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New Plasma Membrane Proteins Expressed by a High Metastatic Variant of a Chemically Induced Tumor*

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Abstract—*The composition of plasma membrane proteins of related low and high metastatic tumor cells were compared in order to elucidate possible genetic changes which may occur during progression of tumor cells towards metastatic capacity. For this purpose a procedure was worked out which allows the purification of mouse plasma membrane (PM) proteins from biosynthetically labeled cells. PM isolated from the two related T lymphoma lines Eb and ESb, which differ greatly in metastatic capacity, as well as from Con A-activated T cell blasts displayed similar degrees of purification as shown by marker enzyme analysis. When [^{35}S]-methionine-labeled proteins of such PM preparations from cloned Eb and ESb cells were compared by 2-D gel electrophoresis it was found that maps of the highly metastatic variant ESb cells permitted detection of about 50 new protein spots which were not found on the 2-D gel maps of the parental Eb cells. Approximately 80% of these new spots were also found on maps derived from syngeneic splenic Con A blasts. This observation indicated that most of the newly expressed membrane proteins of ESb cells were possible differentiation or cell activation-related and not tumor cell-specific.*

INTRODUCTION

TUMOR progression is considered to be the result of random generation of tumor cell variants within a growing tumor and the exertion of selective pressure from the host [1]. Progression towards metastatic capacity may proceed stepwise but the details of this process are far from being understood. Metastatic tumor cell variants have been shown to differ from non-metastatic parental tumor cells in antigenic and functional properties [2, 3]. An important issue of tumor variant generation is the distinction between phenotypic and genotypic variants. While the former can arise as a result of epigenetic changes the latter must involve changes in gene expression.

We have studied cell surface changes associated with tumor progression in a model system which consists of a chemically induced murine T cell lymphoma with two distinct sublines [4]. One, Eb, is the parental line and has low metastatic capacity (after s.c. transplantation); the other, ESb, is a spontaneous variant thereof with high metastatic capacity. In previous studies we have shown that Eb and ESb tumor cells differ in the expression of Lyt differentiation antigens [5], Fc receptors and specific tumor-associated transplantation antigens (TATAs) [6]. The change from low to high malignancy in the Eb/ESb system involved loss of some surface markers and gain of others [4]. The analysis of glycoproteins and glycolipids [5, 7] and lectin-binding studies [8] also revealed differences in the composition of glycoconjugates between the different cell types.

In this report we present biochemical evidence that ESb cells differ from Eb cells not only in some serological markers and glycosylation but also in the protein composition of the plasma membrane. Plasma membranes of metabolically labeled

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Abbreviations: Con A: concanavalin A; H-BSS: Hank's balanced salt solution; PM: plasma membranes; PHA: phytohemagglutinin from *Phaseolus vulgaris*; D-PBS: Dulbecco's phosphate-buffered saline; FCS: fetal calf serum; IEF: isoelectric focusing; SDS: sodium dodecyl sulfate; PAGE: polyacrylamide gel electrophoresis; 2-D: two-dimensional.

cloned tumor cell lines were purified and analyzed by 2-D gel electrophoresis. The study also involves a comparison of plasma membrane proteins from the two T lymphoma sublines with those of normal T lymphocytes activated by Con A.

MATERIALS AND METHODS

Cells

Eb is the Heidelberg subline of the methylcholanthrene-induced T cell lymphoma L5178Y of DBA/2 mice and ESb is a spontaneous high metastatic variant thereof. The etiology of the tumor lines and their functional and cell surface characteristics have been described in detail elsewhere [4].

The cells were maintained in tissue culture medium RPMI 1640 supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (5 mM), HEPES (50 mM), 2-mercaptoethanol (5×10^{-5} M) and 5 or 10% fetal calf serum.

Twice cloned lines of Eb (clone 34.2 = No. 736) and ESb (clone 18.1 = No. 721) were established by limiting dilution [4]. Con A blasts from DBA/2 were prepared by activating splenic lymphocytes with concanavalin A (5 μg/ml) at $2–5 \times 10^6$/ml for 3 days. Blasts were separated by Ficoll-Hypaque centrifugation. Recovered cells stained to more than 85% positive with monoclonal anti-Thy 1.2 antibodies as assessed by indirect immune fluorescence and cytofluorographic analysis.

