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ALTERED SURFACE GLYCOPROTEINS IN MELANOMA CELL VARIANTS WITH REDUCED METASTASIZING CAPACITY SELECTED FOR RESISTANCE TO WHEAT GERM AGGLUTININ

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SUMMARY: Variants of B 16-F 1 mouse melanoma cells selected for resistance to wheat germ agglutinin (WGA) toxicity in vitro were found to have undergone a stable surface change correlated both to lectin resistance and reduced metastasizing potential. The surface alteration, as indicated by the increased electrophoretic mobilities of several lactoperoxidase-iodinated cell surface proteins in SDS-PAGE, was restricted to polypeptides able to interact with WGA. The availability of lectin-resistant melanoma cell variants having altered metastasizing behavior provides a promising approach to studies of the role of specific cell surface components in the metastasizing process.

Tumor metastasis is a multistep process in which the tumor cell surface is thought to play an important role (1,2). We have recently isolated B 16 mouse melanoma cell variants (designated Wa-4) resistant to WGA toxicity in vitro (3). Although Wa-4 cells showed no great alterations in growth rate and plating efficiency in culture, they were found to metastasize poorly in the animal in comparison to parental F-1 cells (3,4). A cell surface change is suggested by their decreased WGA binding and agglutinability (3,8). In the present study, the major surface glycoproteins capable of interacting with WGA are shown to be modified in the less metastatic Wa-4 cells.

EXPERIMENTAL PROCEDURES: Mouse B 16-F 1 cells, adapted to grow in tissue culture, were supplied by Dr. I.J. Fidler. Wheat germ agglutinin-resistant Wa-4 cells were isolated from F1 cells and cloned as described previously (3). R4c-a cells, selected from F 1 cells for resistance to Ricinus communis agglutinin (RCA), were isolated by similar procedures (4). All cells were main-

*Abbreviations: WGA=wheat germ agglutinin; Con A=concanavalin A; SDS-PAGE=sodium dodecyl sulfate polyacrylamide gel electrophoresis; MEM=minimal essential medium; FCS=fetal calf serum; PBS=phosphate buffered saline; LPO=lactoperoxidase; PMSF=phenylmethylsulfonylfluoride.

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tained in Eagle's MEM supplemented with MEM vitamins, non-essential amino acids and 10% FCS.

For surface radioiodination, cells were grown in 35 mm Falcon tissue culture dishes to near confluency. The cell monolayers were washed with PBS and radioiodinated by addition of 1 ml PBS containing $Na^{125}I$ (500 µCi), 5 mM glucose, 0.84 u. lactoperoxidase (Calbiochem, B grade) and 0.1 u. glucose oxidase (Worthington), followed by incubation for 10 min. at room temperature. They were then washed in PBS containing 50 mM NaI and dissolved in PBS-1 mM PMSF containing 1% SDS or 1% Triton X-100. The radioiodination procedure performed with lactoperoxidase conjugated to Sepharose 4B (9.61 U/ml packed beads) was similar to the above, except that LPO-bead conjugate was substituted for soluble LPO in the labeling mixture (5).

Coupling of the plant lectins concanavalin A (Con A) and WGA to CNBr-activated Sepharose beads was performed by the method of Cuatrecasas (6). Ten ml of washed, conjugated beads (1.8 mg Con A/ml or 2.1 mg WGA/ml, packed volume (Pharmacia)) were placed in small columns and equilibrated with PBS, 0.5% Triton X-100 and 1 mM PMSF. For affinity chromatography, 1 ml samples of Triton extracted proteins (after exhaustive dialysis) were applied to the columns and eluted with 10-20 ml of 100 mM α-methyl mannoside (for Con A) or 200 mM N-acetylglucosamine (for WGA). Peak fractions were pooled and concentrated by ultracentrifugation for SDS-PAGE analysis.

SDS-PAGE on slab gels was performed by the method of Laemmli (7). Following electrophoresis, gels were fixed, stained, dried on Whatman filter paper, and autoradiographed using Kodak no screen X-ray films.

