Int. J. Cancer: **36,** 623–628 (1985)

IMPROVED DETECTION OF LABILE CELL-SURFACE COMPONENTS WITH ZINC CHLORIDE-APROTININ: DEMONSTRATION OF GLYCOPROTEIN DIFFERENCES IN K-1735 METASTATIC MELANOMA VARIANTS

Manuel RIEBER and Mary S. RIEBER

Centre of Microbiology and Cell Biology, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas 101, Venezuela.

Metabolic labelling of K-1735 melanoma variants with ^{3}H-glucosamine and cell harvesting with the commonly used protease inhibitor phenylmethylsulfonylfluoride revealed a Triton-insoluble fibronection-like 230 kd component in poorly metastatic cells. This component was not evident in highly metastatic cells. Significantly improved surface labelling and detection of the 230 kd glycoprotein in the highly metastatic variant was achieved by zinc chloride-aprotinin treatment of cells prior to harvesting. This procedure also revealed an increase in a trypsin-sensitive glycoprotein of higher molecular weight in the Triton-insoluble fraction of the highly metastatic cell variant. Glycoprotein labelling in this fraction showed an electrophoretic pattern strongly resembling that reported by others for the high-molecular-weight human melanoma-associated glycoprotein complex. The differential detection of the high-molecular-weight glycoprotein species in melanoma variants with differing metastatic abilities in an animal model provides a means of studying their possible relevance to metastatic melanoma. Our data also suggest that zinc chloride-aprotinin can be used to improve the detection of labile cell-surface components.

The invasiveness of metastatic cells may be related to their ability to survive following detachment from the primary tumor, a property which may be mediated by cell-surface and matrix-associated components. Recent *in vivo* experiments support a role for the surface membrane in metastatic behavior by showing that membrane vesicles from highly metastatic cells can influence the invasive ability of poorly metastatic subpopulations when fused with the latter (Poste and Nicolson, 1980). Other correlations of metastatic potential with external protein sialylation (Yogeeswaran and Salk, 1981) and cell-surface protein alterations (Rieber and Rieber, 1981) also suggest a role for the cell surface in tumor-cell metastasis. However, in addition to the known differences between normal and transformed cells, it is important to develop methods of revealing differences between cells from the same tumor, since metastasis presumably results from preexisting tumor variants (Fidler and Kripke, 1977).

In recent years there has been considerable interest in the role of proteases in tumor growth. In the particular case of cultured melanoma cells, elevated protease levels have been found to correlate with metastatic ability (Liotta *et al.*, 1980; Wang *et al.*, 1980; Kramer *et al.*, 1982). Hence, it may well be that a number of reports concerning the absence of specific surface or matrix components from tumor variants may be due to their degradation during *in vitro* processing. In such a context, it is worth emphasizing that cells which possess fibronectin lose this protein following brief formaldehyde treatment, as detected by immunofluorescence (Hsieh *et al.*, 1980) or by biochemical means (Rieber and Rieber, 1982). Since we are interested in the biological relevance of matrix and cytoskeleton association of cell-surface proteins, we have carried out sequential isolations of Triton-soluble membrane proteins and those surface components which remain linked to the detergent-insoluble cytoskeleton. During the course of previous studies on differential matrix association, it has been observed that cytoskeletal matrices from tumor, but not from normal cells, detach from their growth substratum (Brown *et al.*, 1976; Rieber *et al.*, 1984). This effect may be due to the absence or degradation of macromolecules which usually anchor the matrix from normal cells to the substrate (Carter, 1982). Since our preliminary studies on surface labelling of K-1735 melanoma metastatic variants usually gave poor labelling, and since our previous observations have indicated that tumor matrix detachment and partial cleavage of detergent-insoluble proteins could be prevented by zinc ion pretreatment (Rieber *et al.*, 1984), we decided to test the effect of zinc ions on the detection of glycoprotein differences in the K-1735 variants. The results reported here suggest that zinc chloride in combination with the protease inhibitor, aprotinin, can reveal novel differences in high-molecular-weight glycoproteins from tumor cells with different metastatic potentials.