Labeling with [^{35}S]-methionine

Tumor cells or Con A blasts (100×10^6) were biosynthetically labeled with [^{35}S]-methionine ([^{35}S]-Met, SJ204, sp. act. 1070 Ci/mmol Amersham Buchler, Braunschweig, F.R.G.). For labeling, cells were washed twice with D-PBS and once with RPMI 1640 without methionine supplemented with 2% dialyzed FCS (Met labeling medium). Cells were kept in labeling medium for 1 hr, then spun and resuspended in 10 ml fresh labeling medium in the presence of 0.5 mCi [^{35}S]-methionine. Labeling was done overnight. Next morning the cells were washed three times in prewarmed H-BSS and subjected to glycerol lysis as described by Jett *et al.* [9].

Isolation of plasma membranes

Briefly, cells were suspended in 1 ml warm H-BSS and 0.5 ml of 90% (w/v) glycerol in H-BSS was added to yield a final concentration of 30% glycerol. After 10 min at 37°C the cells were pelleted and put on ice. Ten milliters of ice-cold 10 mM Tris–HCl buffer, pH 7.4, supplemented with 1 mM $MgCl_2$ and 1 mM phenylmethylsulfonylfluoride (PMSF) (Cell lysis buffer), was added under vigorous vortexing. Disrupted cells were left on ice for 10 min and then pottered with 10 strokes in a Potter glass homogenizer (Braun, Melsungen). The homogenate was spun for 10 min at 800 *g* to remove nuclei and intact cells.

The supernate was transferred to centrifuge tubes (Beckman, No. 331374), underlayed with 3 ml of 42% sucrose solution made up in cell lysis buffer ($n = 1.3950$) and spun in a Beckman Spinco L265B centrifuge at 95,000 *g* using an SW 40 TI rotor. Material floating on top of the 42% sucrose cushion was collected (2 ml) and 4 ml of 38% sucrose solution in cell lysis buffer ($n = 1.3850$) were added. The density was readjusted with 80% sucrose to $n = 1.3850$ and the mixture transferred into fresh centrifuge tubes (Beckman No. 331374). Five milliliters of 42% sucrose solution were carefully underlayed to the 38% layer and the tubes were filled by overlayering with 15% sucrose solution ($n = 1.3520$). The stepwise gradient was spun at 205,000 *g* for 16 hr at 4°C and then harvested from the bottom using a 0.8×120-mm needle and a peristaltic pump. Fractions of 0.4 ml were collected and the gradient was assayed for sucrose density. The 38%/15% interphase fractions containing PM were pooled and diluted with lysis buffer. PM (500 μg) derived from human PHA blasts were added as cold carriers (only when radiolabeled PM were used) and the mixture was pelleted at 95,000 *g* for 1 hr. Pelleted PM were stored at – 70°C for further analysis.

Protein and enzymatic assays

For protein determination the Coomassie blue method of Bradford [10] was used. Enzyme assays were carried out according to standard procedures. 5'Nucleotidase (E.C. 3.1.3.5) served as marker for PM and was determined according to the protocol of Weaver and Boyle [11]. Glucose-6-phosphatase (E.C. 3.1.3.9) (for ER) was carried out as outlined by Swanson [12]. Inorganic phosphatase was determined according to Anner and Moosmeyer [13]. The assay for β-*N*-acetylglucosaminidase (E.C. 3.2.1.30) (lysosomal marker) was done as described by Kornfeld and Siemers [14] using *p*-nitrophenylglucosaminide as substrate. Succinate dehydrogenase (E.C. 1.3.99.1) was used as marker for mitochondria. The assay of Earl and Korner [15] was used as modified by Weaver and Boyle [11]. For the assay routinely 70 μl of each fraction were used.

2-D gel electrophoresis

[^{35}S]-Methionine-labeled PM were extracted for 30 min with O'Farrell lysis buffer [16] containing 9.5 M urea, 2% ampholines (pH 3–5, 0.8%; pH 5–7, 0.8%; pH 2–11, 0.4%; Serva, Heidelberg, F.R.G.), 5% 2-mercaptoethanol and 2% NP40.

Insoluble material was pelleted by spinning for 15 min in a table-top centrifuge (Eppendorf, Hamburg) at 10,000 *g*. Equal amounts of TCA-precipitable counts were applied to 130 × 3 mm IEF gels. After focusing gels were extruded from the glass tubes and equilibrated in 10 ml of O'Farrell's equilibration buffer for 2 hr and applied to 7.5–15% polyacrylamide gradient slab gels using Laemmli's buffer system [17]. Gels were run at 20 mA constant current; thereafter they were fixed and processed for fluorography as previously described [5].

