Scanning Microscopy

Volume 3 | Number 3 Article 21

10-9-1989

Surface Heterogeneity of Tumor Cells and Changes Upon Ionizing Radiation

Z. Somosy
"Frédéric Joliot-Curie"National Research Institute for Radiobiology and Radiohygiene

Orsolya Csuka
National Institute of Oncology

Tamara Kubasova
"Frédéric Joliot-Curie"National Research Institute for Radiobiology and Radiohygiene

J. Kovács
Eötvös Lorand University

G. J. Köteles
"Frédéric Joliot-Curie"National Research Institute for Radiobiology and Radiohygiene

Scanning Microscopy, Vol. 3, No. 3, 1989 (Pages 895-906)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/89$3.00+.00

SURFACE HETEROGENEITY OF TUMOR CELLS AND CHANGES UPON IONIZING RADIATION

Z. Somosy*, Orsolya Csuka+, Tamara Kubasova, J. Kovács++, G.J. Köteles

"Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, Budapest
+National Institute of Oncology, Budapest
++Department of General Zoology of Eötvös Lorand University, Budapest

(Received for publication February 16, 1989, and in revised form October 9, 1989)

Abstract

Heterogeneous distribution of surface domains is a characteristic feature of the tumor cell surface and the distribution differs from that of normal cells. During the malignant transformation the heterogeneity may change or disappear. Cell lines with various metastasizing capacities show different distributions of membrane domains or other differences in membrane or surface organization. We have demonstrated that the amount and distribution of negatively charged sites of B 16 melanoma membranes changed upon ionizing radiation (X-ray, ^{60}Co-gamma). In the case of the P 388 lymphoma, however, only the amount of negatively charged sites change after irradiation, the distribution remains unaltered. Both features proved to be radioresistant in human lymphoid leukemic cells.

Key words: membrane domains on tumor cells, B 16 melanomas, human lymphoid leukemic cell, P 388 lymphoma, cationic ferritin binding, X-ray and gamma irradiation, electron microscopy.

*Address for correspondence:
Z. Somosy, "Frédéric Joliot-Curie" National Research Institute for Radiobiology and Radiohygiene, H-1775 Budapest
POBox 101, Hungary, Phone No:36-1-1730026

Introduction

Heterogeneous distribution of membrane domains is a characteristic feature of living cells (55,59,74,86). Some domains belong to special areas of plasma membranes as cell contacts, microvilli, ruffles or all apical, basolateral surface regions and they are stabilized by elements of cytoskeleton (6,12,14,74). Distribution of membrane domains are dependent on the functional state, shape, locomotion and confluency of cells (45, 48,55,74,87).

The tumor cells are different in many cell surface properties (composition, structure, function) from the normal cells (1,2,11,15,17,24,27-29,36,39,43,44,65,77, 92) and even the cell lines originating from the same tumors having different metastasizing ability may display different membrane properties (4,8,22,28,33,35,66, 68,70-72,82,88,90,92,93).

The first part of the present work is a brief survey of the relevant data reported in the literature on nonhomogeneous and polarized distribution of membrane domains in tumor cells and on the changes in domain localization in normal and tumor cell pairs, and in neoplastic cell lines of different metastasizing abilities.

In the second part of the review, we present our data on the topology of negatively-charged surface domains of cultured B 16 melanoma cells, P 388 lymphoma cells and a human leukemia line as well as their changes upon irradiation with X- and ^{60}Co gamma rays.

Heterogeneous distribution of membrane domains on the tumor cells

The morphologically distinct surface regions of tumor cells (microvilli, contacts, ruffles, trailing and leading edges) may differ from each other in the amount and types of ligand binding sites. For example, specific binding of cationized colloids (cationized ferritin, colloid iron) was observed on the microvilli of Ehrlich ascites carcinoma cells

Table 1.

Heterogeneous distribution of membrane domains on tumor cell surfaces

Cell types	Membrane domain	Distribution	References
A 431 cell	epidermal growth factor	heavily on villi	(10)
L 1210 cell	transferrin receptor	heavily on villi	(50)
L 1210 cell	transcobalamin receptor	heavily on villi	(50)
Human colon carcinoma	heparan sulphate	heavily on villi	(44)
Ehrlich ascites	negatively charged sites	heavily on villi	(85)
P 388 ascites	negatively charged sites	heavily on villi	present paper
Mouse mammary tumor cells	Con-A binding sites	heavily on villi	(64)
13762 rat mammary ascites	Con-A binding sites	heavily on villi	(89)
JEG 3 chorio-carcinoma	75 000 Mw protein	heavily on villi	(67)
HeLa	negatively charged sites	apical	(60)
HeLa	WGA binding sites	apical	(60)
B 16 melanoma	negatively charged sites	apical	present paper
PYS -2 cell	laminin, heparan sulphate	basal	(38,58)
M 21 melanoma	GD2, GD3 gangliosides	cell contacts	(20,21)
M 21 melanoma	fibronectin, vitronectin binding sites	cell contacts	(75)
AH 136 rat ascites	cell surface adhesive factor	cell contacts	(46)
F-9 embryonal carcinoma	galactosyl transferase	cell contacts	(73)
Giant HeLa cells	transferrin, LDL receptor	leading edge	(13)

(85), human colon carcinoma (43), SV 40 transformed fibroblasts (11) and the ascitic form of P 388 lymphoma cells, as shown on Fig. 1. The microvilli may contain binding sites for Concanavalin-A (64, 65), receptors for epidermal growth factor (EGF) (10), transferrin and transcobalamin (50); the microvillar localization of a 75000 D protein on JEG 3 chorioncarcinoma cells was also reported (67). Localization of gangliosides and glycoproteins in the cell contacts was observed in several types of cells (20,21,33,46, 58,73). The dominance of negative charged sites was found on the apical surface of HeLa cells (60) and we report here that the same phenomenon is characteristic for the B16 melanoma cells and its low metastasizing ability line as shown in Figs. 5,8,9.

The basal localizations of laminin and heparan sulphate on M 21 melanoma (75) and on teratocarcinoma-derived endothermal (PYS 2) cells (38) were observed. The uneven distribution of receptors and enzymes on trailing and leading edges of moving tumor cells is also known (5,13, see Table 1 for details).

Changes in the localization of membrane domains during malignant transformation

There are apparently differences in the fate of domains originally localized in cell contacts of normal cells after transformation; they can either remain at contact sites or spread out through the whole surface (15,17,30,36,40,74,76). The localization of membrane domains is deeply altered upon malignant transformation, however, and as a rule, the original distribution of the domains disappear. Villin, carcinoembryonic antigens and secretory components in colon cancers (1,84,92), limulin, leucine aminopeptidase in rat pancreatic acinal carcinoma cells (42,63), transferrin receptors and cell surface attachment (CSAT) complex in transformed cells (3,19,31) are well known examples of this phenomenon. In these cells the originally heterogeneous distribution of receptors becomes uniform during transformation. The randomization of membrane domains following malignant transformation is presumably due to reduction of the cell contacts and/or changing of cell shape. Those tumors or tumor cell lines which kept morphological similarities concerning cell contacts and shapes to that of the starting normal tissue cells usually returned the original distribution of various domains. The original arrangement of membrane domains, however, is usually lost in fully dedifferentiated cells (1,17,18,30,32,37,56,57,84,90,92). This relationship is well demonstrated in HT 29 cells the shape and nature of which can be modified by the culture conditions (30,32,56,57). The distribution of membrane domains on the surface of the undifferentiated form of the cell is usually uniform; however, it becomes polarized on differentiated forms. Membrane domains changing their distribution are the histocompatibility antigens, vasoactive intestinal peptide receptors, adenylate cyclase, villin and a 28 kD glycoprotein

Table 2.