MATERIAL AND METHODS

Cells

(1) The K-1735 melanoma metastatic variants (Fidler *et al.*, 1981) were kindly given by Dr. I.J. Fidler (Houston, TX). The cell variants were grown as monolayers in Dulbecco's medium supplemented with 4.5 g/l glucose, 1× non-essential amino acids and 10% fetal calf serum (FCS), either for surface labelling or for *in vivo* assay. In the latter case, cells were detached with 0.5 mM EDTA in medium without serum, for intravenous injection. Inoculation of 2×10^5 cells into 6- to 9-week-old C_3H mice revealed after a 30-day period a median of 200 (170–285) lung colonies when the *met* highly metastatic variant was used, whereas the median of lung colony formation was 7 (1–19) when the same number of *low* poorly metastatic variant cells was similarly injected, in agreement with others (Fidler *et al.*, 1981; Kripke, 1979). (2) Normal rat kidney (NRK) fibroblasts were grown in the same medium as used for the melanoma cells, as previously described (Rieber and Rieber, 1982).

Received: April 11, 1985 and in revised form June 15, 1985.

In vitro *labelling*

Surface labelling of cell monolayers was carried out after extensive washing of the cells with medium to remove serum-adsorbed components, using 40 μg of lactoperoxidase (14.7 IU/mg, Behring Diagnostics, Marburg, FRG), 5μg glucose oxidase (110 U/mg; Worthington, Freehold, NJ) and 1.5 ml NaI^{125} (NEZ-033H; New England Nuclear, Boston, MA) in PBS with 5 mM glucose, using 1.8×10^6 cells per iodination for 10 min at 22°C. Cells were then washed in PBI in which NaCl was replaced by the same molar concentration of NaI, and cultures were harvested with a rubber policeman in PBS containing 0.002 M phenylmethylsulfonyl-fluoride (PMSF) (Rieber and Rieber, 1981). Metabolic labelling with ^3H-glucosamine in glucose-free medium supplemented with sodium pyruvate, non-essential amino acid and 10% FCS was carried out with an addition of bromodeoxyuridine (2.5 μg/ml whenever indicated) as described previously (Rieber *et al.*, 1984). Zinc treatment was carried out with serum-free monolayers which were exposed to zinc chloride 0.01 M in 0.15 M NaCl or to 0.15 M NaCl prior to iodination, unless otherwise indicated. Subsequently, cells were surface-labelled and sequentially solubilized with 1% Triton in TNM buffer (Tris-HCl 0.05 M, NaCl 0.15 M, $Mg(OAc)_2$ 0.005 M, pH 7.4) containing 0.002 M phenylmethylsulfonyl fluoride (PMSF). The detergent-insoluble components were collected at 12,000 × for 5 min for further solubilization with 8 M urea-25 mM β-mercaptoethanol, 0.002 M PMSF, and subsequently with dissociating buffer (2% SDS, 0.1 M β-mercaptoethanol, 0.1 M Tris-HCl, pH 6.8, 0.002 M PMSF), or directly in dissociation buffer. In every case, samples received a final concentration of the latter buffer for heating at 90°C for 3 min and subsequent electrophoresis in 7.5% SDS-polyacrylamide gels (Laemmli, 1970) and autoradiography.

RESULTS

Effect of zinc on the differential surface labelling of K-1735 melanoma metastatic variants

Electrophoretic analysis of matrix-associated and Triton-soluble fractions from surface-iodinated melanoma cells without zinc pretreatment usually led to low and diffuse labelling in the poorly metastatic variants, and to an even more diffuse pattern from highly metastatic cells (Fig. 1, lanes A,I,E,M). However, when identical cell numbers from simultaneously seeded cultures were surface-iodinated following zinc chloride pretreatment, we detected well-defined labelling in the 75 and 55-kd region in both Triton-soluble and Triton-insoluble fractions from the more metastatic cells (Fig. 1, lanes G,O). When these cells were iodinated after