RESULTS

Purification of metabolically labeled plasma membranes

In order to analyze plasma membrane proteins from biosynthetically labeled cells a protocol was required that allows the use of minimal cell numbers and a stringent PM purification procedure. To this end a PM purification protocol was established using initially unlabeled cells. The procedure is based on the stepwise sucrose gradient method of Jett *et al.* [9] originally developed for the isolation of PM from the human Burkitt lymphoma line Raji with some modifications most suitable for our purposes. Modifications had to be employed in order to adjust to the differences in PM densities between human and mouse cells as well as to the much lower cell numbers to be used. PM were isolated as described in detail in Materials and Methods. The final centrifugation step consisted of a stepwise sucrose gradient consisting of three layers (42%/38%/15%) of sucrose and the crude PM sample was introduced into the middle layer. After centrifugation the gradient fractions were tested for marker enzyme activity. Figure 1A shows that 5'-nucleotidase activity was highest at the boundary between the 38 and 15% sucrose layers. This activity peak, representing floated PM, also displayed trace amounts of glucose-6-phosphate activity (ER) while other enzyme activities were not detectable. Figure 1B shows the radioactivity profile obtained when PM derived from [^{35}S]-methionine-labeled cells were separated under identical conditions. One of the three radioactivity peaks showed an overlap with the 5'-nucleotidase activity and thus represented plasma membrane proteins. The radioactive fractions of this peak were pooled, diluted in lysis buffer and pelleted in the presence of cold carrier PM by high-speed centrifugation. This material was used for further studies by 2-D gel electrophoresis. This purification procedure resulted in a 37-fold enrichment of PM over total cell homogenate as

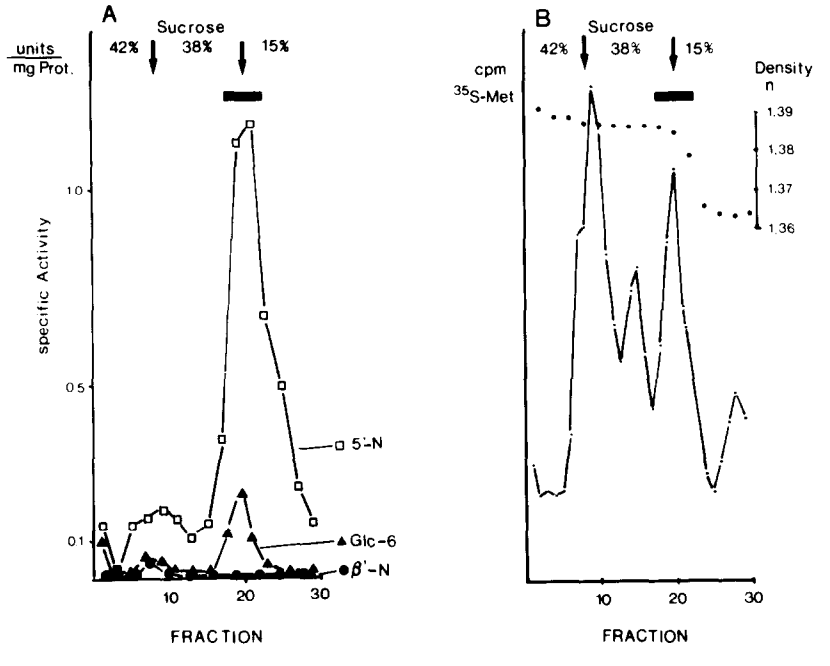

Fig. 1. Elution profiles of a stepwise sucrose gradient for the isolation of PM. Crude PM obtained after an initial centrifugation on a 42% sucrose cushion were introduced into the middle layer of a 42%/38%/15% stepwise sucrose gradient and spun again at 205,000 g for 16 hr. The gradient was harvested from the bottom and 0.4-ml fractions were collected. (A) Specific activities of marker enzymes recovered from a typical gradient: 5'N: 5'-nucleotidase; Glc-6: glucose-6-phosphatase; β-N: β-N-acetylglucosaminidase; succinate dehydrogenase: not detectable. (B) Elution profile of [^{35}S]-methionine-labeled material. Five microliters of each fraction were counted. Data shown were derived from crude PM of ESb cells. Similar results were obtained with Eb cells and Con A-activated T lymphoblasts. Indicated fractions were pooled and pelleted in the presence of carrier PM by high-speed centrifugation.

