Radiol Oncol 1998; 32(1): 109–117

Plasma membrane fluidity alterations in cancerous tissues

Marjeta Šentjurc[1], Miha Sok[2] and Gregor Serša[3]

[1]Jožef Stefan Institute, [2]University Medical Center, [3]Institute of Oncology, Ljubljana, Slovenia

Plasma membrane is a heterogeneous structure with several coexisting domains having different fluidity characteristics. It plays an important role in the control of cell growth, differentiation and transformation. Fluidity of the whole plasma membrane reflects the ordering and dynamics of phospholipid acyl chains in specific membrane domains, as well as the fraction of each domain in the membrane. Changes in the membrane fluidity affect processes on the membrane such as transport, enzyme activities and expression of the receptors.

In this paper we present results of our recent electron paramagnetic resonance (EPR) studies of plasma membrane fluidity characteristics, which take into account heterogeneous nature of the plasma membrane. By the computer simulation of the EPR spectra line-shapes, the number of coexisting domains in the plasma membrane, their relative portion in the membrane as well as the ordering and dynamics of each domain can be determined. Therefore, we could distinguish the contribution of the relative portion of each domain from the contribution of fluidity alterations in the domain to the entire fluidity changes in the membrane.

Two examples will be discussed: membrane fluidity characteristics of excised lung tumor tissues and influence of microtubule depolymerizing agent vinblastine on membrane fluidity of vinblastine sensitive and resistant HeLa cells.

Key words: membrane, fluidity, electron paramagnetic (spin) resonance, cancer

Introduction

Plasma membrane fluidity

Plasma membrane is a heterogeneous structure, composed of lipids and proteins (enzymes, receptors, transport proteins) with or without the attached oligosaharides. It regulates the transport of ions and chemicals and plays an important role in the control of cell growth, differentiation and transformation.[1] Lipids are arranged in lipid bilayer, and can be treated as an anisotropic two-dimensional liquid in which constituent molecules undergo translational and rotational motion at a rate characteristic for viscous oil. It is characterised by membrane fluidity, which is in opposite relation to membrane microvis-

Correspondence to: Dr. Marjeta Šentjurc, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia. Phone: +386 61 1773 689; Fax: +386 61 1235 400; E-mail: marjeta.sentjurc@ijs.si

cosity.[2] In the membrane, where the structure is heterogeneous, fluidity in different regions is different. In such a case the term fluidity is used to describe the motional freedom of lipid-soluble molecular probe within the bilayer. It is described by order parameter (S), time-averaged deviation of the acyl chains from the normal to the bilayer plane, and rotational correlation time (τ_c), time required for the molecules to forget what were their previous spatial orientations.[3] Membrane fluidity regulates the dynamics of conformational changes and the lateral mobility of membrane proteins. Changes in membrane fluidity influence membrane processes such as transport, enzyme activities and receptor expression. Fluidity of plasma membrane seems to be biologically very important since bacteria, yeast and other organisms whose temperature fluctuates with that of the environment, change the fatty acid composition of the membrane in order to maintain a relatively constant fluidity.[1]

Main factors which influence the fluidity are: composition of lipids and distribution of integral and peripheral proteins within the bilayer. Main lipid components of plasma membrane are phospholipids. Membranes are fluid if lipids are in liquid crystalline phase and rigid if lipids are in gel phase. Phospholipids with short acyl chains or non-saturated bonds in the chain make bilayer more fluid. Phospholipids of the same type tend to aggregate and as a consequence a phase separation in the bilayer can occur, therefore domains with different fluidity characteristics are formed. Another important lipid constituent of the plasma membrane is cholesterol, which composes 30 to 50 mol % of all lipids in eucaryotes. In this cholesterol concentration region a new type of phase is obtained in which the acyl chains are more highly orientationally ordered than in the fluid phase of pure phospholipid system (liquid-ordered phase). In this way cholesterol tends to regulate or completely eliminates the possible phase transitions of phospholipids from solid to fluid phase. Besides, it decreases permeability for water molecules and enhances mechanical stability of bilayer.[2] Highly non-saturated acyl chains (20:4 and 20:6) are unlikely to associate with cholesterol, while highly saturated phospholipids, such as sphingomielin preferentially interacts with cholesterol, creating highly saturated and non-saturated lipid domains.[1]

