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MOLECULAR ASPECTS OF THE PLASMA MEMBRANE IN TUMOR CELLS

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INTRODUCTION

It is well known that malignant neoplastic cells are different in their surface properties from their normal counterparts.[1,20] Unusual cell-to-cell interactions in the malignant cells are the most important behavior for distinguishing them from their normal counterparts and also for determining the prognosis of patients suffering from cancer.

Decrease in or loss of the cell's ability of (1) sorting out each other, (2) mutual adhesiveness, (3) intercellular communication by exchange of small molecules, (4) contact inhibition of both growth and movement affecting the cell density of in vitro culture, and changes in the surface antigenisity, including changes in surface arrangement of various receptors, have been considered hitherto as specific abnormalities in the surface properties of malignant cells. A change in the association of plasma membrane with cytoskeletal structures also seems to have a close relation with these abnormalities. Almost all the details of the molecular mechanisms of these abnormalities, however, remain obscure.

In this paper, to clarify some of these molecular mechanisms, we will elucidate some of the molecular events and discuss them in connection with abnormalities of the surface properties in tumor cells based on our analytical results obtained from the plasma memberane of tumor cells.

Specific changes in the plasma membrane components of tumor cells

(I) Lipids

Subcellular fractionation techniques can partially separate and purify several important biologic membranes from many kinds of animal cells. As reported previously, we established a purification method of the plasma membranes from liver and hepatoma cells.[30] Using the purified membrane materials, analytic studies were performed on the membrane lipid composition as well as on the fatty acid composition of phospholipids.[3–24]

Table 1 shows the main changes in the lipid bilayer of the plasma membrane isolated from FAA-induced primary hepatoma, transplantable hepatoma, and hyperplastic nodules which are in a preneoplastic stage in rats. The amount of cholesterol in the membrane of the hepatoma cells examined, in general, tended to decrease, while it markedly increased in the hyperplastic nodule cells. The molar ratio of cholesterol to phospholipid-phosphorous increased in the order of hepatoma < normal liver < hyperplastic nodule, suggesting less fluidity of the plasma membrane in hyperplastic nodule cells. As to the phospholipid composition, the relative amount of choline phospholipids, which are mainly distributed in the outer leaflet of the membrane, were reduced in all tumor membranes as far as examined. The molar ratio of choline phospholipids to

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ethanolamine phospholipids, therefore, showed a lower value in hepatomas than in the liver. Similar trends were also found in the hyperplastic nodule membrane, although the change was intermediate between normal and malignant. These changes in the lipid bilayer may suggest that an asymmetrical distribution of each phospholipid component in the normal plasma membrane is disordered in malignant and preneoplastic cells. Fatty acid analysis by gas-liquid chromatography indicated that the total amount of short fatty-acyl chains of less than 18 carbon chains increased, but that of long fatty-acyl chains of more than 20 carbon chains decreased in the FAA-induced and transplantable hapatoma cells in comparison with that in the adult liver. The same changes were also observed in preneoplastic cells, i.e., the total amount of short fatty-acyl chains was 75% in the induced hepatoma, 73% in the hyperplastic nodule, and 62% in the adult normal liver. Furthermore, the studies also showed that the contents of the saturated fatty acids decreased, and those of the unsaturated ones increased in the hepatoma cells. The molar ratio of 18:1 to 18:0 was three times higher in the hepatoma and two times higher in the hyperplastic nodule as compared with the ancestral cells. Since an elevated molar ratio of 18:1 to 18:0 was reported in other experimental tumor cells, it is suggested that the elevation of that ratio in the plasma membrane may be one of the cellular indicators of neoplastic transformation.

Table 1. Tumor-specific Changes in the Phospholipid Bilayer of the Plasma Membrane

The analytical data on the plasma membrane of hepatoma and hyperplastic nodules were compared with those of normal adult liver membrane.

	Hepatomas	Hyperplastic nodules
Cholesterol		
Amount of cholesterol	no change or increase	increase
Molar ratio of cholesterol to phospholipid	slightly high value	high value
Phospholipids		
Choline phospholipids including sphingomieline	decrease	intermediate decrease between normal and malignant
Ethanolamine phospholipids	increase	intermediate increase between normal and malignant
Molar ratio of choline phospholipids to ethanolamine phospholipids	low value	intermediate value between normal and malignant
Fatty acids		
18:0	decrease	intermediate decrease between normal and malignant
18:1	increase	intermediate increase between normal and malignant
18:2	increase	intermediate increase between normal and malignant
20:4	decrease	intermediate decrease between normal and malignant
Molar ratio of 18:1 to 18:0	high value	intermediate value between normal and malignant
Moloar ratio of 20:4 to 18:2	low value	intermediate value between normal and malignant
Microviscosity of lipid bilayer	low viscosity	high viscosity