revealed by marker enzyme analysis (5′-nucleotidase, 37.4-fold; glucose-6-phosphatase, 1.3-fold; β-*N*-acetylglucosaminidase, 0.2-fold; succinate dehydrogenase, 0.02-fold). The purification method was found to be applicable for both types of tumor cells (Eb and ESb) and also for T lymphoblasts obtained from splenic lymphocytes by Con A activation. All three cell types yielded similar highly purified PM fractions for unlabeled cells. Since in our experiments only five times more cold cells were used than labeled cells the same purification factor can be assumed for biosynthetically labeled cells.

2-D gel electrophoretic comparison of membrane proteins of cloned Eb and ESb tumor cells and of normal Con A-activated T lymphoblasts

Labeled PM were purified as described above and extracted with O'Farrell's lysis buffer for 2-D gel analysis. The 2-D gels are shown in Fig. 2(A–C). A schematic drawing of the spots observed using films of two different exposure times is given in Fig. 2(D–F). The following observations were made:

Quantitative comparison. The 2-D gel patterns from Eb cells (Fig. 2B,E) revealed 169 spots; those from ESb cells (Fig. 2A,D) 203 spots; and those from Con A T lymphoblasts (Fig. 2C,F) 248 spots. Due to the IEF conditions used, this analysis did not include the very basic proteins which remained at the top of the IEF gel and thus underestimates the total number of membrane proteins. There was also a striking difference in the quantitative expression of particular spots. For example, Eb cells possessed a prominently labeled cluster of spots in the mol. wt range 44–70 kd, which constitutes a major proportion of the labeled material of Eb cells. Some spots of this cluster were also visible in the ESb material but to a much lesser extent [note as reference the actin spots (arrows), which were labeled to a similar degree in all three cell types].

Qualitative comparison between Eb and ESb membrane proteins. Of the 169 spots on the gel patterns of Eb type cells, 60% were also detected on the gels from ESb cells. There were some protein spots apparently unique to Eb type cells which were not detectable on ESb cells even when the films were overexposed (see, for instance, spots 8–12, Fig. 2E). Similarly, new spots were detected on ESb maps which were not at all detectable on Eb 2-D gel maps (see, for instance, the spots 1–7, Fig. 2D). Altogether, the ESb spot pattern contained approximately 50 additional spots compared with that of Eb cells.

Comparison with normal T lymphoblasts. A careful comparison of the maps from the tumor cells with the map of membrane proteins from normal splenic T lymphoblasts revealed that about 41 (82%) of the 50 new spots from the ESb type cells were also detected on normal T cells. For example, the ESb-derived protein spots 1–3 and 5–7 were found on the Con A blasts while the Eb-derived spots 8–12 were undetectable. Thus many of the new plasma membrane proteins expressed by the high metastatic variant seem to represent differentiation- or activation-related proteins of normal cells rather than being tumor cell specific.

DISCUSSION

The introduction of 2-D gel electrophoresis techniques has made it possible to analyze complex protein mixtures. The technique has been successfully applied to cell surface glycoproteins of mammalian cells [18–22]. In order to analyze mechanisms of tumor progression towards metastatic capacity we have used the 2-D gel method to study proteins on the cell surface of slightly vs highly metastatic cells and have compared them to those derived from corresponding normal cells. Since we decided to use biosynthetic labeling instead of external labeling procedures it was at first necessary to design an isolation procedure for plasma membranes suitable for our purposes. With the modified procedure described here, PM proteins of high purity can be obtained from relatively small amounts (100×10^6) of biosynthetically labeled cells suitable for analysis by 2-D gel electrophoresis. In spite of the high purity of the plasma membranes obtained (37-fold enrichment), we are not suggesting that there were absolutely no contaminating proteins present in the preparations. The marker enzyme analysis revealed the presence of ER-derived proteins so that it cannot be ruled out that perhaps some of the 2-D gel spots could be due to this contamination. Cell surface properties of L5178 lymphoma cells have been studied previously and found to change as a function of position in the growth curve and cell cycle [23, 24]. It seems unlikely, however, that the differences observed in this study between Eb and ESb membrane proteins are due to differences in cell growth or cell cycle because both cell lines showed similar growth characteristics in tissue culture [25].