Another factor which contributes to membrane heterogeneity is specific interactions between lipids and proteins.[4] Lipid-protein interactions influence the distribution of lipids around proteins which cause the formation of domains with different fluidity characteristics. The important parameters for the interaction are polar heads (charge, size) and matching of acyl chains with the hydrophobic span of proteins (length and fluidity of acyl chains). Influence of polar heads is stronger as that of the chains. It is also influenced by pH, temperature and ionic strength.

Fluidity in malignant cells

There are some typical cellular alterations observed after neoplastic transformation which are in close relation to plasma membrane fluidity. Transport of ions and chemicals is altered as well as membrane permeability, phagocytosis or endocythosis, etc. Typically is increased lectin agglutinability, which is generally correlated with enhanced ability of lectin binding sites on transformed cells to move laterally through the plasma membrane. This enhanced mobility may be a consequence of the increased plasma membrane fluidity.[5]

However, plasma membrane fluidity measurements in malignant cells are controversial.[5] According to the literature most tumor cells *in vitro* exhibit higher membrane fluidity than their normal progenitors. For example, such differences were observed in Mal-

oney sarcoma-virus transformed murine tumor cells,[6] in leukemic T lymphocytes,[7] in human CLL lymphocytes,[8] in mouse malignant lymphoma cells,[9] in rat colon tumor cells,[10] in mouse Morris 7288C hepatoma cells,[11] in human ovarian carcinoma cells.[12] However, in KiMSV transformed rat kidney cells,[13] in virally, chemically or tumorigenically transformed 3T3 cells,[14] in hepatoma cells[15] and in T lymphocytes from untreated patients with Hodgkin's disease[16] decreased membrane fluidity was found, in comparison to corresponding controls. In freshly excised tumors only a few studies have been performed. In excised local and metastatic LM cell tumor tissues[17,18] and in malignant brain tumors[19] more fluid plasma membranes were observed as in the corresponding non-malignant samples.

One possible reason for the measured differences in plasma membrane fluidity is the technique of measurements. Most investigations were performed with EPR or fluorescence polarisation method. In both methods a probe, which measures the properties of its surrounding, has to be introduced into the membrane. It should be stressed, that in the measurements reported, heterogeneous membrane structure composed of several coexisting domains with different fluidity was not taken into account. Only an average order parameter and correlation time were measured, which include the contribution of different domains and could be biased in favour of more fluid domains. Besides, the parameters could be biased by preferential distribution of the probe in certain type of domains.

Having this in mind the model for computer simulation of the EPR spectra line shape was developed,[20] which takes into account heterogeneity of the plasma membrane. It will be described in more details in the next section. We present two examples where this method has been applied. One is *in vitro* model where fluidity of two types of HeLa cells was compared: wild type and the type resistant to the chemotherapeutic drug vinblastine. In the other example the study was performed on excised human lung tumor tissue and fluidity characteristics for different types of tumors were determined.

EPR method

In our electron paramagnetic resonance (EPR) studies lipophilic spin probe methylester of 5-doxyl palmitate (MeFASL (10,3)) was used, which is sparsely dissolved in water solutions but very well dissolved in lipids. It is incorporated preferentially into the membrane bilayer of cells and/or tissues. Since it is reduced very fast in hypoxic conditions by oxy redoxy systems in cells and tissues, which are primarily located at the site of ubiquinone in mitochondria[21] we believe that the main EPR signal observed corresponds to the EPR spectrum in the plasma membranes.

