heterogeneity. New bands and peaks, numbered 2, 6, 7, and *8*, appear, and *bands* and *peaks* numbered 2 and *10* are lost. *E*, enzymes; *abscissa*, relative mobility; *ordinate*, relative absorbance.

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amide gel in all the melanoma lines that they examined; the molecular weight range of the glycoproteins was 22,000 to 110,000. In these studies, forms of other individual glycoproteins in the particular lines of melanoma were found.

However, at present, it is difficult to explain the significance of this heterogeneity of surface glycoproteins on different melanoma cell lines and to correlate this phenomenon with such biological properties of melanoma as an acceleration of growth or metastasizing capacity.

Our previous study of the antigenicity and immunogenicity of both kinds of melanoma indicated that the change of a less malignant melanoma into a more malignant amelanotic form was accompanied by changes in antigenicity and a pronounced immunogenicity (13).

Since the separated surface material has an antigenic activity, it is presumed that the changed electrophoretic pattern of proteins and glycoproteins is the reflection of a changed antigenicity. However, this phenomenon requires further studies.

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