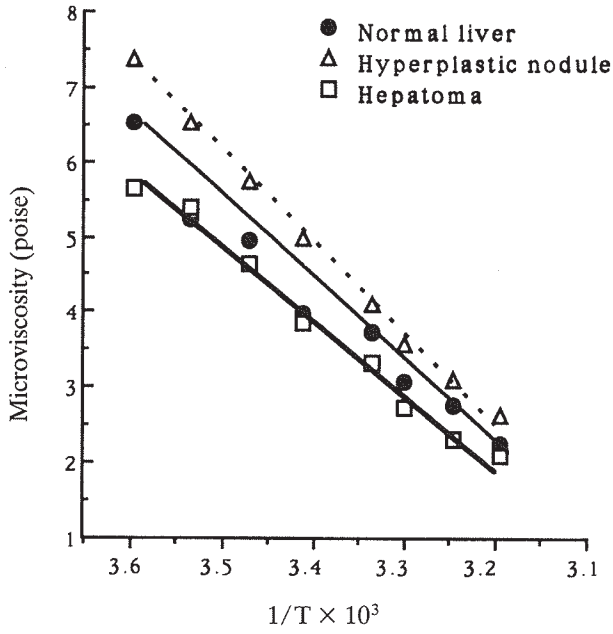


Fig. 1. Changes in microviscosity of hepatocyte plasma membrane during carcinogenesis.
The microviscosities of plasma membranes isolated and purified from normal liver, hyperplastic nodule, and hepatoma were calculated from the P value (fluorescence polarization) determined by an Elscint microviscosimeter, model MV-1a (Elscint, Israel) using 1,6-diphenyl-1,3,5-hexatriene as a fluorescence polarization probe.

Arachidonic acid (20:4) is synthesized de novo from linoleic acid (18:2), an essential fatty acid in mammalian cells, and the former plays an important role in cell growth control through its metabolites, prostaglandins, after its liberation by phospholipase A_2. In this connection, an alteration in the ratio of 20:4 to 18:2 might reflect a cellular response to growth stimuli. In our experiments, the molar ratio of 20:4 to 18:2 decreased by 60% in malignant cells, suggesting an elevated consumption of arachidonic acid in the tumor cells. A similar tendency in the fatty-acyl chains was also observed in the hyperplastic nodule cells, but the magnitude of their change was intermediate between normal and malignant cells.

It is well known that fluidity of the plasma membrane is largely dependent on its lipid composition and cholesterol content. In general, lipids with short or unsaturated fatty acyl chains undergo the phase transition at lower temperatures than do lipids with long or saturated chains. An increase of short and unaturated acyl chains in the lipid bilayer of the membrane may increase the fluidity in the membrane of tumor cells. Fig. 1 shows the microviscosities measured by a microviscosimeter in the separated plasma membranes. Microviscosity of the plasma membrane was slightly reduced in the primary hepatoma cells, suggesting that the membrane is more fluidal in the malignant growth than in the normal growth. This change was remarkable in transplantable ascites hepatoma cells as well as in other experimental tumor cells. In contrast, less fluidity was shown in hyperplastic nodule cells. Since cholesterol tends to make the membrane less fluidal at physiological temperature, this fact may reflect increased cholesterol content in the preneoplastic membrane. It is suggested that less fluidity in the preneoplastic plasma membrane may have an inhibitory effect on cell growth.

(II) Proteins

Since it has been difficult to obtain sufficient amounts of pure membrane proteins to determine their sequences, a number of membrane proteins have remained to be elucidated with respect to their real functions as well as their structures, though some of them have now been clarified from the sequences of cloned c DNA. The plasma membrane proteins are now classified into five groups according to their putative functions[25]: the receptor proteins to various extracellular ligands such as growth factors and hormones, the channel proteins for transportation of ion and small molecules across the membrane, the various enzyme proteins such as phospholipases and phosphatases, the regulatory proteins associated with functional proteins such as p21 and, finally, the cellular adhesion proteins such as cadherines, cell-CAMs, integrin family, and others. Although fragmental knowledge on alterations of the membrane proteins in tumor cells has been obtained, the information is insufficient to generalize about tumor-specific changes.

(III) Saccharides

Oligosaccharide chains are found outside of the plasma membrane, attached to both proteins and lipids. Polysaccharide chains are also found on the surface of mammalian cells except for erythrocytes.[26] These saccharides are organized on the cell surface to form the "glycocalyx" and may play important roles in cell-to-cell and cell-to-matrix interactions.[1,27] The polysaccharide organisation varies according to the cell types. However, the architecture of the glycocalyx has been obscure in spite of its importance for cell interaction because there have been few methods whereby to analyse it with intact cells. We developed a method to analyse the acidic polysaccharide organisation in the glycocalyx, using the cell-electrophoresis coupled with the treatment of cells with specific enzymes that release saccharide chains.[28–30] Cell-electrophoresis has produced the new finding that proteoglycans are components of the plasma membranes in almost all mammalian cells inculding tumor cells.[31–51]