For rough estimation of relative changes in plasma membrane fluidity a maximal hyperfine splitting $2A_{II}$, which is related to the average order parameter (S_{ef}), and/or empirical correlation time τ_{ep} can be determined from the EPR spectra.[22]

$$S_{ef} = k(A_{II} - A_{\perp})/(A_{zz} - A_{xx}) \qquad (2)$$

k = correction due to the polarity of spin probe environment

A_{II} and $A_{\perp}$ = measured maximal and minimal hyperfine splitting (Figure 2. A).

A_{zz} and A_{xx} = hyperfine coupling constants of MeFASL(10,3).

Empirical correlation time τ_{ep} is given by the expression:

$$\tau_{ep} = k\Delta H_o(h_o/h_{-1} - 1)^{1/2} \qquad (3)$$

where ΔH_o, h_o, and h_{-1} are parameters which can be measured from the EPR spectra. ΔH_o is the line-width of the middle line of the EPR spectra, while h_o, and h_{-1} are amplitudes of

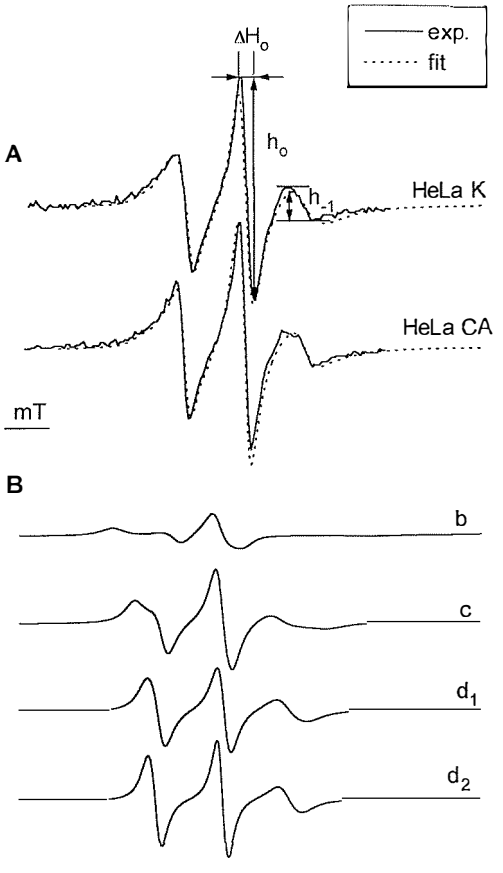

Figure 1. Typical EPR spectra of MeFASL(10,3) in the membrane of HeLa K, and HeLa CA cells.
A. Full line: experimental spectra, dotted line: the best fit to the experimental spectra, taking into account that the experimental spectra are superimposition of the spectra of three coexisting domains (b,c,d), which are presented in B together with the corresponding fluidity parameters S and τ_c.
B. Computer simulation of the EPR spectra of three coexisting domains in HeLa K and HeLa CA cells: domain b (spectrum b), domain c (spectrum c). Spectrum b and c are the same for HeLa K and HeLa CA (S = 0.7 and 0.32, respectively and τ_c = 4.0 ns and 2.0 ns, respectively) and domain d (spectrum d1 for HeLa K, S = 0.15, τ_c = 1.5 ns and spectrum d2 for HeLa CA with S = 0.10 and τ_c = 1.0 ns).

middle- and low-field line (Figure 1.A.), k is constant typical of the spin probe used. The relation is valid only for fast isotropic motion of spin probes and can be used as a rough approximation to estimate relative membrane alterations under different influences.[22]