RESULTS: Several prominently labeled cell-surface polypeptides appear modified in WGA-resistant Wa-4 cells, as revealed by their increased electrophoretic mobilities during SDS-PAGE (Fig. 1; A,B). These qualitative changes were not present in RCA-resistant R4c-a cells (Fig. 1; C), which were also similar to parental F 1 cells in their sensitivity to WGA *in vitro* (3). Thus, the surface alteration seen in Wa-4 cells is associated with the lectin used for selection, rather than the selection procedure *per se*. A similar analysis of seven other WGA-resistant clones, originating from the same cell line Wa-3, all showing reduced metastasizing capacity and tumorigenicity, revealed the same surface changes.

Since lactoperoxidase itself comigrated with some of the major labeled bands in several SDS-PAGE systems, a solid-state labeling procedure was performed to control for possible contamination of cells by self-iodinated LPO (5). This method,

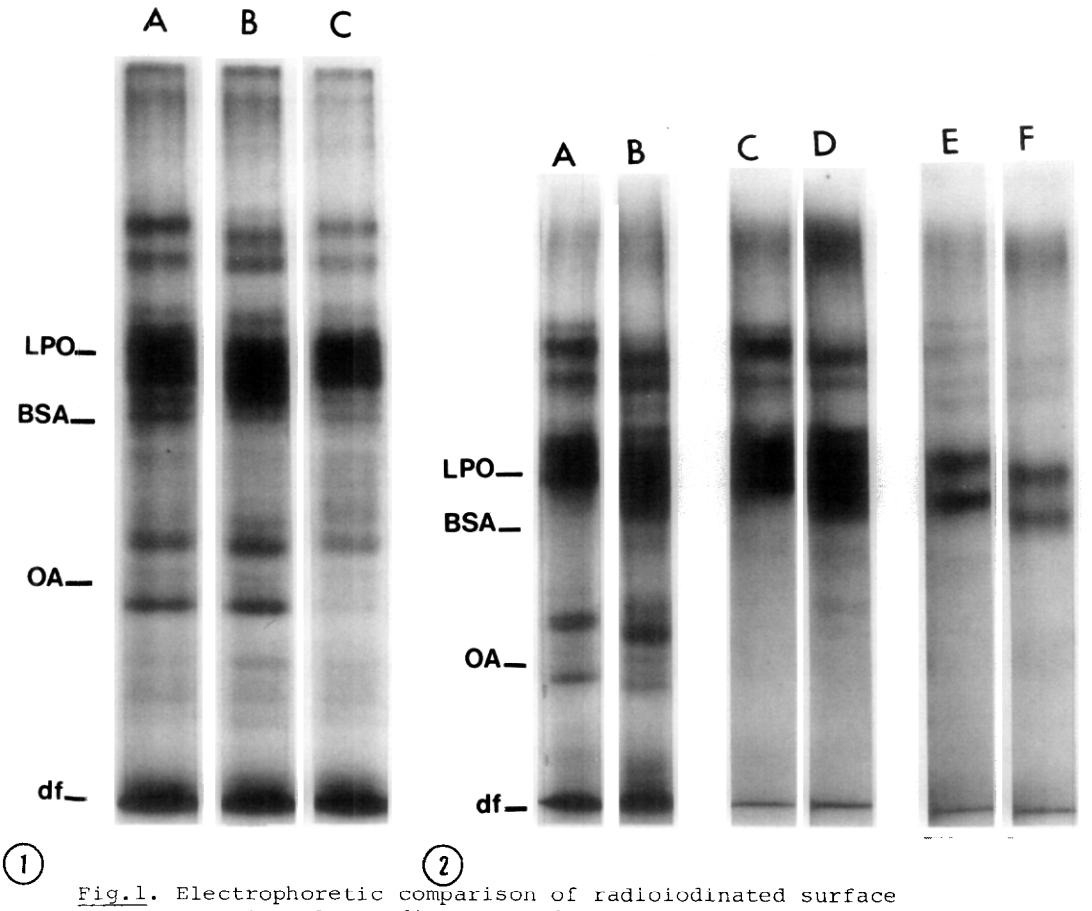

Fig.1. Electrophoretic comparison of radioiodinated surface proteins. Autoradiogram of 8% gel in SDS showing (A) F 1 cells, (B) Wa-4 cells, (C) R4c-a cells. Positions of molecular weight markers - lactoperoxidase (LPO, 75K), bovine serum albumin (BSA, 68K), and ovalbumin (OA, 45K) as indicated, df= dye front.