As shown in Table 2, the chemical analyses of the purified plasma membranes indicated that chondroitin 4-sulfate appeared specifically in malignantly transformed cells, while preneoplastic cells showed the normal pattern.[19,27] Since chondroitin 4-sulfate is also seen in the plasma membranes of embryonic or neonatal hepatocytes, a new appearance of chondroitin 4-sulfate in the membrane may indicate one of the embryonic alterations in malignant tumor cells. On the other hand, the electrokinetic charge in the free-cell-type cells as well as in the normal peripheral white blood cells is mainly due to carboxylate residues of NANA of oligosaccharide chains. The difference in the electrokinetic charge between the free-cell-type and the island-forming-type cells of rat hepatomas may reflect some differences in organisation of the polysaccharide chains on the cell surface. Since ascites hepatoma islands, which proliferate in the form of cell aggregates in the abdominal cavity, could be considered a primitive tissue-forming ability of the cells,[40] the difference in the surface organisation of polysaccharide chains may imply a general difference between free cells, including peripheral blood cells, and tissue-forming cells. Indeed, even the electrokinetic charge of the fibroblast in the primary culture was independent of NANA, but many other cultured cell lines were dependent on it, suggesting that the cell lines are not the same as in vivo cells in their surface organisation.

To clarify any difference of polysaccharide organisation in the glycocalyx among the different types of cells, cell-electrophoretic analysis was conducted with island-forming-type and the free-cell-type hepatoma cells after sequential treatment with the specific enzymes for NANA, chondroitin sulfates, and hyaluronic acid.[37] As shown in Table 3, when the cells were treated initially with chondroitinase and subsequently with neuraminidase, an additional reduction in electrophoretic mobility was observed in the island-forming-type cells. When the cells were treated

Table 2. Characteristics of Saccharide Chains in the Glycocalyx of Hepatoma Cells in Rats

The architecture of the glycocalyx of the hepatoma cell was characterized in comparison with that of the hyperplastic nodule.

	Ascites hepatomas		FAA-induced hepatomas	Hyperplastic nodules
	Free-cell types	Island-forming types		
Electric negative surface charge	dependent on NANA[a)]	dependent on chond-4S[b)]	dependent on chond-4S heparan S[c)]	dependent on heparan S
Polysaccharide chains in the plasma membrane	chond-4S HA[d)]	chond-4S heparan S HA	chond-4S (trace) heparan S dermatan S[e)] HA	haparan S dermatan S HA
Oligosaccharide chains in the plasma membrane				
Accumulation of short saccharide chains	yes	yes	yes	no
Unusual saccharide chains	yes	no	no	no
Higher gangliosides	no	no	no	yes
Poly-branched saccharide chains in N-linked sugars	yes	yes	yes	no

[a)] N-acetyl neuraminic acid, [b)] Chondroitin 4-sulfate, [c)] Heparan sulfate,
[d)] Hyaluronic acid, [e)] Dermatan sulfate.

Table 3. Difference in Surface Architecture Detected by Cell-electrophoretic Studies Between Two Cell Types of Rat Ascites Hepatomas

The changes of electrophoretic mobility of the cell after treatment with various enzymes or chemicals were compared between two cell types of rat ascites hepatoma.

Treatment	Electrophoretic mobility of the cell	
	Island-forming type	Free-cell type
NA[a)]	no change	decrease
CH[b)]	intermediate decrease	similar with NA alone
CH+NA	additional decrease	no additional decrease
NA+CH	similar with CH+NA	similar with CH+NA
HA[c)]	intermediate decrease	intermediate decrease
HA+NA	similar with HA alone	additional decrease
HA+CH	additional decrease	similar with HA+NA
HA+CH+NA	more additional decrease	no additional decrease
0.1N H_2SO_4	no change	decrease
Methylation of acidic residues with diazomethan		
Electrophoretic condition at pH6.0	null mobility	null mobility
The condition at pH7.0	no recovery of mobility	recovery of mobility

[a)] Neuraminidase treatment, [b)] Chondroitinase ABC treatment, [c)] Hyaluronidase treatment