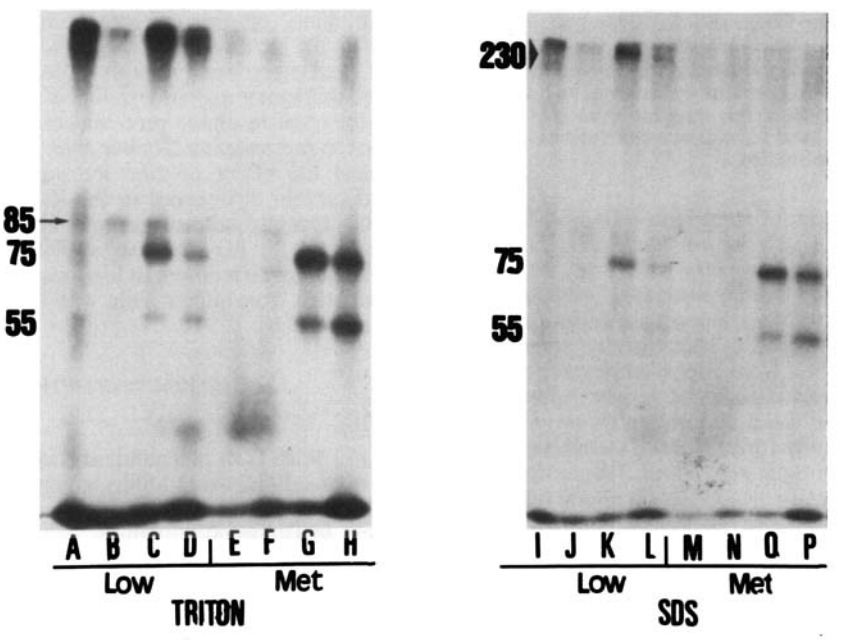

FIGURE 1 – *Effect of zinc on the surface labelling of K-1735 melanoma metastatic variants.* Cell monolayers were labelled by enzymatic radioiodination and exposed to $ZnCl_2$ 0.01 M in 0.15 M NaCl for 10 min whenever indicated for sequential solubilization and electrophoretic analysis, as indicated in "Material and Methods". A-D, Triton-soluble surface proteins from poorly metastatic cells (*low*); E-H, Triton-soluble surface proteins from highly metastatic cells (*met*); I-L, matrix-associated proteins from poorly metastatic cells; M-P, matrix-associated proteins from highly metastatic cells. Lanes A,E,I and M represent surface proteins from control cells. Lanes B,F,J and N; surface proteins from cells iodinated and then exposed to zinc chloride. Lanes C,G,K and O; surface proteins from cells exposed to zinc chloride *prior* to iodination. Lanes D,H,L, and P; surface protein from cells iodinated after exposure to zinc chloride and then exposed in monolayers to 1 μg/ml 5 × recrystallized trypsin for 10 min at 37°C in a reaction stopped by 20 μg/ml soybean trypsin inhibitor. All surface labelling experiments were carried out with identical numbers of cells (1×10^6/9-cm tissue culture dish) simultaneously seeded for 24 hr for subsequent parallel surface labelling.

exposure to zinc chloride and then treated with 1 μg/ml crystalline trypsin in PBS for 10 min, the corresponding patterns revealed an increase in the 55-kd region (Fig. 1, lanes G,H,O,P). This suggests that the 55-kd surface component may originate from trypsin-like activity in the highly metastatic cells.

Examination of the patterns from poorly metastatic cells also revealed a favorable effect of zinc chloride pretreatment. The corresponding Triton-soluble fraction showed relatively increased surface iodination of an 85-kd component compared with the *met* cells (lanes C,G) as well as preferential trypsin susceptibility in the 75-kd and 85-kd species (lanes C,D). Analysis of the Triton-insoluble surface molecules showed components of higher molecular weight preferentially in the poorly metastatic low variant which resembled similarly migrating components in the 230-kd region observed in the Triton-soluble fraction of the same cells. In addition, the SDS-soluble fraction of poorly metastatic cells also exhibited a trypsin-sensitive 75-kd species, whereas the *met* variant showed labelling of 75 and 55-kd components in the same fraction variant (lanes K,O). Finally, it is of interest that the effect of zinc chloride was manifested optimally prior to iodination. Studies carrying out surface iodination and then exposing cells to zinc chloride gave weaker and more diffuse labelling than that obtained when cells were pretreated with zinc chloride prior to iodination (lanes B,F,J,N). Moreover, lanes A *vs.* B and I *vs.* J show a decreased labelling in slowly migrating species, which may be due to the fact that after enzymatic iodination by oxidation reactions, cells are further incubated in serum-free medium prior to harvesting. Since cells are washed repeatedly before and after iodination and exposure to zinc, to remove extraneous proteins and non-incorporated label, this may lead to destabilization of surface components, which seems to be prevented if membrane components are stabilized prior to surface iodination (Warren *et al.*, 1966).