Distribution of membrane domains on normal and tumor cell pairs

Membrane domain	Normal cell	Domain distribution	Tumor cell	Domain distribution	References
Milk fat globule membrane antigen (MFGM-A)	mammary epithelial cells	apical	T 470 breast carcinoma	uniform	(90)
Limulin	rat pancreas	apical	pancreatic carcinoma	uniform	(63)
Villin	colon	apical	colon carcinomas, HT 28 cell linea	uniform uniform	(92) (30)
Apical antigens	colon	apical	colon cancers HT 28 cell linea	uniform	(1,84) (56,57)
Basolateral antigens	colon	basolateral	colon cancers HT 28 cell linea	uniform uniform	(1,84) (56,57)
Leucin aminopeptidase	rat pancreas	basolateral	pancreatic carcinoma	uniform	(42)
Acetylcholine receptor	muscle fibers	clustered	RSV transformed muscle fibers	uniform	(3)
CSAT antigen	fibroblasts	clustered	transformed fibroblasts	uniform	(19)
Transferrin receptor	fibroblasts	leading lamella	A 8387 fibrosarcoma	uniform	(31)
Submembraneous cytoskeletal proteins	fibroblasts	focal contacts	RSV and VSV transformed cells	uniform at podosomes	(27,36,76) (15,75)
Cell surface urokinase	fibroblasts	focal contacts	transformed cell	uniform	(40)
Cationized ferritin binding sites	fibroblasts	clustered	SV-40 transformed cells	microclusters, increased on villi	(11)
Con-A binding sites	3T3 fibroblasts	randomly, small number clusters	SV-3T3 cells	large, randomly clusters on villi	(65)
Nucleoside triphosphatase	breast cells	uniform	breast cancer	clustered	(24)
La^{3+} binding sites	lymphocytes	uniform	leukemic cells	clustered	(2)
Cationized ferritin binding sites	lymphocytes	uniform	leukemic cells	clustered	present paper
Phospholipase A_2	rat kidney cells (CRL 1570)	ruffles	MSV transformed cells (CRL 1526)	ruffles	(5)
75 kD protein	fibroblasts	villi	carcinoma cells	villi	(67)
Alkaline phosphatase	placental cells	clustered	transformed placental cells	clustered	(47)

(30,32,37,56,57).

In some cases the uniform distribution of domains on normal cell surfaces disappears during the process of malignant transformations. Thus the disappearance of nucleoside triphosphatase on the surface of breast cells (24) and La^{+} and cationized ferritin binding as shown on Fig. 2 were reported [2, 52].

On the other hand, membrane domains and/or proteins exist which do not change localization during transformation. The microvillar 75 kD protein (67), the membrane-bound alkaline phosphatase (47) and the phospholipase A2 (5) of membrane ruffles are prominent examples of this type of behaviour. Data related to this topic are summarized in Table 2.

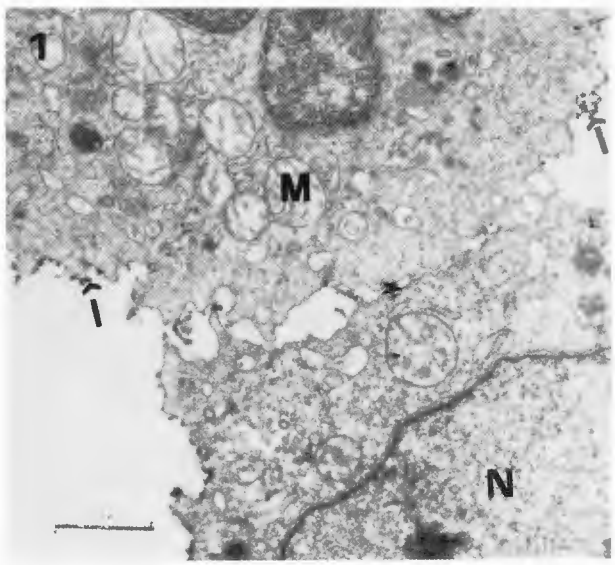


Figure 1. Cationized ferritin binding on P 388 lymphoma cells. The CF localized heavily on microvilli (➡). The contacts at adherent cells (*) are usually free of CF. M = mitochondria, N = nucleus. Bar = 0.5 µm.

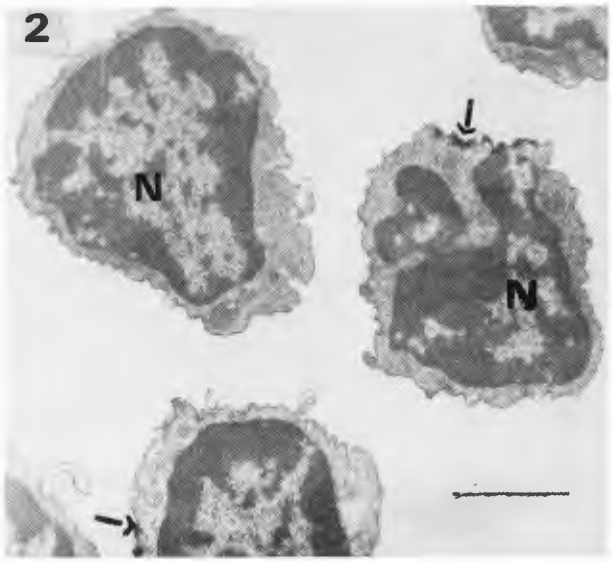


Figure 2. Cationized ferritin binding on human lymphoid leukemic cells. Some cells bind the ferritin uniformly, while others bind it in patches (➡). N = nucleus. Bar = 1 µm.

Positions of membrane domains on the surfaces of cells of different metastatic capabilities originating from the same tumor

Cell lines of different metastatic capabilities are also different in their cell surface organization (9,16,25, 33,35,49,66,68,70,72,82,93). Thus, increasing metastasizing capability is associated with the loss of binding sites for fibronectin and wheat germ agglutinin (WGA) in human melanoma cell lines (8, 35). A similar observation was made on colon and breast cancers where the localization of carcinoembryonic antigen, the secretory component, villin and fibronectin were investigated (1,22,92).

This phenomenon was studied in the following experiments in which the distribution of negative charges and Con-A binding sites were detected on B 16 melanoma cell lines of different metastatic capacities.

Materials and Methods

Cells and their treatments

Three types of tumor cells were used in our experiments, the B 16 melanomas, P 388 lymphoma and a human lymphoid leukemic cell type.

For the selection of differently metastasizing cell lines B 16 melanoma cells from "mixed" culture were inoculated into the foot pad of C 57 male mice, 200,000 cells per inoculation.

The pulmonary metastases were transmitted to culture and stabilized. The metastasizing capacities of stabilized cell lines were tested with reinoculation into the C 57 Black male mice. The metastatic potential was determined by counting the number of pulmonary colonies. The average numbers of colonies were 61, 47, and 7 for the cell lines "12.2". "10a", and "10" respectively. The cells lines were cultivated in RPMI (Rosewell Park Memorial Institute) medium supplemented with 20 per cent calf serum and antibiotics.

P 388 lymphoma cells (100,000 per inoculum) were inoculated into intraperitoneum of C 57 Black and DBA/2 hybrid male mice.