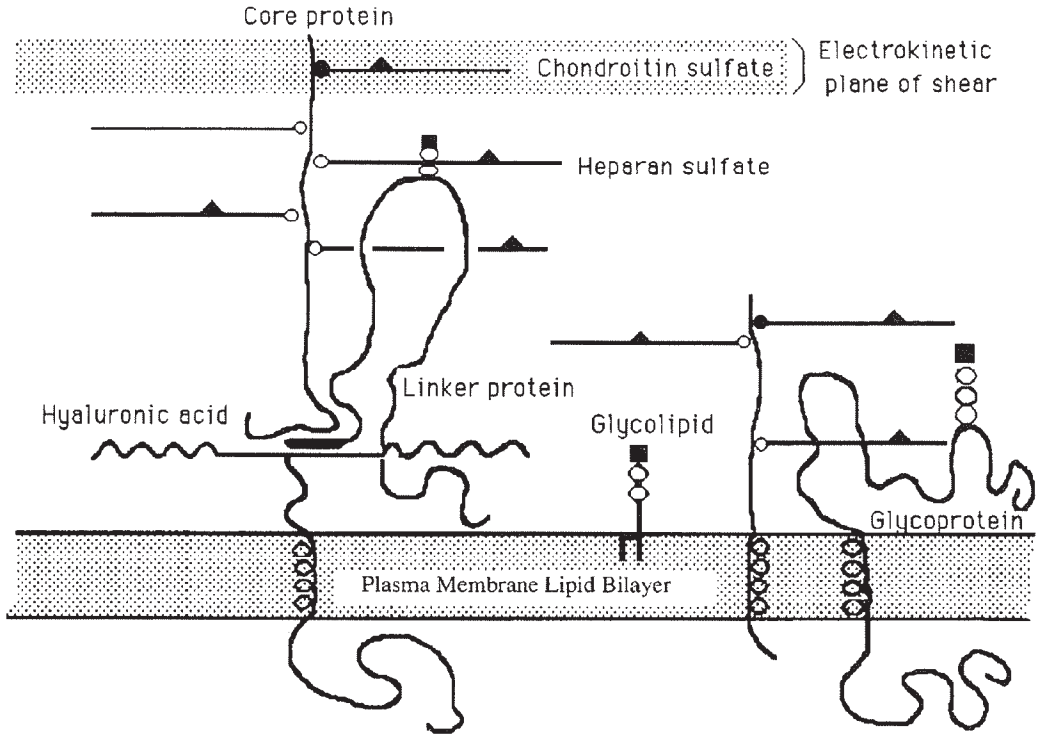
initially with hyaluronidase and subsequently with chondroitinase, an additional reduction in mobility was seen, and the experiment revealed that NANA of glycoproteins appears on the cell surface after removal of almost all polysaccharide chains. In contrast, with the free-cell-type hepatoma cells, NANA was shown to be exposed at the most external portion of the glycocalyx, and the polysaccharide chains were at its inner portion. The removal of polysaccharide chains resulted in disappearance of NANA residues from the electrokinetic plane of the shear electrophoretically. This disappearance of NANA residues from the surface may indicate their dislocation from the electrokinetic plane of the shear to the internal deep portion of the cell surface by conformational changes of glycoproteins, since no liberation of NANA was detected. This may also suggest that polysaccharide chains of proteoglycans play an important role in the maintenance of physiological conformation of glycoproteins on the cell surface. We have electrophoretically found a similar conformational change in glycoproteins on the cell surface of X-irradiated or UV-irradiated cells[26–30,41–42,45–46,49,63] and of anesthesized cells[68–69], although the mechanism might be different in each case. It has been reported that hyaluronic acid in proteoglycans on the cell surface was situated in the inner portion of the glycocalyx in mouse lymphocytes.[41–42]

A highly organized structure has also been reported in proteoglycan aggregate from cartilage.[52] This architecture may lead us to similarly organized architecture in proteoglycans of the cell surface. Recently, it has been reported that CD44,[53] a polymorphic integral membrane glycoprotein of the lymphoid cell, is the principal cell-surface receptor for hyaluronate, and is a 37Kd polypeptide homologous to a cartilage-link protein in a physiologically conserved amino-terminal domain. Thus, we could propose here some difference in the surface organisation between tissue-forming-type and free-cell-type cells as shown in Fig. 2. Some chondroitin and heparan proteoglycans bind to make a complex with a hyaluronate and an integral glycoprotein homologous to a cartilage-link protein, while other proteoglycans bind directly to membrane lipid bilayer. Liberation of hyaluronic acid by hyaluronidase may partially remove the binding proteoglycans, which have an affinity to hyaluronate, and a conformational change of the surface glycoproteins may be induced by loss of supporting hyaluronate. This model explains our electrophoretic results obtained in various cells, without contradiction.[31–51,54–73]

An alteration of oligosaccharide chains in the hepatoma membranes[74–91] is also summarized in Table 2. Accumulations of short oligosaccharide chains, unusual oligosaccharides such as asialo GM1 and GM1b, loss of higher gangliosides, and appearance of poly-branched saccharide chains in N-linked oligosaccharides were detected and agree with previously reported results in other tumors. The unusual saccharide chains of glycolipids, galactosyl-N-acetlygalactosyl-lactosyl ceramide (asialo-GM1), and sialyl-galactosyl-N-acetyl-galactosyl-lactosyl ceramide (GM1b), were seen only in free-cell-type hepatoma cells. It has been demonstrated that these unusual oligosaccharides are accumulated by lack of two specific sialyl transferases, lactosylsialyl transferase and N-acetyl-galactosyl-lactosyl-sialyl transferase, which synthesize sialyl-lactosyl ceramide (GM3) from lactosyl ceramide and N-acetyl-galactosyl-sialyl-lactosyl ceramide (GM2) from N-acetyl-galactosyl-lactosyl-ceramide (asialo-GM2), respectively.[19,78,80,84,85] This demonstration of the formation of GM1b was the first report of such in mammalian cells. In island-forming-type cells, no unusual sugars were detected, although the pathways from lactosyl ceramide to asialo-GM2 and from asialo-GM2 to GM2 were switched off. Since asialo-GM1 on the cell surface was observed in immature T lymphocytes in mouse and in immature bone marrow cells in rat, the appearance of unusual sugars may reflect the "switch-on" of the embryonic pathway in the oligosaccharide synthesis.