Changes in Triton-soluble and matrix-associated glycoproteins

We also carried out metabolic labelling of glycoproteins by growing the metastatic cell variants with ^{3}H-glucosamine to determine whether the differences in iodination found in the metastatic variants could be also correlated to glycoprotein changes. We recently demonstrated that in B16 melanoma metastatic variants, differential glycoprotein patterns were more easily demonstrated by comparison of bromodeoxyuridine-grown and control cells by sequential solubilization (Rieber *et al.*, 1984). No significant effect of bromodeoxyuridine or zinc chloride pretreatment was evident with Triton-soluble glycoproteins which revealed essentially similar patterns with or without zinc chloride. Nevertheless, a 100-kd glycoprotein and a minor 190-kd component appeared to be increased in the Triton-soluble fraction from highly metastatic cells compared to the corresponding fraction from poorly metastatic cells (Fig. 2, lanes 1-4). Analysis of the Triton-insoluble fractions revealed mostly the presence of a 230-kd matrix-associated glycoprotein in poorly metastatic cells, even when these fractions were prepared from cells grown without bromodeoxyuridine or collected without treatment with zinc chloride (Fig. 2, lanes 5-8). However, the corresponding fraction from highly metastatic cells obtained

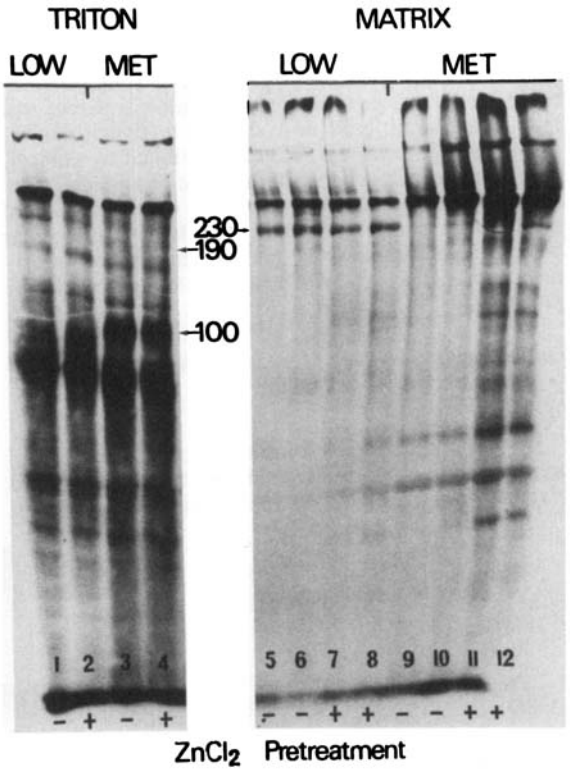

FIGURE 2 – *Differences in metabolic labelling of glycoproteins from K-1735 melanoma variants.* Control and bromodeoxyuridine-grown cells were metabolically labelled with ^{3}H-glucosamine for 48 hr, treated with zinc chloride whenever indicated and solubilized with Triton-containing solution or with 8 M urea-β-mercaptoethanol 25 mM buffer, for electrophoretic analysis as described in "Material and Methods". Lanes 1 - 4, Triton-soluble glycoproteins from poorly metastatic (*low*) and highly metastatic (*met*) cells. Lanes 5-8, matrix-associated glycoproteins from poorly metastatic cells. Lanes 9 - 12, matrix-associated glycoproteins from highly metastatic cells. Lanes with even numbers correspond to fractions from bromodeoxyuridine-grown cells.

without zinc pretreatment showed greater labelling in the very high molecular weight region, but very weak labelling in the 230-kd region in bromodeoxyuridine-grown cells compared to control cells (Fig. 2, lanes 9,10). Significant labelling at the top of the gel but diffuse labelling in the 230-kd region and in faster migrating components was observed in Triton-insoluble preparations obtained with zinc chloride treatment, in both bromodeoxyuridine-grown and control cells (Fig. 2, lanes 11,12).