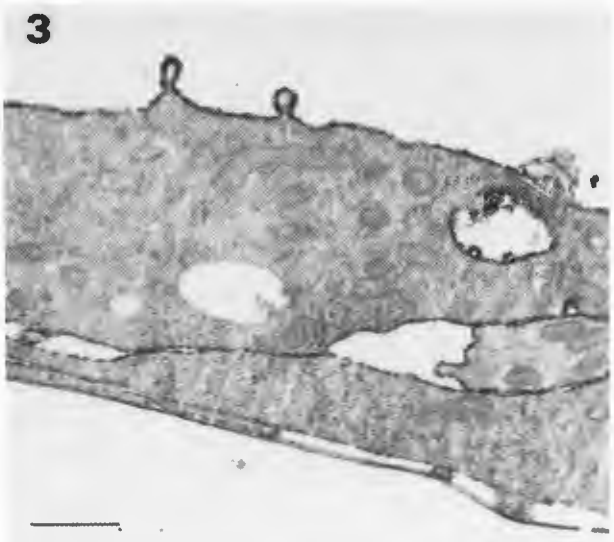
Human lymphoid leukemic cells were separated from a human leukemic patient's blood by ficoll density gradient centrifugation (54).

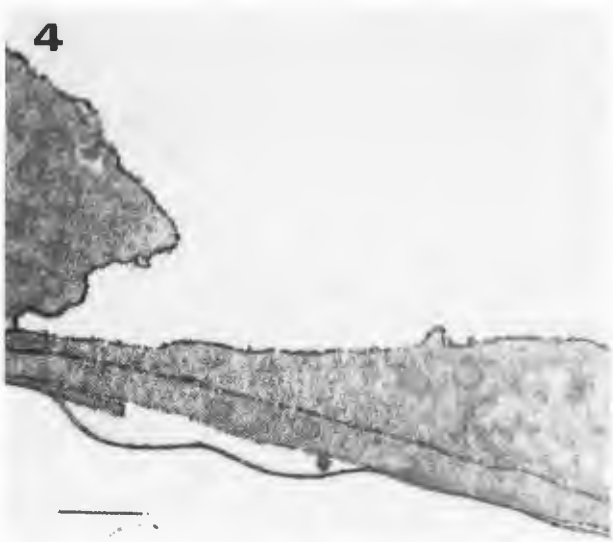
➡

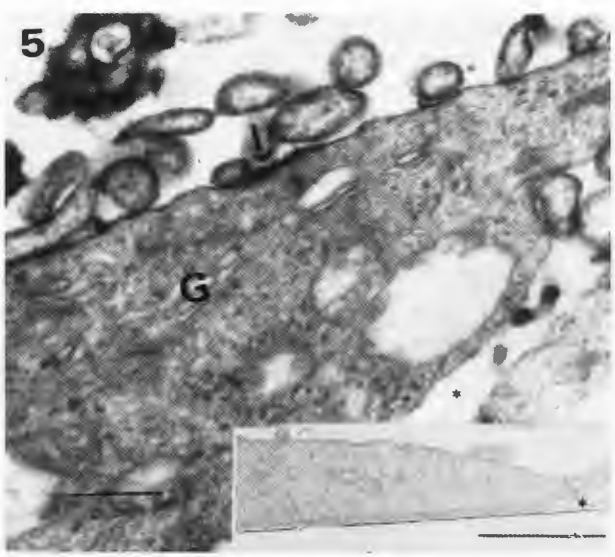
Figure 3. ConA binding on low metastasizing "18" B 16 melanoma cells. The reaction is uniform all over the surface. Bar = 0.5 µm.

Figure 4. ConA binding on high metastasizing "12/2" B 16 melanoma cells. The reaction can be seen on basal or apical surfaces. Bar = 0.5 µm.

Figure 5. Cationized ferritin binding on low metastasizing "18" B 16 melanoma cells. Negatively charged sites occur on the apical surface (➡). Basal surface = * , G = Golgi complex. Bar = 0.5 µm, insert = 1.5 µm.







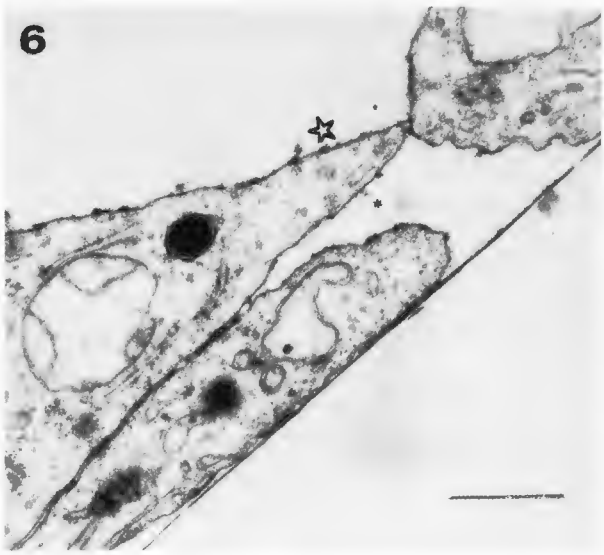


Figure 6. Cationized ferritin binding on high metastasizing "10a" B 16 melanoma cells. The small clusters of marker distributed uniformly over the whole surface. Apical (☆), basal surface (*). Bar = 0.5 /um.

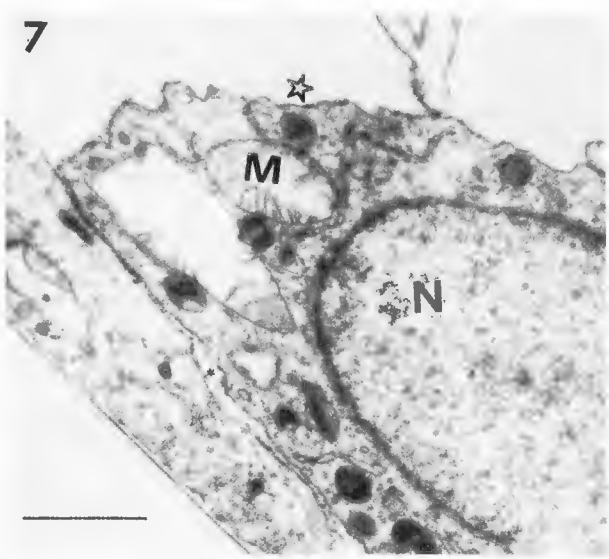
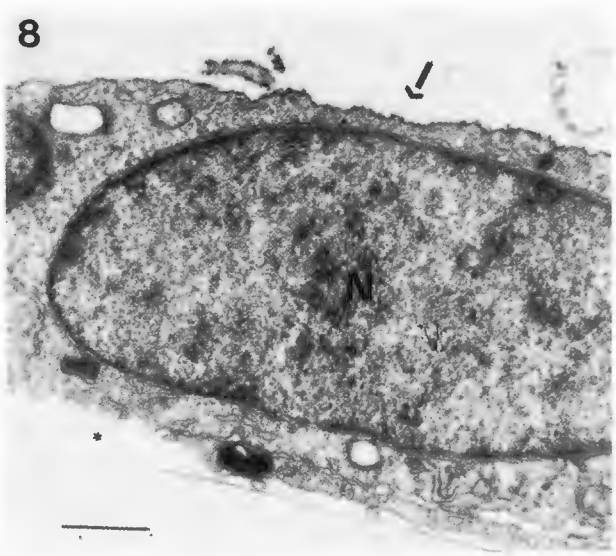
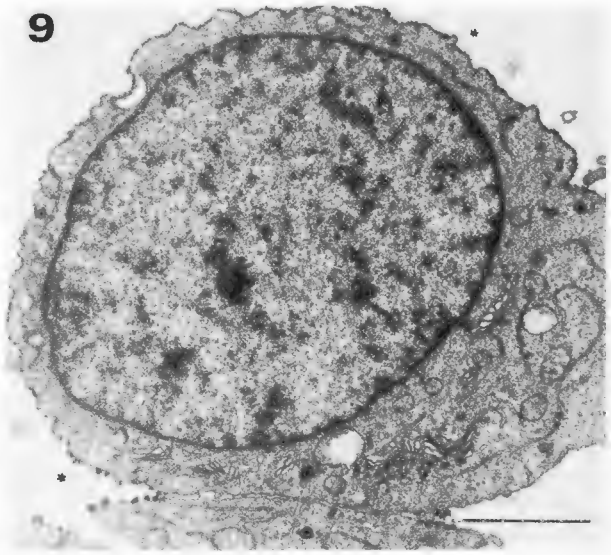