However, to obtain more precise information about the heterogeneous structure of bilayer with several coexisting domains with different order parameter (S) and correlation time (τ_c) the line shape of the experimental EPR spectra should be compared with the spectra calculated by the model which takes into account heterogeneous bilayer structure with several coexisting domains. In the model an isotropic motion of spin probe molecules around the long molecular axis and the restricted motion in the direction parallel to the long molecular axis of lipids is used. In the calculation the number of domains and the relative portion of each domain (W), which is related to the area occupied with the particular domain, is varied as well as the fluidity parameters S and τ_c of each domain. Beside the ordering parameter and correlation time the line width of the middle line is also included as a variable which describes the anisotropy of the two parameters, as well as the ratios g/g_i and a/a_i, which include the difference in the polarity of the spin probe surroundings.[20] From the best fit with experimental spectra relative portion of each domain in the membrane as well as their ordering and dynamics could be determined. Therefore, we could distinguish the contribution of the relative portion of each domain from the contribution of fluidity alterations within the domain, to the entire fluidity changes in the membrane.

Membrane fluidity of vinblastine sensitive and resistant HeLa cells

Depolymerization of microtubules by microtubule depolymerizing drugs, such as vinblastine (VLB), colchicine and vincristine, the antimitotic alkaloids commonly used as chemotherapeutic drugs that arrests many mammalian cells in the metaphase of mitosis

by its action on microtubules, were shown to be responsible for changes in the plasma membrane fluidity.[23,24] On the other hand, several studies indicate that pre-treatment of tumors with a low VLB dose can facilitate uptake of other chemotherapeutic drugs. With respect to these observations we suppose that, among the other factors, facilitated uptake of drugs after VLB treatment could be associated with increased fluidity of the plasma membrane of VLB sensitive cells. On the basis of these findings it would be interesting to compare the fluidity characteristics of cells which are sensitive to VLB with those which are resistant to VLB.

Membrane fluidity of human uterine carcinoma cells (HeLa K cells) was compared with the fluidity of a subclone HeLa CA cells, which was proved to be resistant against vinblastine (VLB)(our results, sent for publication). The cells were grown in Eagle minimal essential medium (EMEM) supplemented with 10 % FCS. In some experiments the cells were incubated for 1 hour with VLB (1 ng/ml). For EPR measurements the cells were trypsinized 48 hours after removal of VLB and spin labelled with MeFASL(10,3) as described elsewhere.[23] The EPR spectra were recorder on Bruker ESP 300 X-band EPR spectrometer.

EPR spectra of HeLA K and HeLa CA cells are presented in Figure 1. For a rough estimation of membrane fluidity changes, empirical correlation time τ_{ep} was calculated from the EPR spectra in Figure 1 using the expression *(3)*. Empirical correlation time in HeLa CA cells (τ_{ep} = 2.08 ns) was significantly lower than in HeLa K cells (τ_{ep} = 2.43 ns, $p < 0.001$). A significant decrease in τ_{ep} was also observed after treatment of HeLa K cells with VLB (τ_{ep} = 2.16 ns). However, treatment of HeLa CA cells with VLB did not significantly affect τ_{ep}, compared to the untreated HeLa CA cells. Similar results were already obtained previously for Chinese hamster ovary cell lines [24] where a decrease of order parameter of resistant mutant was observed in comparison to the wild type CHO cells with 5-doxyl stearic acid as a spin probe. Treatment with microtubule depolimerizing agents have the same influence as on HeLa cells. Also in our previous work on spindle cell sarcoma similar results were obtained.[23] In these studies only an average fluidity characteristics were measured, (an average order parameter S or average correlation time τ_{ep}.[23,24]. In the present experiment heterogeneity of plasma membrane was taken into account and by computer simulation of the EPR spectra line-shape, the information about the domain structure alteration in the plasma membrane was obtained, additionally to the average τ_{ep} alterations.