Differential detection of high-molecular-weight glycoproteins in melanoma metastatic variants

Although growth in bromodeoxyuridine and zinc chloride treatment prior to harvesting of ^{3}H-glucosamine-labelled cells improved the detection of the high-molecular-weight glycoprotein appearing at the top of the gel in the *met* variant cells, neither treatment permitted the clearer detection of the 230-kd glycoprotein in these cells. Hence, we investigated whether this was due to insufficient glycoprotein stabilization related to protease activity manifested during processing of the

Triton-insoluble fraction. To test this, cell variants were labelled with ^{3}H-glucosamine and processed for matrix preparation and analysis using zinc chloride-pretreated cells lysed in the presence of 1 mg/ml aprotinin. Under these conditions it became apparent that highly metastatic cells now showed a 230-kd matrix-associated glycoprotein like that exhibited by control, poorly metastatic cells in the absence of zinc chloride-aprotinin. However, even with the combined use of zinc chloride and aprotinin, the higher molecular weight glycoprotein was not evident in the poorly metastatic cells even with aprotinin and zinc chloride processing (Fig. 3 A-D).

Zinc chloride-aprotinin decreases protease degradation of high-molecular-weight glycoproteins

We attempted to gain some insight into the mode of action of zinc chloride in stabilizing matrix-associated glycoproteins from highly metastatic cells. For this, matrix fractions were prepared from cultures briefly exposed to low trypsin levels and from control cells, including a zinc chloride pretreatment prior to exposure to protease whenever indicated. It became apparent that mild trypsin treatment decreased the high-molecular-weight glycoproteins compared with fractions not exposed to trypsin or to similar fractions exposed to trypsin after zinc chloride pretreatment (Fig. 4, lanes A-C). The reason why the apparent stabilizing effect of zinc chloride against trypsin treatment was more pronounced in these experiments than in those shown in Figure 1, is attributed to the absence of aprotinin during harvesting of the cells in the surface iodination experiments.

Stabilization of Triton-insoluble components by zinc ions in normal rat kidney cells

An additional indication of a stabilizing effect of zinc on detergent-insoluble components came from experiments in which NRK cells were labelled with ^{35}S-methionine, and extracted with 1% Triton in TNM