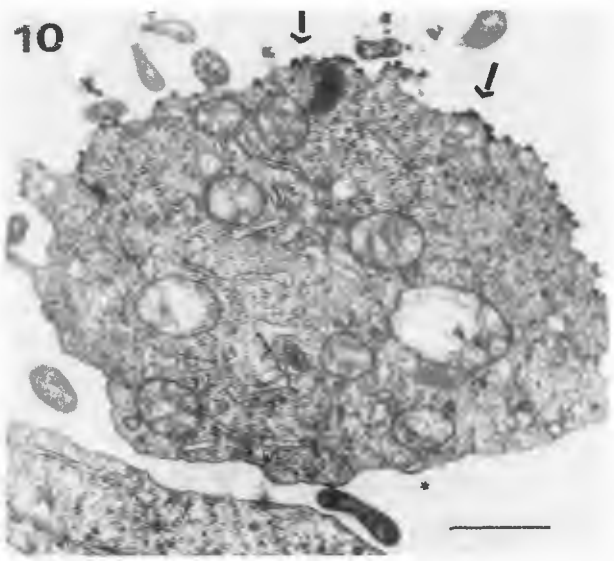
Figure 7. Cationized ferritin binding on high metastasizing "12/2" B 16 melanoma cells. The small clusters of marker distributed uniformly similarly to "10a" cells. M = mitochondria, N = nucleus, basal region = *, apical region = ☆. Bar = 0.5 /um.

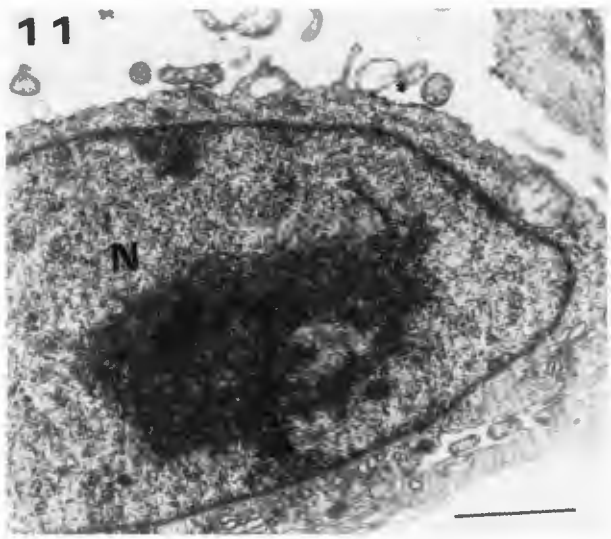
X-irradiation was described in our previous work (80). ^{60}Co gamma-irradiation was performed with a Siemens Gammatron-3.

Cytochemistry: Cationized ferritin was used for the detection of negatively charged sites (26). For the detection of Concanavalin-A binding sites the Concanavalin-A peroxidase reaction was applied (7).









Electron microscopy (EM): The cells were fixed in phosphate (0.1M) buffered 2.5 per cent glutaraldehyde (pH 7.3; 4 °C) for 1 hour, postfixed in 1 per cent OsO_4, dehydrated with acetone and embedded in Durcupan AC (Fluka). The fixation was carried out either on coverslips or in suspension. The ultrathin sections were cut with diamond or glass knives on an LKB ultratome, and examined in JEM 6C or Tesla EMs.

Concanavalin-A binding of B 16 melanoma cell lines

As shown by the ConA-peroxidase reaction, the lectin binding sites are evenly distributed on the whole surfaces of cells of the three B 16 melanoma cell lines investigated (Figs. 3 and 4).

Cationized ferritin binding of B 16 melanoma cell lines

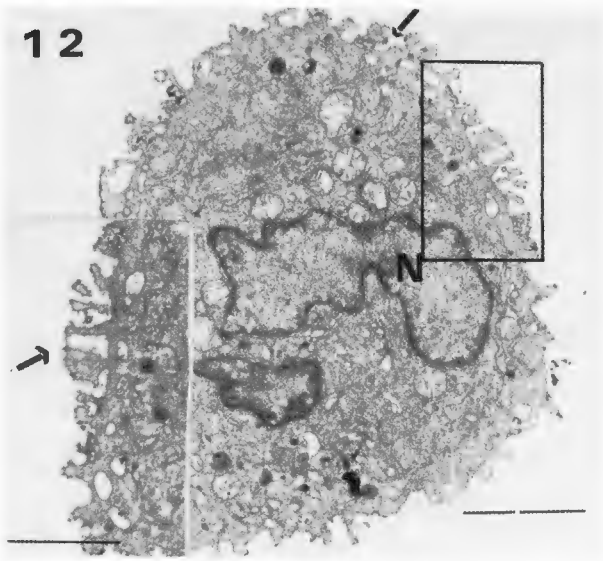
The cationized ferritin (CF) is bound

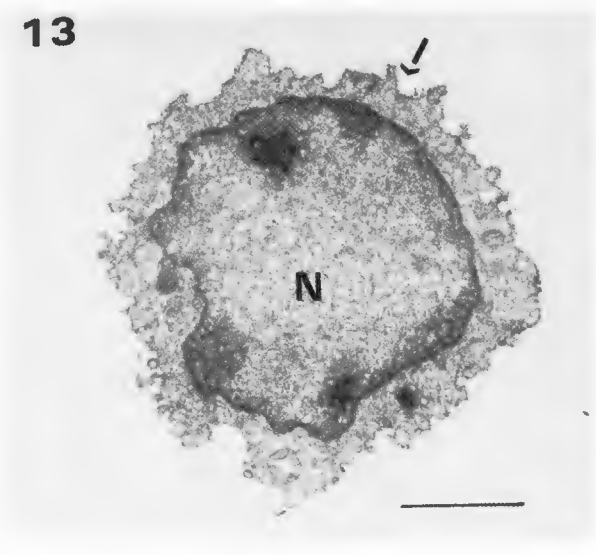
Figure 8. Cationized ferritin binding on B 16 melanoma cell. Bindings are seen exclusively on apical regions (➡). The asterisks indicate the basal region. Bar = 0.5 μm.

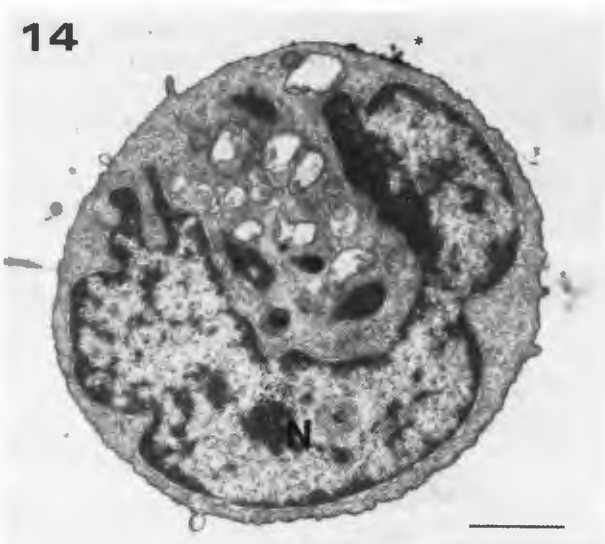
Figure 9. Cationized ferritin binding on B 16 melanoma cell. The ferritin particles are present on the apical and lateral surface (*) of rounded cells, too. Bar = 0.5 μm.