The basic observation described in this report is that the transition from the low metastatic Eb tumor line to its highly metastasizing ESb variant is accompanied by a profound change in total membrane composition. Gain and loss of particular membrane proteins during this transition could be caused by true genctic changes. Such genetic changes were indeed found when the chromosomes of Eb and ESb cells were compared by the G-banding technique [26]. These studies

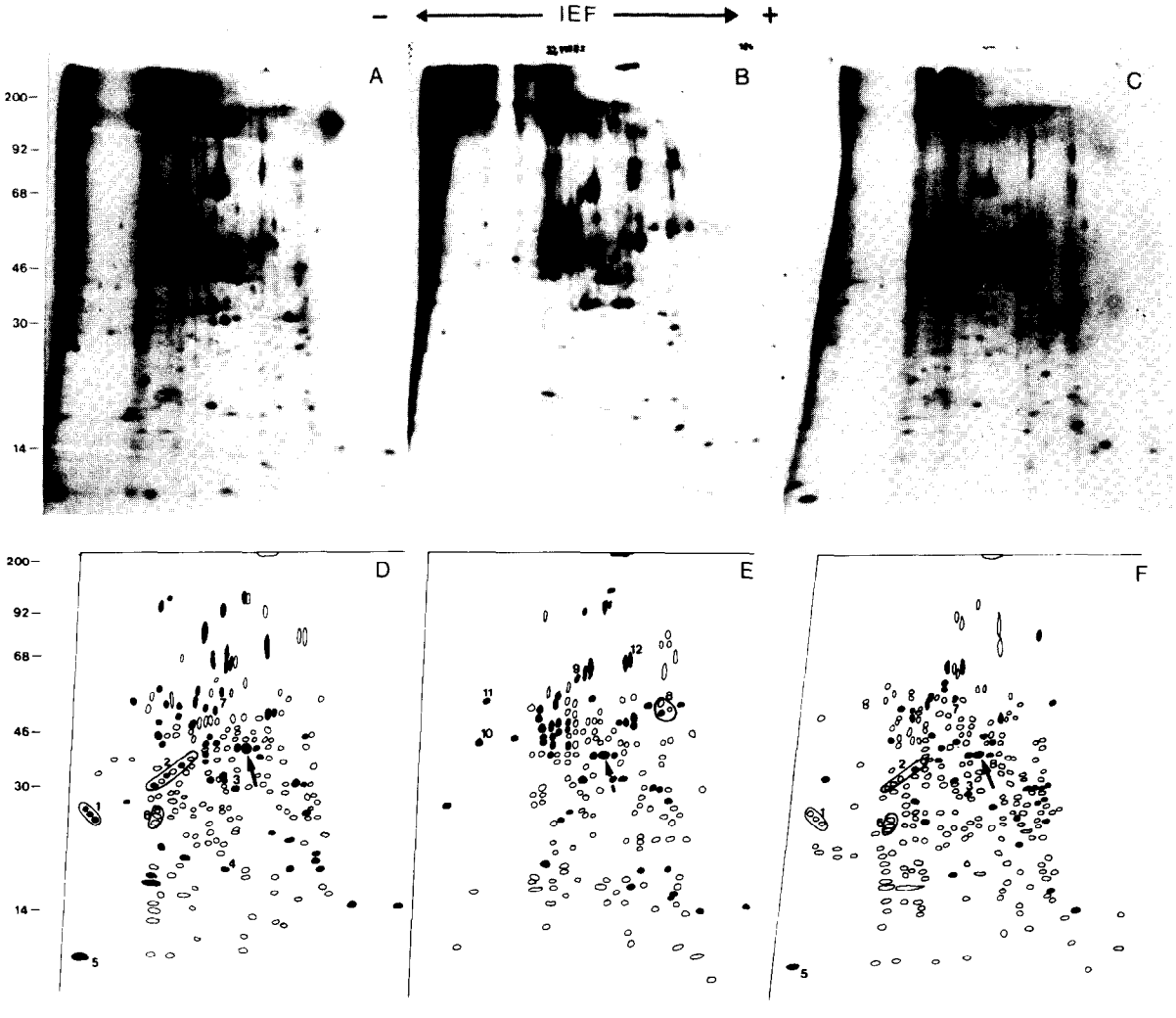

Fig. 2. 2-D gel electrophoretic separation of purified PM derived from [^{35}S]*-methionine-labeled cells. (A) ESb clone* 18.1; *(B) Eb clone* 34.2; *(C) DBA/2 Con A T blasts. PM were treated with O'Farrell's lysis buffer and the extracts* (600,000 counts/min *TCA-precipitable counts) separated by IEF* (1-*D ampholines*: 0.4%, *pH* 2–11; 0.8%, *pH* 3–5; 0.8%, *pH* 5–7). *IEF gels were applied to slab gels for SDS-PAGE* (2-*D* 7.5–15% *linear gradient gels). Gels were exposed to preflashed Kodak X Omat films for* 1 month. *D–F represent schematic drawings of gels A–C.*

first of all revealed three common chromosome markers in Eb and ESb cells, thus confirming their common origin and genetic relationship. In addition, a karyotypic progression from Eb towards ESb type cells was observed which involved an increasing number of numerical and structural chromosomal aberrations. It is possible that such chromosomal changes facilitate the generation of cellular variants and tumor heterogeneity. *In vivo* tumor growth and metastasis might select from such variants only those which have the proper malignant phenotype.

This idea of apparent random tumor variant generation and host selection may be quite attractive but it does not seem sufficient to explain all observed phenomena. The main obstacle is the reproducibility of the transition of Eb type cells to the ESb phenotype [27, 28]. A similar observation was made by Vaage [29], who found in repeated serial transplantation experiments that individual C3H mammary tumors undergo 'progression' in a highly reproducible way so that certain characteristics appeared in the same generations as if on schedule.

It is possible that the Eb to ESb transition has similarities with processes in normal cells leading to changes in the state of differentiation and/or activation (for a more detailed discussion of this point see ref. [30]), although in normal cells such changes are not associated with chromosomal aberrations. It remains to be elucidated to what extent the changes in membrane protein composition between Eb and ESb cells are tumor specific (possibly due to particular caryotypic changes) or differentiation related. The latter process could possibly account for the similarities observed between newly observed 2-D gel spots of ESb-type cells and spots of activated normal peripheral T cells. The 80% similarity found could even be an underestimate because not all differentiation-related membrane proteins of T cells may be expressed on Con A lymphoblasts.