In Figure 1 (dotted lines) the best fits to the experimental spectra are presented. The calculated spectra which fitted the best to the experimental spectra of HeLa CA and HeLa K cells (Figure 1, full line) were superimposition of three spectra (Figure 1.B). These spectra correspond to the spin probe molecules in three different types of domains (b, c, d) with different order parameter (S) and correlation time (τ_c). The spectrum of VLB resistant HeLa CA cells could not be fitted adequately if only altered ratio between the domains was taken into account. For good fit it was necessary to decrease the order parameter S, of the most fluid domain (d) (spectrum d2 in Figure 1.B.). The portion of this domain in HeLa CA was slightly smaller (W = 25 %) than in HeLa K cells (W = 30 %). On the other hand, treatment of HeLa K cells with VLB did not change fluidity characteristics (S and τ_c) of membrane domains; only the portion of different domains was changed. In HeLa K portion of domain b and d was 30 % and in HeLa K treated with VLB portion of domain b was 20 % and of domain d it was 40 %. The portion of the middle domain c remained unchanged (40 %).

From the results obtained it could be concluded that the reason for the average fluidity

decrease, observed from τ_{ep} measurements for HeLa CA cells and VLB treated HeLa K cells is not of the same origin. The altered ordering and dynamics of the most fluid domain (d) in HeLa CA cells indicates that the composition of domain d is altered in comparison to HeLa K cells, while after treatment of HeLa K cells with VLB the composition of domains remain the same, but the portion of domains in the membrane change. An increased content of non-saturated acyl chains, and/or decreased cholesterol content may be the reason for the increased fluidity of the most fluid domain (d) in HeLa CA in comparison to HeLa K cells. On the other hand it seems that the treatment of HeLa K cells with VLB, which causes depolymerization of microtubules, triggers some rearrangement of membrane constituents in a way which favours the less ordered regions in the membrane.

Membrane fluidity characteristics of excised lung tumor tissues

In this study we used EPR with spin probes to investigate membrane fluidity in human lung cancer tissues specimen, cut at the time of resection. By computer simulation of the EPR spectral line-shape we wanted to distinguish the contribution to the EPR spectra of the membrane fluidity alterations within domains, from the alteration in the portion of each domain in the membrane.

Fiftyone lung cancer samples from patients with predominantly non small lung cancer, who were operated at Department of Thoracic Surgery, University Medical Centre in Ljubljana from June 1988 to June 1990 were analysed by EPR. From lung resectat the samples of cancer and histologicaly normal tissue were cut for EPR analysis, which was performed not more then two hours after operation. The samples were cut to cca 0.5 mm thick slices, weighting from 10 to 20 mg.

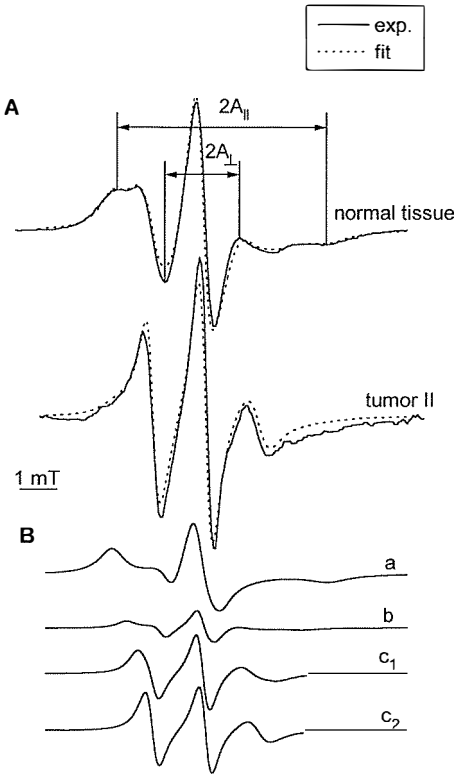

Figure 2. Typical EPR spectra of MeFASL(10.3) in the membrane of normal lung tissue, and in lung cancer tissues (Cancer II). Full line: experimental spectra, dotted line: calculated spectra, taking into account that the experimental spectra are superimposition of EPR spectra of three coexisting domains (a, b, c), with fluidity parameters (S and τ_c) presented in Table 1. Computer simulation of the EPR spectra of MeFaSL(10,3) in three coexisting domains in normal lung tissue and in lung cancer (cancer II), calculated with the parameters presented in Table 1. Spectra a and b, which correspond to the domain a and b are the same for both tissues, while spectra c1 and c2 correspond to the most fluid domain in normal lung tissue and in malignant tissue, respectively.