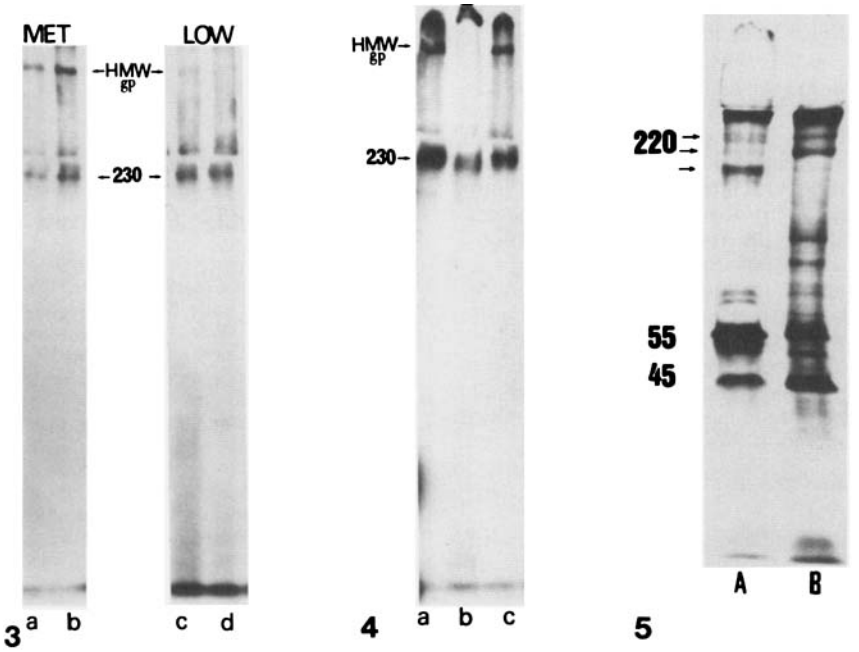

FIGURE 3 – *Zinc and aprotinin permit the detection of high-molecular-weight matrix-associated glycoproteins in highly metastatic cells.* Cells were metabolically labelled with ^{3}H-glucosamine for matrix preparation, being harvested with aprotinin (1 mg/ml) and zinc chloride pretreatment prior to cell lysis. Subsequently, Triton-insoluble fractions were analyzed by SDS-polyacrylamide gel electrophoresis. A and B, Matrix-associated glycoproteins from different passages of highly metastatic cells (*met*). C and D, Matrix-associated glycoproteins from different passages of poorly metastatic cells (*low*). Note the presence of a high-molecular-weight glycoconjugate (HMW gp) in highly metastatic cells.

FIGURE 4 – *Trypsin susceptibility of the high-molecular-weight glycoproteins from metastatic cells is decreased by zinc chloride.* Cultures of the highly metastatic cell variant were metabolically labelled with ^{3}H-glucosamine including a mild exposure to trypsin as described in Figure 1 prior to zinc chloride treatment, harvesting with aprotinin and analysis as described in Figure 3. A, Control matrix fraction without exposure to protease; B, matrix fraction exposed to protease prior to zinc chloride; C, matrix fraction exposed to zinc chloride and then to protease.

FIGURE 5 – *Zinc-mediated changes in matrix-associated polypeptides.* NRK cells were metabolically labelled with ^{35}S-methionine and extracted with TNM containing 1% Triton X-100, for solubilization in dissociation buffer and electrophoretic analysis, as indicated in "Material and Methods". A, Triton-insoluble polypeptides from control cells; B, Triton-insoluble polypeptides from zinc-treated cells.

buffer prior to electrophoretic analysis. Although the SDS-soluble residual components of high molecular weight were detected in both zinc-pretreatment and control cells, the latter revealed a relative weakening of their 220-kd doublet and an extra band in the 190-kd region which may correspond to cleavage of fibronectin-like components (Fig. 5A,B). Similar results were obtained with rat-1 fibroblasts, which also revealed a stabilization of their fibronectin-like component, which migrated like a single 220-kd fibronectin when prepared from zinc + aprotinin or zinc-pretreated cells, in contrast to the presence of a doublet of about 210 kd in cells not pretreated with zinc chloride prior to processing (not shown).