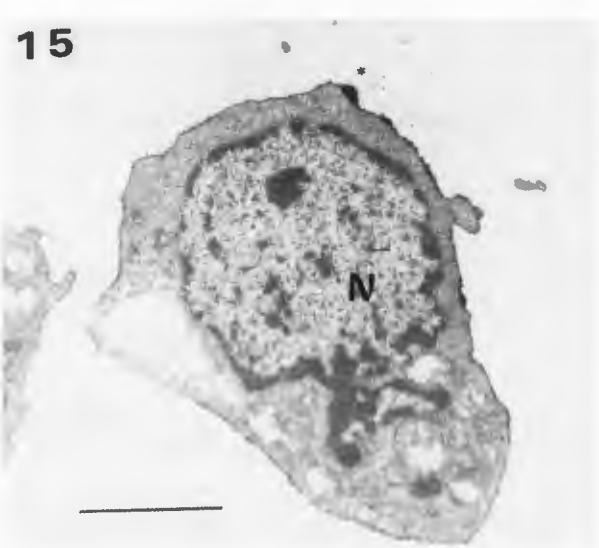
Figure 10. Cationized ferritin binding on B 16 melanoma cell 1 h after 2.5 Gy X-irradiation. The CF distributed on cell surface in clusters and patches (➡). Basal region = *. Bar = 0.5 μm.

Figure 11. Cationized ferritin binding on a B 16 melanoma cell 30 min after 2.5 Gy ^{60}Co gamma irradiation. The ferritin particles localized in small clusters and patches (*) on apical region of the cell. N = nucleus. Bar = 0.5 μm.









to the apical and lateral parts of the surfaces of cells of mixed B 16 melanoma cell cultures (Figs. 8 and 9) and the same distribution is seen on cells of the "18" line, which is a poorly metastasizing one (Fig. 5). In cell clones of high metastasizing capacities (in the "10a" and "12/2" clones) the polarity is less pronounced and this is manifested by a rearrangement of binding sites between the apical and basal surfaces (Figs. 6 and 7).
On the cells of these clones, CF is localized in clusters over the whole surface. Clustered distribution of CF was also observed by others on a breast adenocarcinoma (4) and also on B 16 melanoma cells (70).

High metastasizing capacity is usually accompanied by increased motility of

Figure 12. Cationized ferritin binding on P 388 lymphoma cell. Microvilli and other cytoplasmic projections are more pronounced (➡). N = nucleus. Bar = 0.5 μm, insert 1.5 μm.

Figure 13. Cationized ferritin binding on P 388 lymphoma cell 1 hour after 2.5 Gy X-ray irradiation. Number of ferritin particles decreased but the microvillar localization (➡) did not change. N = nucleus. Bar = 1 μm.

Figure 14. Cationized ferritin binding on human lymphoid leukemic cell. The CF bound in patches (*). N = nucleus. Bar = 1 μm.

Figure 15. Cationized ferritin binding on human lymphoid leukemic cell 1 h after X-irradiation with 2.5 Gy. The patched localization of CF (*) did not change. N = nucleus. Bar = 1 μm.

cells, decrease of their adherence to substrates and by increased numbers of rounded cells (6,33,35,66,68,71,91,93). These morphological alterations could also be observed in our material. Thus, the cells of poorly metastasizing melanoma clones were grown in multilayer. The culture contains mainly flat stretched-out cells among which numerous fibroblastic spindle-like bipolar cells are dispersed. There are a few blebbed cells. The shapes of cells of highly metastasizing clones are more variable. There are more non-adherent rounded cells here than in less metastasizing cultures (82).

As the shape and contacts determine the distribution of domains (23,34,48,55, 61,74,83) we suggest that the alterations of these features might explain the observed phenomena.

Effects of ionizing radiation on the distribution of membrane domains on the surfaces of tumor cells

The plasma membrane is sensitive to ionizing radiation (41,51-54,62,79-81) which is often used in the therapy of tumors. As mentioned above, the heterogeneous distribution of domains is a characteristic property of tumor cell membranes. It is known that irradiation alters the distribution of membrane domains in normal cells (41,53,54,80,81). Therefore, we raised the question whether this phenomenon can be induced in tumor cells, too.

In our experiments we used B 16 melanoma, P 388 lymphoma cells, and acute lymphoid leukemic lymphocytes and examined the distribution of sites of surface negative charges following X- and ^{60}Co-irradiation.

The B 16 melanoma cells have a considerable dominance of negatively charged sites on their apical and lateral surfaces (Figs. 8. and 9). Following irradiation the negatively charged sites decreased in a dose range between 0.5-2.5 Gy, similarly to the decrease that was observed on normal cells (Figs. 10-11) (78-80). It is often observable that the remaining number of charges is localized in apical regions in patch forms (Fig. 10 and 11).

It could be observed that, presumably due to radiation effects, the P 388 cells more or less lost their microvilli, but the microvillar dominance of negative sites did not change (Figs. 1, 12 and 13). It should be noted that the overall amount of surface negative charges also decreased (Fig. 13).

The distribution of surface charges on the lymphoid leukemic cells examined did not change in the dose range studied (Figs. 2,14 and 15). Radiation resistance of lymphoid leukemic cells is also indicated by their unchanged ConA binding capacity following irradiation (52,54).

These results suggest that as a result of the effect of radiation, the change of negative surface charge depends on the type of tumor cell, unlike various types of normal cells (53,80).

On the B 16 melanoma cell surfaces there is a special change in distribution of negative charges. The patched distribution of their membrane domains suggest a topological disorganization caused by radiation. Such changes in distribution have not been experienced in our previous studies with primary human fibroblasts and Green Monkey kidney cells (78-80).

Acknowledgements

The authors acknowledge highly the interest and support of Professor Dr. L.B. Sztanyik, Director-General of the Institute.

References

1. Ahnen DJ, Kinoshita K, Nakane PK, Brown WR (1987) Differential expression of carcinoembrionic antigen and secretory component during colonic epithelial cell differentiation and in colonic carcinomas. Gastroenterology 93, 1330-1338.
2. Anteunis A, Vial M (1975) Cytochemical and ultrastructural studies concerning the cell coat glycoproteins in normal and transformed human blood lymphocytes. II. Comparison of lanthanum-retaining cell coat components ant T and B lymphocytes transformed by various kinds of stimulating agents. Exp. Cell Res. 90, 47-55.
3. Anthony DT, Schuletz SM, Rubin LL (1984) Transformation by Rous sarcoma virus prevent clustering of acetylcholine receptors on cultured chicken muscle fibers. Proc. Natl.Acad.Sci. USA 81, 2265-2269.
4. Bal de Kier Joffé E (1984) Ultrastructural study of membrane anionic sites on two related murine mammary adenocarcinomas with different metastasizing ability. Comm.Biol. 2, 371-379.
5. Bar-Sagi D, Suhan JP, McCormick F, Feramisco JR (1988) Localization of phospholipase A_2 in normal and rastransformed cells. J. Cell Biol. 106, 1649-1658.
6. Ben-Ze'ev A (1987) The role of changes in cell shape and contacts in the regulation of cytoskeletal expression during differentiation. J. Cell Sci.Suppl. 8, 293-312.
7. Bernhardt W, Avremas S (1971) Ultrastructural visualization of cellular carbohydrate components by means of Concanavalin A. Exp. Cell Res. 64, 232-236.
8. Berthier-Vergens O, Deugnier MA, Reano A, Doré J (1985) Surface distribution of Wheat Germ Agglutinin sites of human melanoma cell lines with low and high tumorgeneicity. Biochem.Intern. 11, 669-675.
9. Berthier-Vergens O, Réano A, Doré JF (1986) Lectin binding glycoproteins in human melanoma cell lines with high or low tumorgeneicity. In.J. Cancer 37, 747-751.
10. Boonstra J, VanBelzen N, VanMaurik P, Hage WJ, Blok FJ, Wiegant FAC, Verkleit AJ