They were immersed for 30 min into 0.1 mmol/l solution of MeFASL(10,3) in phosphate buffered saline (PBS) (0.01 ml of 10 mmol/l MeFASL(10,3) in ethanol solution was added to 1 ml of PBS).

Typical EPR spectra of normal lung tissue and of lung cancer are shown in Figure 2A. The calculated line shapes of such heterogeneous EPR spectra, which fitted the best the experimental spectra are superimposition of three spectra (Figure 2.B), which correspond to the spin probe molecules in three different types of domain with different order parameters (S). The parameters, by which the best fits of the calculated spectra to the experimental spectra were obtained, are presented in Table 1 for normal tissue and for two different lung cancer samples (cancer I and cancer II).

Our results demonstrate that plasma membranes in lung cancer samples are more fluid than the membranes in normal lung tissue. The alterations observed are connected with an enlargement of the most fluid and less ordered domains as well as with an increased fluidity of these domains in malignant tissues. This is reflected in a decrease of order parameter S from 0.25 in normal tissue to 0.05 in malignant tissues. This indicates that the lipid composition of the most fluid domains is altered, and could be most probably ascribed to a decreased cholesterol content in plasma membrane of tumor tissue. This is in accordance with other studies which showed that the membranes of tumor cells were more fluid then the membranes of normal cells.[7,8,9] According to Vitols [25] there are two possible explanations for enhanced plasma membrane fluidity of tumor cells: rapid cholesterol turnover and plasma membrane shedding in tumor cells. Adequate cholesterol/phospholipid molar ratio in plasma membrane is maintained by cell's own cholesterol biosynthesis and intense receptor mediated endocytosis of serum cholesterol.[25,26] The consequence could be a decreased serum cholesterol level in tumor bearing subjects, which was found in tumor bearing mice [26] and in cancer patients with tumor of different origin described in many epidemiological and clinical studies.[27,28,29] On the other hand, cholesterol is lost from plasma membrane by continuous exfoliation or shedding of membrane vesicles, which are reach in antigens and cholesterol.[30,31,32]

From Table 1 it is also evident that two cancer types differ only in the ratio of domains with different fluidity, while the ordering and dynamics of these domains does not change significantly. Enlargement of the most fluid domain and a decrease of the area with the most ordered domain could be attributed to the protein and/or antigen redistribution in the membrane. This could be connected with bulbs formation in tumor cells, which could be the first step in plasma membrane shedding.

Table 1. EPR fluidity parameters S, and τ_c and the portion of each domain in the membrane W used in the calculation of the EPR spectra of MeFASL(10,3) in the membrane of lung tissues presented in Figure 1, which gave the best fit to the experimental spectra

	Normal* tissue			Cancer I			Cancer II		
Domain	a	b	c	a	b	c	a	b	c
S	0.8	0.5	0.25	0.8	0.5	0.05	0.8	0.5	0.05
τ_c(ns)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
W (%)	0.72	0.10	0.18	0.43	0.27	0.3	0.3	0.15	0.55

* normal tissue was taken from the periphery of resected lung, far away from tumor

Conclusions

In this review article we showed two examples of EPR investigation: on cultured malignant cells and on non-treated human tissues taking into account heterogeneity of plasma membrane. With this approach the fluidity changes within certain membrane domain were distinguished from the alteration in the portion of domains in the membrane. In this way we could obtain additional information about the modification of lipid and/or protein composition in the domain and about the redistribution of membrane components within the membrane.