DISCUSSION

We have now shown that exposure to zinc ions prior to enzymatic surface iodination can be used to improve significantly the external labelling of K-1735 melanoma cells which are very poorly labelled by the usual lactoperoxidase-mediated iodination procedures. We have also shown that zinc chloride-aprotinin permits the detection of novel surface glycoprotein differences between melanoma metastatic variants. Although it is premature at this stage to relate the differences now described for the K-1735 melanoma variants with their different *in vivo* lung colonizing behavior, our approach should be useful in further studies of tumor and organ cell heterogeneity (Fidler and Kripke, 1977) in which protease degradation during isolation may mask the identification of surface and matrix-associated macromolecules. That protease activity may partly explain the differences in surface glycoproteins, such as those now found between K-1735 melanoma metastatic variants, was supported by studies using mild trypsin treatment. These experiments revealed that brief exposure to low levels of protease led to an increase of a 55-kd surface protein which was present in the highly metastatic cells, even without trypsin treatment, but absent in poorly metastatic counterparts. Also, metabolic labelling with ^{3}H-glucosamine suggested a differential protease digestion of matrix-associated components from the melanoma variants. This was shown by the fact that the presence of a glycoprotein of about 230 kd in the Triton-insoluble fraction from poorly metastatic cells could be shown under all conditions, whereas a comparable component and glycoproteins of higher molecular weights could only be detected in highly metastatic cells when the cells were processed with zinc chloride and aprotinin. In contrast, use of only PMSF was insufficient to prevent protease degradation in highly metastatic cells. Although the electrophoretic properties of the Triton-insoluble glycoproteins now detected with zinc-aprotinin treatment in highly metastatic cells are remarkably similar to those found in human melanoma cells (Natali *et al.*, 1981; Bumol and Reisfeld, 1982; Hellström *et al.*, 1983), the human antigen can be extracted by non-ionic detergents (Hellström *et al.*, 1983) or by salt procedures that extract peripheral membrane proteins (Natali *et al.*, 1981) in contrast with the non-ionic detergent insolubility of our high-molecular-weight glycoprotein. Further studies with specific antibodies should clarify whether there are different forms of this molecule in melanoma metastatic variants and whether matrix-anchoring molecules for this glycoprotein have some significance in melanoma tumor growth. Also, if this high-molecular-weight glycoprotein proves to be related to the human melanoma-associated antigen (Bumol and Reisfeld, 1982), it may be useful to elucidate its role in experimental metastasis in animals, which cannot be tested with humans. With regard to the matrix-associated 230-kd glycoprotein now detected in K-1735 cells, its Triton insolubility, gelatin-binding properties (not shown) and its molecular weight make it similar to a fibronectin-like component also reported to be decreased in highly metastatic K-1735 melanoma variants (Volk *et al.*, 1984). We believe that the latter finding may be partly due to the susceptibility of this 230-kd glycoprotein to undergo degradation when cells are processed without zinc-aprotinin treatment or when melanoma fibronectin is shed into the medium (Bumol and Reisfeld, 1983).

Our study shows that proper control of the conditions used to lyse and process cell extracts can permit the isolation of otherwise undetectable surface and matrix-associated components which may be shed at significant rates in metastatic cells (Bumol and Reisfeld, 1982; Wilson *et al.*, 1981). A zinc inhibition of the shedding of surface molecules may play a role in its effect. Another possible mechanism for the zinc chloride effect on surface iodination may be an interaction of Zn^{2+} ions with external anionic groups on the cells. However, the fact that zinc chloride also promotes the detection of high-molecular-weight glycoproteins in cells metabolically labelled with ^{3}H-glucosamine and the observation that the zinc effect is further enhanced in some cases by aprotinin, strongly suggests that it may be stabilizing external components by preventing their loss or degradation during isolation. This loss may be partly explained by a possible zinc-mediated inhibition of proteases which are known to be present in increased amounts in metastatic cells (Liotta *et al.*, 1980; Wang *et al.*, 1980; Kramer *et al.*, 1982). Support for this possible role of zinc chloride came from the experiments in which the degradation of high-molecular-weight glycoproteins by mild protease was decreased by zinc pretreatment of the cells.

In summary, these results now show that it may be useful to monitor surface protein differences using zinc chloride to facilitate detection of labile cell-surface components such as those extracted from tumors grown *in vivo*. Our data also suggest that differential turnover of surface and matrix components in metastatic melanoma variants may be of importance in their invasive behavior. Finally, these observations suggest that the combined use of zinc chloride and aprotinin may be of more general utility in the identification and isolation of cell surface antigens and matrix-associated components.

ACKNOWLEDGEMENTS

This work was completed and written while the authors were Visiting Scientists at the La Jolla Cancer Research Foundation. We thank Dr. E. Ruoslahti for his hospitality, Ms. N. Beddingfield for excellent typing skills and the International Union Against Cancer for providing an Eleanor Roosevelt Cancer Research Fellowship for this visit.

REFERENCES

BROWN, S., LEVINSON, W., and SPUDICH, J.A., Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. *J. supramolec. Struct.*, **5,** 119–120 (1976).

BUMOL, T.F., and REISFELD, R.A., Unique glycoprotein-proteoglycan complex defined by monoclonal antibody on human melanoma cells. *Proc. nat. Acad. Sci. (Wash.)*, **79,** 1245–1249 (1982).