In hyperplastic nodule cells, the alterations of oligosaccharide chains, as detected in hepatoma cells, were not observed, and the polysaccharide pattern was similar to that in adult hepatocytes.[19,91] Therefore, the hyperplastic nodule cells (premalignant cells) retain the organisation

A: Island-forming types



B: Free-cell types

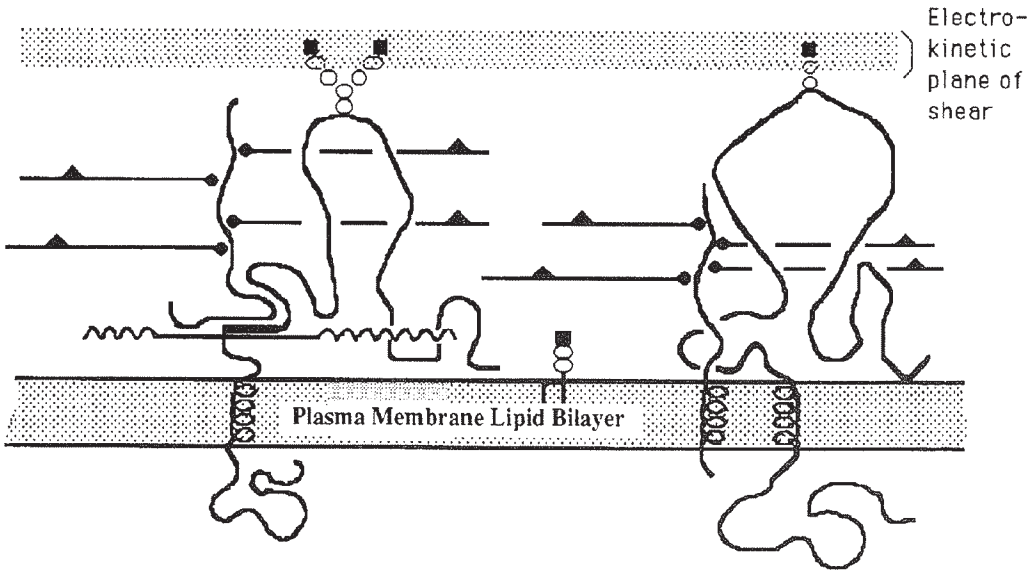


Fig. 2. A schematic representation of the glycocalyx organisation in ascites hepatoma cells of rats. The meaning of the symbols in the B panel is the same as that of those in the A panel.

of the glycocalyx similar to that of normal hepatocytes. Since hyperplastic nodule cells proliferate following partial hepatectomy as detected by DNA synthesis (Fig. 3), the normal glycocalyx may participate in the response to proliferation stimuli during regeneration. The irreversible alteration in glycocalyx organisation might be responsible, at least in part, for malignant transformation of cells.