The general observation was that in tumor tissues the fluidity of the most fluid domain is increased, what could correspond to the decreased cholesterol concentration in plasma membrane. In different tumors only the area of domains is altered, while the fluidity of domain is basically unchanged. Similar observations were also obtained for two HeLa cell lines. They differ in the fluidity characteristics of certain domains, while the treatment of VLB sensitive cells with VLB, only altered the area of domains.

In further membrane fluidity investigations it should be also taken into account that different probes could preferentially be dissolved in different domains, more or less reach with cholesterol or proteins. Therefore different probes should be applied.

References

1. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. *Molecular Biology of the Cell.* London: Garland Publishing, Inc, 1989: 275-341.
2. Bloom M. The physics of soft, natural materials. *Physics in Canada* 1992; **48:** 7-16.
3. Alvarado Cader A, Butterfield DA, Watkins BA, Hong Chung B, Hennig B. Electron spin resonance studies of fatty acid-induced alterations in membrane fluidity in cultured endothelial cells. *Int J Biochem Cell Biol* 1995; **27:** 665-73.
4. Marsh D. Lipid-protein interactions and heterogeneous lipid distribution in membranes. *Mol Membr Biol* 1995; **12:** 59-64.
5. Ruddon RW. *Cancer biology.* Oxford: Oxford University Press, 1995: 103-7.
6. Taraboletti G, Perin L, Bottazzi B, Mantovani A, Giavazzi R, Salmona M. Membrane fluidity affects tumor cell motility, invasion and lung colonizing potential. *Int J Cancer* 1989; **RR44**: 707-13.
7. Yang YZ, Hao TL, Qian M, Dai WX, Huangfu YM, Zhang ZH. Normal and leukemic lymphocyte membrane fluidity and response to stimulation with ConA and PHA. *J Tongji Med Univ* 1989; **9:** 143-7.
8. Deliconstantinos G. Physiological aspects of membrane lipid fluidity in malignancy. *Anticancer Res* 1987; **7:** 1011-21.
9. Inbar M. Fluidity of membrane lipids: A single cell analysis of mouse normal lymphocytes and malignant lymphoma cells. *FEBS Lett* 1976; **67:** 180-5.
10. Dudeja PK, Dahiya R, Brasitus TA. The role of sphingomyelin synthetase and sphingomyelinase in 1,2- dimethylhydrazine induced lipid alterations of rat colonic plasma membranes. *Biochim Biophys Acta* 1986; **863:** 309-12.
11. Benko C, Hilkmann H, Vigh L, van Blitterswijk WJ. Catalytic hydrogenation of fatty acyl chains in plasma membranes; effect on membrane lipid fluidity and expression of cell surface antigens. *Biochim Biophys Acta* 1987; **896:** 129-35.
12. Mann SC, Andrews PA, Howell SB. Comparison of lipid content, surface membrane fluidity, and temperature dependence of cis-diamminedichloroplatinum(II) accumulation in sensitive and resistant human ovarian carcinoma cells. *Anticancer Res* 1988; **8:** 1211-5.
13. Schara M, Šentjurc M, Cotič L, Pečar S, Palčič B, Monti-Bragadin C. Spin label study of normal and Ki-MSV transformed rat kidney cell membranes. *Stud Biophys* 1977; **62:** 141- RR50.
14. Parola AH. Membrane dynamic alteration associated with cell transformation and malignancy. In: Galeotti T, Cittadini A, Neri G, Papa S, eds. *Membranes in tumor growth.* Amsterdam: Elsevier biomedical press, 1982: 69-81.
15. Mahler SM, Wilce PA, Shanley BC. Studies on regenerating liver and hepatoma plasma membranes. Membrane fluidity and enzyme activity. *Int J Biochem* 1988; **20:** 613-9.
16. Aleksijevic A, Cremel G, Mutet C, Giron C, Hubert P, Waksman A, Falkenrodt A, Oberling F, Mayer S, Lang JM. Decreased membrane "fluidity" of T lymphocytes from untreated patients with Hodgkin's disease. *Leuk Res* 1986; **10:** 1477-84.
17. Kier AB, Parker MT, Schroeder F. Local and metastatic tumor growth and membrane proper-

ties of LM fibroblast in athymic (nude) mice. *Biochim Biophys Acta* 1988; **938:** 434-46.