Fig.2. Lectin binding fractions of iodinated surface proteins. Autoradiograms of 8% gels of (A) F 1 cells labeled with LPO-agarose conjugate, (B) Wa-4 cells labeled with LPO-agarose. Fractions bound to WGA affinity columns from (C) F 1 and (D) Wa-4 cells. Fractions bound to Con A affinity columns from (E) F 1 and (F) Wa-4 cells.

however, yielded labeled polypeptide profiles (Fig. 2,A) which were qualitatively similar to the results obtained using soluble LPO (Fig. 1).

To identify potential cell surface receptor proteins for WGA and Con A, LPO-iodinated cells were extracted with Triton X-100, and the solubilized material was analyzed by affinity chromatography on WGA and Con A-Sepharose columns. Wa-4 and F 1

TABLE I
Binding of Radioiodinated Surface Proteins to Lectin-Agarose Affinity Columns

Cell Type	Lectin Affinity Column	Specifically Bound Fraction[a] (% of total c.p.m.[b] applied to column)
F 1	Con A	22
Wa-4	Con A	28
F 1	WGA	34
Wa-4	WGA	38

[a]Bound material eluted with specific hapten sugar (see Methods)

[b]TCA precipitable counts

cells did not appear to differ in the fraction associated specifically with the WGA or Con A affinity columns (Table I), arguing against any large differences in the number of lectin binding sites. On the other hand, binding experiments (8) have shown a dramatically reduced affinity of WGA binding to intact Wa-4 cells, suggesting that WGA receptors are probably modified in these cells. SDS-PAGE analysis of WGA-binding and Con A-binding surface polypeptides (Fig. 2, C,D) shows that the surface proteins modified in Wa-4 cells are potential WGA-binding sites. Some of these sites may interact with Con A as well (Fig. 2, E,F).

When co-cultured Wa-4 and F 1 cells were radioiodinated with LPO, a mixed cell-surface labeling profile was obtained (Fig. 3, B), which reflected the co-dominant expression of both cell surface phenotypes (Fig. 3, A,C). This result suggests that the surface change in Wa-4 cells is not produced by glycosidic or proteolytic activity present in the culture medium. However, treatment of iodinated F 1 cells with Vibrio cholera neuraminidase produced electrophoretic shifts in several surface glycoproteins shown to be modified in Wa-4 cells (Fig. 3,D), suggesting that differences in sialic acid moeities may partly account for the changes observed in the variant cells.

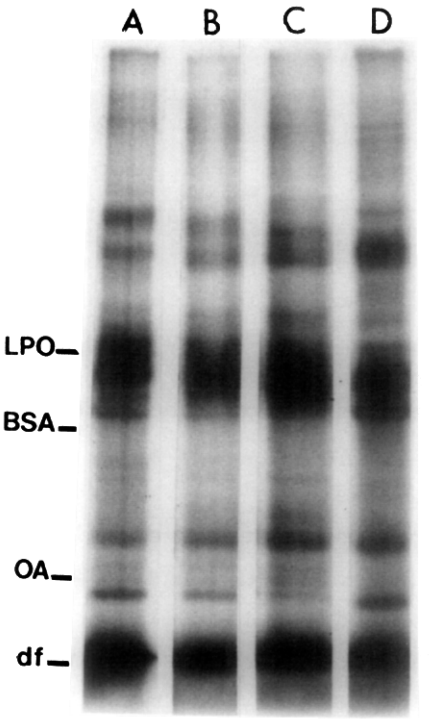

Fig.3. Surface polypeptide profiles in co-cultured F 1 and Wa-4 cells. Autoradiograms of 8% gels in SDS showing (A) F 1 cells, (B) mixed culture of F 1 and Wa-4 cells (approx. 1:1), (C) Wa-4 cells, (D) iodinated F 1 monolayer treated with V. cholera neuraminidase (50 µg/ml, 20 min., room temp.