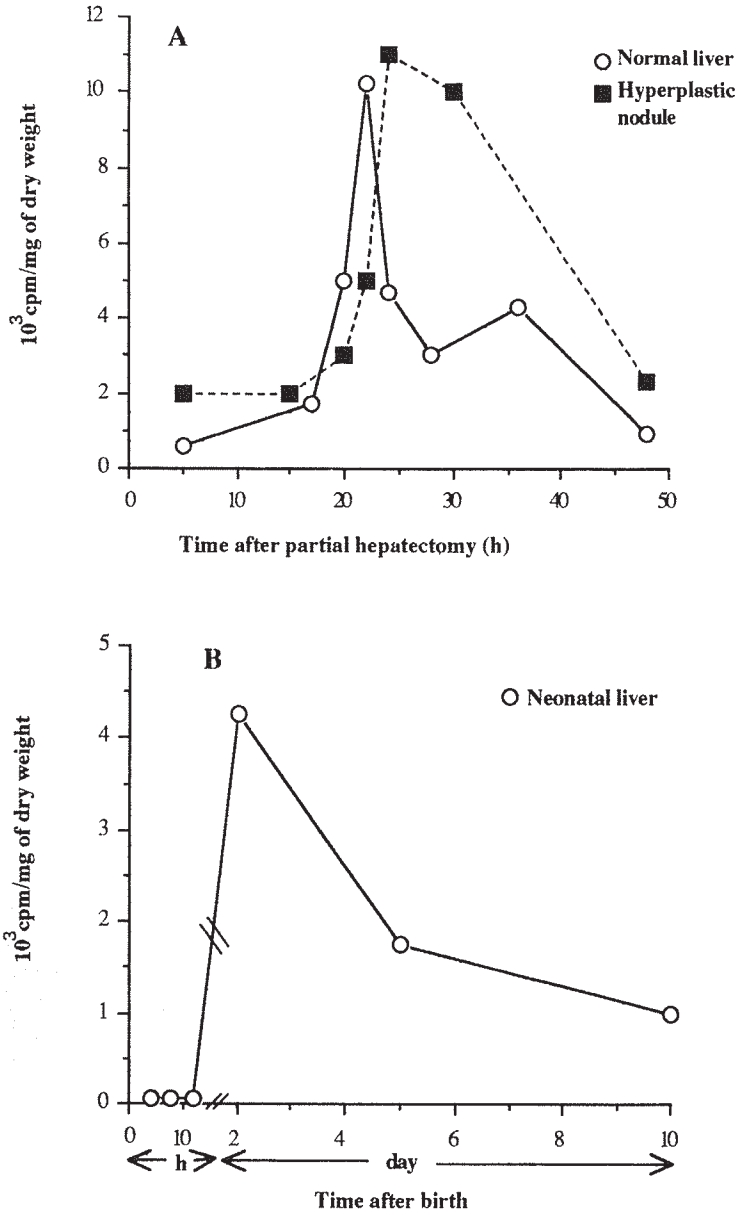


Fig. 3. Change of ^{3}H-thymidine incorporation into acid-insoluble fraction in the growing liver.
A: The regenerating livers at various times after partial hepatectomy of normal adult and hyperplastic nodule-bearing rats were subjected to the assay of ^{3}H-thymidine incorporation into acid-insoluble fraction.
B: The neonatal livers of rats within 24 h and 2, 5 and 10 days after birth were examined for their uptake of ^{3}H-thymidine.

Dynamics of the plasma membrane during cell cycle

It is known that growing cells run the cell cycle, while resting cells have exited from the cycle before the S phase. Almost all adult mammalian cells in vivo are in the resting phase, but they can enter the cell cycle again in case of need. The regulatory mechanism that guides the cell from one phase to another during the cell cycle has remained a major challenge to us. In this context, alterations in the plasma membrane during cell cycle were studied using regenerating and neonatal hepatocytes.[3,5–9,16,19,27,81,83] Fig. 3 displays a time course of ^{3}H-thymidine incorporation into DNA in partially hepatectomized adult (A) and neonatal (B) rat liver. It was shown that hepatocytes proceeded into S phase at 20 to 24 h after partial hepatectomy. In the FAA-induced hyperplastic nodule cells, S phase started at 20 h, as normal hepatocytes, but ^{3}H-thymidine incorporation continued until 28 h or more after the operation, suggesting that growth control was rather loose compared with that of normal cells. It is remarkable that no thymidine incorporation was observed until 12 h after birth in the neonatal livers, and a sharp recovery in DNA synthesis was seen at more than 24 h after birth, and then the activity gradually decreased.

Table 4 shows some alterations in the plasma membranes of the regenerating and neonatal growing hepatocytes in rats. The most outstanding changes were observed in early G1, in which both cholesterol and sphingomyelin dramatically decreased. The decrease of cholesterol contents results in an increase in membrane fluidity without any change in fatty acid components of the phospholipids.[9,16,91] Furthermore, a decrease of sphingomyelin contents suggests an activation

Table 4. Changes of the Plasma Membrane Responsible for Hepatocytic Growth

The contents of lipids, proteoglycan and heparan sulfate of the plasma membranes isolated from hepatectomized and neonatal livers were compared with those of normal adult liver membrane. Chondroitin 4-sulfate was detected by chemical analysis. The change of surface charge by treatment with hyaluronidase or neuraminidase was detected by cell-electrophoresis.

Events	Hepatectomized liver			Neonatal liver		
	5h	17h	28h	1 day	5 days	10 days
Lipid bilayer						
Relative cholesterol contents	less	nd[a)]	slightly less	less	less	slightly less
Relative sphingomyelin contents	less	nd	normal	less	slightly less	normal
Sum of choline phospholipids	less	nd	less	less	less	less
Molar ratio of choline phosphoglyceride to sphingomyeline	high	nd	normal	high	normal	normal
Membrane fluidity	more fluidic	nd	slightly fluidic	more fluidic	more fluidic	slightly fluidic
Glycocalyx						
Proteoglycan contents	less	nd	more	less	normal	more
Heparan sulfate content	less	nd	more	less	normal	more
Appearance of chondroitin 4-sulfate chain	−	nd	−	+	+	−
Hyaluronidase-sensitive surface charge	+	+	−	nd	nd	nd
Exposure of N-acetyl neuraminic acid of glycoproteins at the cell surface	−	+	−	nd	nd	nd