We would like to propose that the differences in the composition of total membrane proteins observed between Eb and ESb cells represent changes in the gene activation state between the two cell types. The formation of the metastatic variant seems to be accompanied with the acquisition of new cell surface determinants. The activation of silent genetic information in the metastatic variant might also explain the gain of certain functional activities such as invasive capacity and expression of new degradative enzymes [31].

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REFERENCES

1. Nowell PC. The clonal evolution of tumor cell populations. *Science* 1976, **194**, 23–28.
2. Poste G, Fidler IJ. The pathogenesis of cancer metastasis. *Nature* 1980, **283**, 139–146.
3. Fidler IJ, Nicholson GL. The immunobiology of experimental metastatic melanoma. *Cancer Biol Rev* 1981, **2**, 171–234.
4. Schirrmacher V, Fogel M, Russmann E, Bosslet K, Altevogt P, Beck L. Antigenic variation in cancer metastasis: immune escape versus immune control. *Cancer Met Rev* 1982, **1**, 241–274.
5. Altevogt P, Kurnick JT, Kimura AK, Bosslet K, Schirrmacher V. Different expression of Lyt differentiation antigens and cell surface glycoproteins by a murine T lymphoma line and its high metastatic variant. *Eur J Immunol* 1982, **18**, 300–307.
6. Schirrmacher V, Bosslet K, Shantz G, Clauer K, Hübsch D. Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice. IV. Antigenic differences between the parental tumor line and its metastasizing variant. *Int J Cancer* 1979, **23**, 245–252.
7. Schwartz R, Schirrmacher V, Mühlradt P. Glycoconjugates of murine tumor lines with different metastatic capacity. I. Differences in fucose utilization and in glycoprotein patterns. *Int J Cancer* 1984, **33**, 503–509.
8. Altevogt P, Fogel M, Cheingsong-Popov R, Dennis J, Robinson P, Schirrmacher V. Different patterns of lectin binding and cell surface sialylation detected on related high and low metastatic tumor lines. *Cancer Res* 1983, **43**, 5138–5144.
9. Jett M, Seed TM, Jamieson GE. Isolation and characterization of plasma membranes and nuclei from lymphoid cells. *J Biol Chem* 1977, **252**, 2134–2142.
10. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal Biochem* 1976, **72**, 248–254.
11. Weaver RA, Boyle W. Purification of plasma membranes of rat liver: application of zonal centrifugation to isolation of cell membranes. *Biochim Biophys Acta* 1969, **173**, 372–389.