(1985) Immunocytochemical demonstrations of cytoplasmic and cell-surface EGF receptors in A 431 cells using cryo-ultramicrotomy, surface replication, freeze-etching and label fracture. J.Microsc. 140, 119-129.
11. Borysenko JZ, Woods W (1979) Density, distribution and mobility of surface anions on a normal/transformed cell pair. Exp. Cell Res. 118, 215-227.
12. Bray D, Heath J, Moss D (1986) The membrane-associated "cortex" of animal cells: its structure and mechanical properties. J. Cell Sci.Suppl. 4, 71-88.
13. Bretscher MS (1983) Distribution of receptors for transferrin and low density lipoprotein on the surface of giant HeLa cells. Proc.Natl.Acad.Sci. USA 80, 454-458.
14. Buck CA, Horwitz AF (1987) Cell surface receptors for extracellular matrix molecules. Ann.Rev. Cell Biol. 3, 179-205.
15. Burridge K (1986) Substrate adhesion in normal and transformed fibroblasts: Organization and regulation of cytoskeletal membrane and extracellular matrix components at focal contacts. Cancer Rev. 4, 18-78.
16. Calorini L, Fallani A, Tombaccini D, Mugnal G, Rugieri S (1987) Lipid composition of cultured B 16 melanoma cell variants with different lung-colonizing potential. Lipids 22, 651-656.
17. Cathcart MK, Culp LA (1979) Phospholipid composition of substrate adhesion sites of normal, virus transformed, and revertant murine cells. Biochemistry 18, 1167-1176.
18. Chantret I, Barbat A, Dussaulx E, Brattain MG, Zweibaum A (1988) Epithelial polarity, villin expression, and enterocytic differentiation of cultured human colon carcinoma cells: A Survey of twenty cell lines. Cancer Res. 48, 1936-1942.
19. Chen W-T, Wang J, Hasegawa T, Yamada SS, Yamada KM (1986) Regulation of fibronectin receptor distribution by transformation, exogenous fibronectin, and synthetic peptides. J. Cell Biol. 103, 1649-1661.
20. Cheresh DA, Harper JR, Schultz G, Reisfeld RA (1984) Localization of the gangliosides GD 2 and GD 3 in adhesion plaques and on the surface of human melanoma cells. Proc.Natl.Acad.Sci. USA 81, 5767-5771.
21. Chreseh DA, Klier FG (1986) Disialoganglioside GD 2 distribute preferentially into substrate associated microprocesses on human melanoma cells during their attachment to fibronectin. J. Cell Biol. 102, 1887-1897.
22. Christensen L, Nielsen M, Anderson J, Clemensen I (1988) Stromal fibronectin staining pattern and metastasizing ability of human breast carcinoma. Cancer Res. 48, 6227-6233.
23. Crawford BJ (1983) Some factors controlling cell polarity in chick retinal pigment eptihelial cells in clonal culture. Tissue Cell 15, 993-1005.
24. Csuka O, Sugár J (1978) Alterations of membrane constituents in carcinomas and drug resistant tumor cells. Arch. Geschwulstforsch. 48, 770-782.
25. Damjanov I (1987) Lectin cytochemistry and histochemistry. Lab.Invest. 57, 5-25.
26. Danon D, Goldstein L, Marikovsky Y, Skutelsky E (1972) Use of cationic ferritin as label of negative charges on cell surface. J.Ultrastruct.Res. 38, 500-510.
27. David-Pfeuty T, Singer SJ (1980) Altered distribution of the cytoskeletal proteins vinculin and alfa-actinin in cultured fibroblasts transformed by Rous sarcoma virus. Proc.Natl.Acad.Sci. USA 77; 6687-6691.
28. Davina JHM, Lamers GEM, VanHaelst JGM, Kenemas P, Stadhouders AM (1984) Tannic acid binding of cell surfaces in normal, premalignant, and malignant squamous epithelium of the human uterine cervix. Ultrastruct.Pathol. 6, 275-284.
29. Dennis JW, Laferte S (1987) Tumor cell surface carbohydrate and the metastatic phenotype. Cancer Metastasis Rev. 5, 185-204.
30. Dudouet B, Robina S, Huet C, Sahuquillo-Merino C, Blair L, Coudrier E, Louvard D (1987) Changes in villin synthesis and subcellular distribution during intestinal differentiation of HT 29-18 clones. J. Cell Biol. 105, 359-369.
31. Ekblom P, Thesleff I, Lehto VP, Virtanen I (1983) Distribution of the transferrin receptor in normal human fibroblasts and fibrosarcoma cells. Int.J. Cancer 31, 111-117.
32. Fogel M, Altevogt P, Scirrmacher V (1983) Metastatic potential severly by changes in tumor cell adhesiveness and cell - surface sialylation. J.Exp.Med. 157, 371-376.
33. Fantini J, Martin JM, Luis J, Rémy L, Tirard A, Marvaldi J, Pichon J (1988) Restricted localization of functional vasoactive intestinal peptide (VIP) receptors in vitro differentiated human colonic adenocarcinoma cells (HT29-D4). Eur. J. Cell Biol. 46, 458-465.
34. Fujimoto T, Ogawa K (1983) Cell membrane polarity in dissociated frog urinary bladder epithelial cell. J.Histochem. Cytochem. 31, 131-138.
35. Geiger B, Volk T, Raz A (1985) Cell contacts and cytoskeletal organization of metastatic cell variants. Exp.Biol.Med. 10, 39-53.
36. Giancotti FG, Comoglio PM, Torone G (1986). A 135,000 molecular weight plasma membrane glycoprotein involved in fibronectin-mediated cell adhesion. Immunofluorescence localization in normal and RSV-transformed fibroblasts. Exp. Cell Res. 163, (1986) 47-62.
37. Godefroy O, Huet C, Blair LAC, Sahuquillo-Merino C, Louvard D (1988) Differentiation of a clone isolated from HT 29 cell line: polarized distribution of