[a)] Not determined

of the neutral Mg^{2+}-dependent sphingomyelinase,[12–18] which liberates ceramide, an important molecule for signal transduction. Similar changes were observed in the newborn hepatocyte membrane in the phase before DNA synthesis, although the increased fluidity may be due to both low cholesterol contents and relative increase of short fatty acid. Another change in the glycocalyx in early G1 phase cells was a partial removal of heparan sulfate proteoglycan from the glycocalyx.[19,40] This produced a reduction of the electric negative net charge of the cells. This change continued at least until late G1 phase, and then the surface charge recoverd gradually through the S phase in company with recovery of heparan sulfate contents in the membrane. Although the mechanism of specific removal of heparan sulfate proteoglycan in the G1 phase is unknown, it may induce a disorganisation of the glycocalyx. In fact, cell-electrophoresis revealed that a change of the electrokinetic charge is produced by treatment with hyaluronidase of the G1 phase cells. Furthermore, in the late G1 phase, oligosaccharide chains were in an exposed form at the most external portion of the glycocalyx as in the free-cell type (Fig. 2B). It may be speculated that under this condition, gorwth factor(s) can access more easily to the functional glycoproteins, which are responsible for leading the cells into S phase. This "exposure" is observed only at the critical point of the cell cycle for 1 to 2 h before DNA synthesis. This speculation is also supported by the observation that mechanically separated hepatocytes did not grow, while the cells separated by proteolytic enzymes did grow in vitro presumably because of this "exposure." Thus, it is suggested that the glycocalyx organisation may also play an important role in growth control in vivo, and the disorganisation of the glycocalyx may trigger G0 cells to enter the G1 phase.

Cellular signaling systems for cell growth

In multicellular organisms, an elaborate cell-to-cell communication net work coordinates the growth, differentiation, and metabolism of the multitude of cells in diverse tissues and organs. Fig. 4 summarizes schematically the signal transmission processes through plasma membrane. When the ligand binds to the membrane receptor, the receptor-ligand complex initiates a sequence of reactions including phospholyration (R1) or dephospholyration (R2) of the functional proteins, followed by the the ligand-triggered activation (R3) of a G protein, which activates second messengers such as c-AMP, inositol 1,4,5-triphosphate and 1,2-diacylglycerol, and the ligand-triggered ion channels are important for cellular growth control.[93] The cytosolic substrate protein(s) to these enzymes may be directly modified in its function by phospholyration or dephospholyration. This is followed by the activation of G protein, which stimulates enzymes involved in the production of the intracellular second messengers listed above, coupled with the action of phospholipase C. On the other hand, diacylglycerol activates the phospholipid-calcium ion-dependent protein kinase (C kinase).[94] c-AMP activates c-AMP-dependent protein kinase (A kinase). Inositol 1,4,5-triphosphate binds to the specific receptor on the endoplasmic reticulum and opens a Ca^{2+} channel. Elevated Ca^{2+} in the cytosol activates the calmodulin-dependent protein kinase. Thus, these activated protein kinases induce various cellular events responsible for cell functions, growth, and differentiation.[2,23] Recently, it has been reported that sphingosin generated by the cooperation with neutral sphingomyelinase and ceramidase in the plasma membrane is a potent inhibitor of C kinase and other protein kinases,[95,96] indicating that sphingosin is also one of the second messengers for signal transmission.[24,97] Furthermore, arachidonic acid generated by the membrane phospholipase A_2 might act as a signal, through the synthesis of various kinds of prostaglandins.

Lack of receptor protein(s), its mutation and its excess production responsible for the signaling pathway have been reported in many tumor cells. However, the details of these molecular events remain to be clarified. On the other hand, the specific interactions between the actin-based cytoskeleton and key molecules of membrane signaling pathways have been identified in