12. Swanson MA. Phosphatases of liver. I. Glucose-6-phosphatase. *J Biol Chem* 1950, **184**, 647–653.
13. Anner B, Moosmeyer M. Rapid determination of inorganic phosphate in biological systems by a highly sensitive photometric method. *Anal Biochem* 1975, **64**, 305–309.
14. Kornfeld R, Siemers C. Large scale isolation and characterization of calf thymocyte plasma membranes. *J Biol Chem* 1974, **249**, 1295–1301.
15. Earl DCM, Korner A. The isolation and properties of cardiac ribosomes and polysomes. *Biochem J* 1965, **94**, 721–734.
16. O'Farrell HP. High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 1975, **250**, 4007–4021.
17. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970, **277**, 680–682.
18. Litin BS, Grimes WJ. Two-dimensional electrophoresis of membrane proteins from normal and transformed cells. *Cancer Res* 1979, **39**, 2595–2603.
19. Clemetson KJ, Capitanio A, Luescher EF. High resolution two-dimensional gel-electrophoresis of the proteins and glycoproteins of human blood platelets and platelet membranes. *Biochim Biophys Acta* 1979, **553**, 11–24.
20. Comings DE, Cohen LW. Two-dimensional gel electrophoresis of ^{125}I-labeled surface proteins of human fibroblasts. *Biochim Biophys Acta* 2979, **578**, 61–67.
21. McGregor JL, Clemetson KJ, James E, Luescher EF, Dechavanne M. Characterization of human blood platelet membrane proteins and glycoproteins by their isoelectric point and apparent molecular weight using two-dimensional electrophoresis and surface-labelling techniques. *Biochim Biophys Acta* 1980, **625**, 473–483.
22. Imada M, Sueoka M. A two-dimensional polyacrylamide gel electrophoresis system for the analysis of mammalian cell surface proteins. *Biochim Biophys Acta* 1980, **625**, 179–192.
23. Bosmann HB. Membrane glycoprotein transferases: time course of activity changes in a synchronized cell population and enzyme activity half-lives in an asynchronous population. *Arch Biochem Biophys* 1971, **145**, 310–318.
24. Gerstein DB, Bosmann HM. Behavior in two phase aqueous polymer systems of L5178Y mouse leukemic cells. *E.., Cell Res* 1974, **88**, 225–230.
25. Schirrmacher V, Hübsch D, Clauer K. Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice. IX. Radioassay analysis of tumor cell spread from a local site to the blood and liver. In: Grundmann E, ed. *Metastatic Tumor Growth Cancer Campaign.* Stuttgart, Gustav Fischer, 1980, Vol. 4, 147–158.
26 Dzarlieva R, Schirrmacher V, Fusenig NF. Cytogenetic changes during tumor progression towards invasion, metastasis and immune escape in the Eb/ESb model system. *Int J Cancer* 1982, **30**, 633–642.
27. Schirrmacher V, Bosslet K. Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice. X. Immunoselection of tumor variants differing in tumor antigen expression and metastatic capacity. *Int J Cancer* 1980, **25**, 781–788.
28. Schirrmacher V, Altevogt P, Bosslet K. Spontaneous phenotypic shifts from low to high metastatic capcity. In: Chandra P, ed. *Proceedings of the NATO ASI on 'Biochemical and Biological Markers of Neoplastic Transformation'.* New York, Plenum Press, 1983, 121–131.
29. Vaage J. Inherent changes in the *in vivo* growth characteristics of C_3H/Hc mammary carcinomas. *Cancer Res* 1980, **40**, 3495.
30. Schirrmacher V. Shifts in tumor cell phenotypes induced by signals from the microenvironment: relevance for the immunobiology of cancer metastasis. *Immunobiology* 1980, **157**, 89–98.
31. Vlodavsky I, Ariav Y, Fuks Z, Schirrmacher V. Lymphoma cell mediated degradation of sulfated proteoglycans in the subendothelial extracellular matrix: relationship to tumor cell metastasis. *Cancer Res* 1983, **43**, 2704–2711.