histocompatibility antigens (HLA) and of transferrin receptors. Biol.Cell. 63, 41-55.
38. Hamada H, Sato M, Ozawa M, Ohi Y, Maramatsu T (1984) Transmembrane control of laminin, lectin receptors and surface villi in teratocarcinoma-derive endodermal cells. Exp. Cell Res. 154, 299-303.
39. Hakomori S (1985) Aberrant glycosylation in cancer cell membranes as focused on glycolipids: Overview and perspectives. Cancer Res. 45, 2405-2414.
40. Hébert CA, Baker JB (1988) Linkage of extracellular plasminogen activator to the fibroblasts cytoskeleton: Colocalization of cell surface urokinase with vinculin. J. Cell Biol. 106, 1241-1247.
41. Hodges GM, Carr KE, Hume SP, Marigold JCL, Southgate JF, Marshall JP (1985) Changes in surface structure and Concanavalin-A binding capacity of urothelium in the mouse bladder after whole-body neutron irradiation. Scanning Electron Microsc. 1985/IV. 1603-1614.
42. Ingber DE, Madri JA, Jamienson JD (1986) Basement membrane as a spatial organizer of polarized epithelia. Exogenous basement membrane reorients pancreatic epithelial tumor cells in vitro. Am. J. Pathol. 122, 129-139.
43. Iozzo RV (1988) Biosynthesis of heparan sulphate proteoglycan and the neoplastic phenotype. J. Cell Biochem. 37, 61-78.
44. Iozzo RV (1984) Biosynthesis of heparan sulphate proteoglycan by human colon carcinoma cells and its localization at the cell surface. J. Cell Biol. 99, 403-415.
45. Ishihara A, Holifield B, Jacobson K (1988) Analysis of lateral redistribution of a monoclonal antibody complex plasma membrane glycoprotein which occurs during cell locomotion. J. Cell Biol. 106, 329-343.
46. Ishimaru Y, Hattori R, Jinyan C, Hayashi H (1985) Cell membrane polarity in rat ascites hepatoma cells. Distribution of a cell surface-associated adhesive factor on the cell surfaces. Cell Tissue Res. 240, 353-359.
47. Jemmerson R, Klier FG, Fishman WH (1985) Clustered distribution of human placental alkaline phosphatase on the surface of both placental and cancer cells. Electron microscopic observations using gold-labeled antibodies. J.Histochem.Cytochem. 33, 1227-1234.
48. Jesaitis AJ, Yugerabide J (1986) The lateral mobility of the (Na^+,K^+) dependent ATPase in Madin-Darby canine kidney cells. J. Cell Biol. 102, 1256-1263.
49. Kimura A, Kawaguchi T, Ono Y, Sakuma A, Yokoya Y, Koch H, Nakamura K (1988) Cell surface heparan sulphate and adhesive property of sublines of rat ascites hepatoma AH 7974. J. Cell Sci. 90, 683-689.
50. Kishimoto, T, Tavassoli M, Green R, Jacobson DW (1987) Receptors for transferrin and transcobalamin II display segregated distribution on microvilli of leukemia L 1210 cells. Biochem.Res.Comm. 146, 1102-1108.
51. Köteles GJ, Kubasova T, Somosy Z, Horvath M (1983) Derangement of cellular plasma membranes due to nonlethal radiation doses. In "Biological Effects of Low-level Radiation". Vienna, IAEA, pp. 115-129.
52. Köteles GJ, Kubasova T, Somosy Z (1987) Radiation targets and dose-effect relationships. In: Proc. 14th. Int. Cancer Cong., ed. S. Eckhardt, Karger, Basel (Akademiai Kiadó, Budapest), pp. 189-196.
53. Köteles GJ, Somosy Z, Kubasova T (1987) Radiation-induced changes on cell surface charges. Radiat.Phys.Chem. 30, 389-399.
54. Kubasova T, Köteles GJ, Somosy Z (1988) Concanavalin-A binding changes after irradiation in vivo and in vitro. Lectins-Biology, Biochemistry, Clinical Biochemistry 6, 523-528.
55. Kupfer A, Kronebusch PJ, Rose JK, Singer SJ (1987) A critical role for the polarization of membrane recycling in cell motility. Cell Motil. Cytoskeleton 8, 182-189.
56. LeBivic A, Arsanto JP (1987) Localization and expression of alkaline phosphatase and ATPase during HT-29.18 differentiation process. Biol.Cell. 60, 41-48.
57. LeBivic A, Bose-Biern H (1988) Characterization of a glycoprotein expressed on the basolateral membrane of human intestinal epithelial cells and cultured colonic cell lines. Eur.Cell.Biol. 46, 113-120.
58. Leivo I (1983) Basement membrane like matrix of teratocarcinoma derived endodermal cells: Presence laminin and heparan sulphate in the matrix at points of attachment to cells. J.Histochem.Cytochem. 31, 35-45.
59. Lentz BR (1988) Organization of membrane lipids by intrinsic membrane proteins. In "Lipid domains and the relationship to membrane function" Alan R. Liss, Inc. New York, pp. 141-161.
60. Mason PW, Jacobson BS (1985) Isolation of the dorsal, ventral and intracellular domains of HeLa cell plasma membrane following adhesion to a gelatin substrate. Biochem.Biophys. Acta 821, 264-276.
61. Maurice M, Rogier E, Cassio D, Feldman G (1988) Formation of plasma membrane domains in rat hepatocytes and hepatoma cell lines in culture. J. Cell Sci. 90, 79-92.
62. Moullier P, Daveloose D, Dubos M, Letterier F, Hoebke J (1986) Effects of in vivo gamma-irradiation on the binding of wheat germ agglutinin on lymphocyte plasma membranes. Biochim.Biophys. Acta 833, 407-412.
63. Muresan Z, Muresan V, Iwanij V, Jamienson JD (1986) Sialoglycoconjugates of a pancreatic tumor: Markers for cell polarity, membrane fluidity, and possible role in exocytosis. J.Histochem.Cytochem.