18. Kier AB. Membrane properties of metastatic and non-metastatic cells cultured from C3H mice injected with LM fibroblast. *Biochim Biophys Acta* 1990; **1022:** 365-72.

19. Šentjurc M, Schara M, Auersperg M, Jezernik M and Kveder M. Characterization of malignant tissue by EPR. *Studia Biophys* 1990; **136:** 201-8.

20. Žel J, Svetek J, Črne H and Schara M. Effects of aluminum on membrane fluidity of the mycorrhizal fungus *Amanita muscaria. Physiologia Plantarum* 1993; **89:** 172-6.

21. Swartz HM, Šentjurc M, Kocherginsky N. Metabolism of nitroxides and their products in cells. In: Kocherginsky N and Swartz HM Eds. *Nitroxide spin labels: Reactions in Biology and Chemistry*. Boca Raton: CRC press, 1995: 113-47.

22. Marsh D. Electron spin resonance: spin labels. In: Grell E, ed. *Membrane spectroscopy*. Berlin: Springer Verlag, 1981: 52-141.

23. Serša G, Čemažar M, Šentjurc M, Us-Krašovec M, Kalebić S, Drašlar K, Auersperg M. Effect of vinblastine on cell membrane fluidity and the growth of SA-1 tumor in mice. *Cancer Letters* 1994; **79:** 53-60.

24. Aszalos A, Yang GC and Gottesman MM. Depolymerization of microtubules increases the motional freedom of molecular probes in cellular plasma membranes. *J Cell Biol* 1985; **100:** 1357-62.

25. Vitols S, Peterson C, Larsson O, Holm P, Aberg B. Elevated uptake of low density lipoproteins by human lung cancer tissue in vivo. *Cancer Res* 1992; **52:** 6244-7.

26. Van Blitterswijk WJ, Damen J, Hilkman H, de Widt J. Alterations in biosynthesis and homeostasis of cholesterol and in lipoprotein patterns in mice bearing a transplanted lymphoid tumor. *Biochim Biophys Acta* 1985; **816:** 46-56.

27. Alexopoulos CG, Blatsios B, Avgerinos A. Serum lipids and lipoprotein disorders in cancer patients. *Cancer* 1987; **60:** 3065-70.

28. Winawer SJ, Flehinger BJ, Buchalter J, Herbert E, Shike M. Declining serum cholesterol levels prior to diagnosis of colon cancer. A time-trend, case-control study. *JAMA* 1990; **263:** 2083-5.

29. Faintuch J, Cobraitz R, Martin Nieto AR, Yagi OK, Zilberstein B, Cecconello I, Pinotti HW, Wesdorp RI. The prognosis value of cholesterol level in malnourished patients with esophageal carcinoma. *Nutricion Hospitalaria* 1993; **8:** 352-7.

30. Black PH. Shedding from the cell surface of normal and cancer cells. *Adv Cancer Res* 1980; **32:** 75-199.

31. Petitou M, Tuy F, Rosenfeld C, Mishal Z, Paintrand M, Jasnin C, Mathe G, Inbar M. Decreased microviscosity of membrane lipids in leukemic cells: Two possible mechanisms. *Proc Natl Acad Sci USA* 1978; **75:** 2306-10.

32. Van Blitterswijk WJ, de Veer G, Krol JH, Emmelot P. Comparative lipid analysis of purified plasma membranes and shed extracellular membrane vesicles from normal murine thymocythes and leukemic GRSL cells. *Biochim Biophys Acta* 1982; **688:** 495-504.