BUMOL, T.F., and REISFELD, R.A., Biosynthesis and secretion of fibronectin in human melanoma cells. *J. cell. Biochem.*, **21,** 129–140 (1983).

CARTER, W.G., The cooperative role of the transformation-sensitive glycoproteins, GP 140 and fibronectin in cell attachment and spreading. *J. biol. Chem.*, **257,** 3249–3257 (1982).

FIDLER, I.J., GRUYS, E., CIFONE, M.A., and BARNES, Z., Demonstration of multiple phenotypic diversity in murine melanoma of recent origin. *J. nat. Cancer Inst.*, **67,** 947–956 (1981).

FIDLER, I.J., and KRIPKE, M.L., Metastasis results from preexisting variant cells within a malignant tumor. *Science*, **197,** 893–895 (1977).

HELLSTRÖM, I., GARRIGUES, J., CABASCO, L., MOSELY, G.H., BROWN, J.P., and HELLSTRÖM, K.E., Studies of a high molecular weight human melanoma-associated antigen. *J. Immunol.*, **130,** 1467–1472 (1983).

HSIEH, P., SEGAL, R., and CHEN, L.B., Studies of fibronectin matrices in living cells with fluoresceinated gelatin. *J. Cell Biol.*, **87,** 14–22 (1980).

KRAMER, R.H., VOGEL, K.G., and NICOLSON, G.L., Solubilization and degradation of subendothelial matrix glycoproteins and proteoglycans by metastatic tumor cells. *J. biol. Chem.*, **257,** 2678–2686 (1982).

KRIPKE, M.L., Speculations on the role of UV radiation in the development of malignant melanoma. *J. nat. Cancer Inst.*, **63,** 541–548 (1979).

LAEMMLI, U.K., Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature (Lond.)*, **270,** 57–59 (1970).

LIOTTA, L.A., TRYGVASSON, L., GARBISA, S., HART, I., FOLTZ, C.M., and SHAFIE, S., Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature (Lond.)*, **284,** 67–68 (1980).

NATALI, P.G., IMAI, K., WILSON, B.S., BIGOTTI, A., CAVALIERI, R., PELLEGRINO, M.A., and FERRONE, S., Structural properties and tissue distribution of the antigen recognized by the monoclonal antibody 653.405 to human melanoma cells. *J. nat. Cancer Inst.*, **67,** 591–601 (1981).

RIEBER, M., and RIEBER, M.S., Metastatic potential correlates with cell surface protein alterations in B16 melanoma variants. *Nature (Lond.)*, **293,** 74–76 (1981).

RIEBER, M., and RIEBER, M.S., Loss of fibronectin among the selective surface proteins changes associated with p-formaldehyde promoted membrane vesiculation. *Biochem. Biophys. Res. Comm.*, **104,** 844–850 (1982).

RIEBER, M., RIEBER, M.S., URBINA, C., and LIRA, R., Relationship of a novel extracellular matrix glycoprotein to cell detachment in highly metastatic B16 melanoma. *Int. J. Cancer*, **34,** 427–432 (1984).

VOLK, T., GEIGER, B., and RAZ, A., Motility and adhesive properties of high- and low-metastatic murine neoplastic cells. *Cancer Res.*, **44,** 811–824 (1984).

WANG, B.S., MCLOUGHLIN, G.A., RICHIE, J.P., and MANNICK, J.A., Correlation of the production of plasminogen activator with tumor metastasis in B16 mouse melanoma cell lines. *Cancer Res.*, **40,** 288–292 (1980).

WARREN, L., GLICK, M.C., and NASS, M.K., Membranes of animal cells. I. Methods of isolation of the surface membrane. *J. cell. Physiol.*, **68,** 269–288 (1966).

WILSON, B.S., BUBERTO, G., and FERRONE, S., Sulfation and molecular weight of fibronectin shed by human melanoma cells. *Biochem. Biophys. Res. Commun.*, **101,** 1047–1051 (1981).

YOGEESWARAN, G., and SALK, P.L., Metastatic potential is positively correlated with cell surface sialylation of cultured murine tumor cell lines. *Science*, **212,** 1514–1516 (1981).