14, 1265-1270.
64. Nemanic MK (1979) Prospective on cell surface labeling using the hapten-sandwich method: an integrated approach. Scanning Electron Microsc. 1979/III. 537-547.
65. Nicolson GL (1971) Difference in topology of normal and tumor cell membranes shown by different surface distribution of ferritin-conjugated Concanavalin-A. Nature (New Biol.) 233, 244-246.
66. Nicolson GL (1984) Cell surface molecules and tumor metastasis. Exp. Cell Res. 150, 3-22.
67. Pakkanen RW, VanBonsdorff C-H, Turunen O, Wahlström T, Vaheri A (1988) Redistribution of Mw75000 plasma membrane protein cytovillin, into newly formed microvilli in Herpes simplex and Semliki Forst virus infected human embryonal fibrolbasts. Eur.J.Cell Biol. 46, 435-443.
68. Parish RW, Schmidhauser C, Schmidt T, Dudler RK (1987) Mechanisms of tumor cell metastasis. J. Cell Sci. Suppl. 8, 181-197.
69. Pöllanen J, Saksela O, Solonen EM, Andersen P, Nielsen L, Dano L, Vaheri A (1987) Distinct localization of urokinase type plasminogen activator and its type 1 inhibitor under cultured human fibroblasts and sarcoma cells. J. Cell Biol. 104, 1085-1096.
70. Raz A, Bucana C, McLellan W, Fidler IJ (1980) Distribution of membrane anionic sites on B 16 melanoma variants with differing lung colonising potential. Nature 284, 363-364.
71. Raz A, Ben-Ze'ev A (1983) Modulation of the metastatic capability in B 16 melanoma by cell shape. Science 221, 1307-1310.
72. Schroeder F, Gardiner JM (1984) Membrane lipids and enzymes of cultured high- and low-metastatic B 16 melanoma variants. Cancer Res. 44, 3262-3269.
73. Shur BD (1984) The receptor function of galactosyl-transferase during cellular interactions. Molec.Cell.Biochem. 61, 143-158.
74. Simons K, Fuller SD (1985) Cell surface polarity in epithelia. Ann.Rev. Cell Biol. 1, 243-288.
75. Singer II, Scott S, Kawka DW, Kazazis DM, Gailit J, Ruoslahti E (1988) Cell surface distribution of fibronectin and vitronectin receptors depends on substrate composition and extracellular matrix accumulation. J. Cell Biol. 106, 2171-2182.
76. Sobune K (1988) Submembraneous cytoskeletal elements including actin, calspectin (fodrin or nonerythroid spectrin), alpha-actinin and vinculin redistribute into focal contacts after cellular transformation. Abstr.Fourth Intern.Cong. Cell Biol. Montreal, Canada, p. 166.
77. Soligo D, Lambertenhi-Deliliers G, DeHarven E (1987) Immuno-scanning electron microscopy of normal and leukemic leukocytes labelled with colloidal gold. Scanning Micr. 1, 719-752.
78. Somosy Z, Kubasova T, Köteles GJ (1984) Cell membrane polarity in primary human fibroblasts. Cell Biol.Intern.Rep. 9, 407-413.
79. Somosy Z, Kubasova T, Ecsedi G, Köteles GJ (1986) Radiation induced changes on the cell surface of primary human fibroblasts. Int.J.Radiat.Biol. 49, 969-978.
80. Somosy Z, Kubasova T, Köteles GJ (1987) The effects of low doses of ionizing radiation upon the micromorphology and functional state of cell surface. Scanning Microsc. 1, 1267-1278.
81. Somosy Z, Takáts A, Kubasova T, Sudár F, Köteles GJ (1988) Effects of X-irradiation on adenylate cyclase activity and cyclic AMP content of primary human fibroblasts. Scanning Microsc. 2, 2165-2172.
82. Somosy Z, Csuka O, Köteles GJ (1985) Distribution of anionic sites on surfaces of various B 16 melanoma cell lines. Acta Biochim.Biophys.Acad.Sci.Hung. 20, 95.
83. Spicer SS, Baron DA, Sato A, Schultz BA (1981) Variability of cell surface glycoconjugates - Relation to differences cell function. J.Histochem.Cytochem. 29, 994-1002.
84. Sugiyama T, Brown WR, Ahnen DJ (1988) Expression of cell surface antigens on rat colonic cancer cells. Gastroenterology 94, 331-342.
85. Subject JR, Weiss L (1974) The binding of cationized ferritin at the surfaces of Ehrlich ascites tumor cells: The effects of pH and glutaraldehyde fixation. J. Cell Physiol. 85, 529-536.
86. Sweet WD, Schroeder F (1988) Lipid domains and enzyme activity. In "Lipid domains and the relationship to membrane function" Alan R. Liss, Inc. New York, pp. 17-42.
87. Taylor EW (1986) Cell motility. J. Cell Sci. Supl. 4, 89-102.
88. Tehshima S, Hirohashi S, Shimosato Y, Kishi K, Ino Y, Matsumoto K, Yamada T (1984) Histochemically demonstrable changes in cell surface carbohydrates of human germ cell tumors. Lab. Invest. 50, 271-277.
89. Vanderpuye OA, Carraway CAC, Carraway KL (1988) Microfilament association of ASGP-2, the Concanavalin-A binding glycoprotein of the cell-surface sialomucin complex of 13762 rat mammary ascites tumor cells. Exp. Cell Res. 178, 211-223.
90. VanDeurs B, Zou ZZ, Briand P, Balsev Y, Petersen OW (1987) Epithelial membrane polarity: A stable, differentiated feature of an established human breast carcinoma cell line MCF-7. J.Histochem.Cytochem. 35, 461-469.
91. Werling H-O, Spiess E, Aulenbacher P, Paweletz N (1985) Adhesion and spreading characterization of a rat tumor cell system exhibiting different metastatic behaviour. Invasion Metastasis 5, 270-294.
92. West AB, Isaac CA, Carboni JM, Morrow JS, Mooseker MS, Barwick KW (1988) Localization of villin, a cytoskeletal protein specific to microvilli, in human ileum

and colon and in colonic neoplasma. Gastroenterology 94, 343-352.
93. Young MR, Diffie GP, Newby M (1987) Adhesiveness and morphology of Lewis lung carcinoma variants influence their sensitivity to natural killer cells and their metastatic capacity. Invasion Metastasis 7, 96-108.

Discussion with Reviewers

T.M. Seed: In the discussion of changes in the localization of membrane domains during malignant transformation, the authors suggest that the apparent randomization of domains is due to reduction of cellular contacts and/or changes in cell shape. The latter is indeed highly probable. However, these changes are "late" effects of underlying subcellular and molecular changes involved in enhanced fluidity of the lipoidal and protein island domains. Would the authors care to briefly comment on this possible subcellular mechanisms?
Authors: Maintanence and development of membrane domain distribution are regulated by microfilament-microtubular system and cell contacts (it is recently summarized in our recent work Z. Somosy et al, 1989, Polarized membrane domains of fibroblasts. Scanning Microsc. 3, 579-590). This regulation may occur directly or indirectly, too. The filaments can attach to some domains causing their active movement or stabilization in determined surface areas (microvilli, contacts) (15). Membrane domaines have a restricted mobility in this areas (Geiger B et al. J. Cell Biol. 93, 595-500). The cell contact creates diffusion barriers also toward other localized membrane domains not involved in contact (Jesaitis AJ and Yguerabide, J 1986, J.Cell Biol. 102, 1256-1263). During malignant transformation the cell contacts disappear or change (15). Thus, the diffusion barriers also cease to exist. For these membrane domains, the diffusion is regulated by cell contacts, thus it became possible for the membrane domains to move freely along whole cell surface.

J.S. Hanker: Don't you feel, however, that we must keep in mind that levels of organization other than membrane domains or the plasma membrane, such as other surface domains or even cytoskeletal organization, are also affected?
G.M. Hodges: Is the change in the distribution of surface membrane domains in the irradiated B 16 melanoma cells associated with changes in cell cytoskeletal elements?
Is it possible that the radiation induced change in membrane domains of the B 16 melanoma cells may be but transient responses related to short-term cell-cell or cell-substrate attachment modifications?
Authors: The B 16 melanoma cells, similar to other cell types, display an increased ruffling activity upon irradiation, of course, these morphological changes show changes in distribution of cytoskeletal elements and modify cell contacts. As is known, there is a relationship between membrane domain mobility, cytoskeleton system and cell contacts (see answer to Dr. T. M. Seed above). This might explain the changes or at least, contribute to the explanation.

G.M. Hodges: What were the experimental conditions used for the cytochemical reactions? Was the cytochemistry carried out on cells in situ or on ultrathin sections?
Authors: The cytochemistry carried out on cells in situ. Previously the cells were fixed for 30 minutes by 0.025 per cent glutaraldehyde in phosphate buffer, at pH 7.3. Cationized ferritin (CF) (Sigma) was used for the detection of negatively charged sites (26). Cell cultures were incubated for 1 min in phosphate-buffered saline containing 0.3 mg/ml CF. The Concanavalin-A - peroxidase reaction was applied according to Bernhard and Avremas (7). For controlling the specificity of Con-A binding alpha-D-mannose (Sigma) was used in the concentration of 0.05 M in phosphate-buffered saline before incubation of lectin with cells.

T.M. Seed: How do the authors rule out the possibility that the noted non-uniform distribution of selected cell surface receptors (charged sialic acid residues) in normal, non-transformed mammalian cells is not simply the result of lack of penetrance of the cytochemical marker into restricted cell surface sites?
Authors: According to our experiments the polarized or non-uniform localization of studied membrane domains could not be explained by permeability barrier. The particles of cationized ferritin bound on a polarized manner on the fibroblast membrane while the native form of ferritin bound uniformly (80). Similar difference was observed with Concanavalin-A peroxidase and Wheat Germ Agglutinin-peroxidase reactions (80).

G.M. Hodges: At what culture time-points were the observations made?
Authors: For the experiments, semiconfluent asynchronous cultures were used, i.e. depending on the number of cells this level was reached within 48-72 hours.

G.M. Hodges: Were the radiation-induced changes retained over a number of passages in culture?
Author: The cells were not cultured in consequent passages after irradiation.