DISCUSSION: Variants of melanoma cells selected for resistance to WGA are found to contain modified surface glycoproteins which represent potential WGA receptor sites on the intact cells. WGA-binding studies to intact cells showed reduced binding to WGA-resistant cells (3, 8). The WGA affinity chromatography studies reported here argue against major differences in the number of potential binding sites. However, SDS-PAGE analysis showed that the WGA-binding proteins in the WGA-resistant cells had increased electrophoretic mobilities, i.e., the binding sites may be modified. These results agree well with binding studies which showed that there were no great differences in the number of binding sites and that the major difference was in the binding affinity (8). That the chemical basis for these differences could involve changes in sialic acid-containing oligosaccharides was indicated by the observation that electro-

phoretic shifts of comparable magnitude could be produced by enzymatic removal of sialic acid. Analysis of the glycopeptides prepared from cellular glycoproteins indicates indeed a decrease of sialic acid but an increase in the fucose content of the oligosaccharide chains (9). Altered glycosylation has been observed in lectin-resistant variants of other cultured cells (10). Anomalies in the electrophoretic behavior of glycoproteins in SDS-PAGE due to differences in glycosylation are well documented (11).

Terminal sialic acid residues have been implicated in WGA binding to other eukaryotic cells (12), and have been suggested to play a role in metastasis (13). Using in vivo selection techniques, Fidler reported to have isolated cell lines from B 16-F 1 cells with increased metastasizing potential when injected into the host circulation (1, 2, 14). Although these cells lacked surface modifications of the type seen in the present study using SDS-PAGE, the more "metastatic" cells were reported to be enriched in sialic acid and were more agglutinable by WGA (1, 14). Comparisons of these separate studies are complicated due to the different definitions and methods in assaying metastasis. Further investigations, employing relevant assays for the various stages of metastasis, such as cell motility, invasiveness, adhesion, and tissue recognition, will be needed to evaluate the role of specific cell surface components in the metastatic process.

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REFERENCES:

1. Nicolson, G.L., Birdwell, C.R., Brunson, K.W., Robbins, J.C., Beattie, G., and Fidler, I.J. (1977) in Cell and Tissue Interactions (M.M.Burger and J. Lash, eds.), pp. 225-241. Raven Press, N.Y.
2. Fidler, I.J., Gersten, D.M., and Hart, I.R. (1978) Adv. Cancer Res. 28, 149-250.
3. Tao, T.W., and Burger, M.M. (1977) Nature 270, 437-438.
4. Tao, T.W., unpublished data.

5. David, G., and Reisfeld, R.A. (1974) Biochem. 13, 1014-1021.
6. Cuatrecasas, P. (1970) J. Biol. Chem. 245, 3059-3065.
7. Laemmli, U.K. (1970) Nature 227, 680-685.
8. Haskovec, C., Tao, T.W., and Burger, M.M. manuscript in preparation.
9. Finne, J., Tao, T.W., and Burger, M.M., Cancer Res., in press.
10. Stanley, P., Narashimhan, S., Siminovitch, L., and Schachter, H. (1975) Proc. Nat. Acad. Sci. 72, 3323-3327.
11. Bretscher, M.S. (1971) Nature N.B. 231, 229-232.
12. Robinson, P.J., Bull, F.G., Anderton, B.H., and Roitt, I.M. (1975) FEBS Lett. 58, 330-333.
13. Bosmann, H.B., Bieber, G.G., Brown, A.E., Case, K.K., Gersten, D.M., Kimmerer, T.W., and Lione, A. (1973) Nature 246, 487-489.
14. Fidler, I.J. (1973) Nature 242, 148-149.