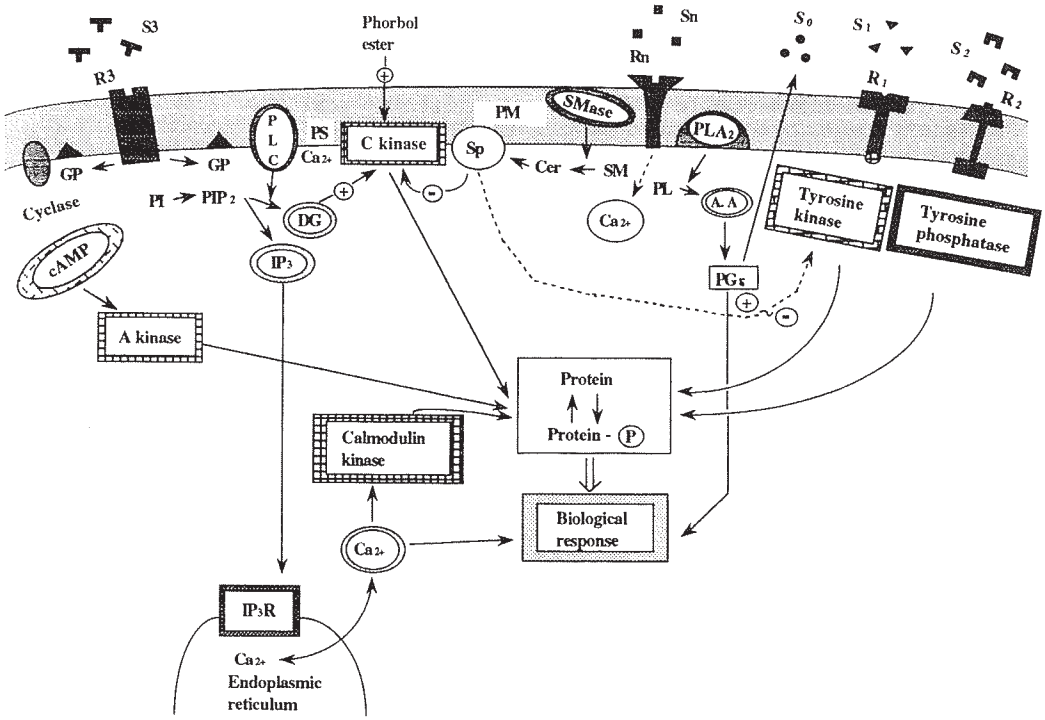


Fig. 4. A shematic representation of the signal transmission process.
S, signal; R, receptor; GP, G protein; PLC, phospholipase C; PI, phosphatidylinositol; PIP_2, phosphatidylinositol 4,5-diphosphate; PS, phosphatidylserine; DG, diacylglycerol; IP_3, inositol triphosphate; Sp, sphingosine; Cer, ceramide; SM, sphingomyelin; PL, phospholipid; PLA_2, phospholipase A_2; A.A, arachidonic acid; PGs, prostaglandines; PM, plasma membrane; +, stimulative effect; −, inhibitory effect; IP_3R, inositol triphosphate receptor.

vitro and in vivo.[98] The actin-binding protein profillin[99,100] may block phospholipase C to access with the substrate PIP_2, by binding to PIP_2. A similar function has been also found with other actin-binding proteins such as gelsolin,[101] destrin, and cofilin,[102] which is transported into the nucleus in heat-shocked cells.[103] Thus, the cytoskeleton organisation also has a close relationship with cellular signaling.

From the view point of cell growth control, it may be essential to clarify the mechanisms of signal transduction from the cytosol to the nucleus. Recently, we found that the nucleus contains large amounts of phospholipids, i.e., ten times more than expected for the nuclear envelope, and the nuclear phospholipids are localized not only in the nuclear membrane but also in the nuclear matrix.[104] It was also suggested that nuclear phospholipases are responsible for cell proliferation.[105–112] The activities of both nuclear phospholipase C and A_2 increased in the early S phase in the regenerating hepatocytes.[109,111–112] Partial purification of the nuclear phospholipases has demonstrated that they are different from those of either the plasma membrane or the cytosol in their substrate specificities and Ca^{2+} requirements. Nuclear localization of phospholipase C_β has been reported recently in Swiss 3T3 cells.[113] Since the activity of protein kinase C exists also in the nucleus,[114] these findings may suggest the existence of signaling pathways in the nucleus similar to those in the cytosol. Since a direct relationship between nuclear phospholipid and

DNA synthesis has also been reported, [115,116] the nuclear phospholipids may be responsible for the nuclear function in cell growth control.

Metastatic growth and the plasma membrane

Metastatic expansion is the most important biological character of malignant tumors. Metastatic foci of tumors are the final result of complicated interactions among tumor and host cells.[92,117] Now, discussion will mainly focus on the lodgement of tumor cells in the peripheral blood vessel wall when the cells enter the circulating blood. The passage potential of a tumor cell through a capillary vessel may be dependent on the surface rigidity of the cell coupled with cellular deformability as well as cell size. The cellular stickiness to the venous vessel wall is also important for lodgement of the tumor cell after capillary passage.[118]

It is known that constituent fatty acids and the molar ratio of cholesterol to phospholipid in the lipid bilayer are major factors in the regulation of the membrane fluidity on which cellular rigidity is dependent.[92,119] This fact leads us to assume that modulation of lipid fluidity in the plasma membrane by substituting constituent fatty acids, if possible, might produce a change in the ability of tumor cells to pass through a capillary vessel and might affect their blood-borne metastasis. This possibility was examined using Yoshida sarcoma cells in rats.[120,121] The results

Table 5. Effects of Exogenously Added Fatty Acids on Membrane Microviscosity[120]

Added fatty acid ester		Microviscosity ($\overline{\eta}$) at 25°
None[a]		1.69 ± 0.06[b]
Saturated		
14:0		1.71 ± 0.07 NS[e]
16:0		1.59 ± 0.11 NS
18:0		1.81 ± 0.03 NS
20:0		1.68 ± 0.08 NS
Unsaturated		
16:1	cis 9 (n-7)	1.08 ± 0.09 P < 0.001[c]
16:1	trans 9 (n-7)	1.55 ± 0.09 P < 0.010
18:1	cis 9 (n-9)	1.59 ± 0.10 NS
18:1	trans 9 (n-9)	1.61 ± 0.08 NS
18:1	cis 11 (n-7)	1.59 ± 0.10 NS
18:2	cis 9-12 (n-6)	1.03 ± 0.05 P < 0.001
18:2	trans 9-12 (n-6)	1.56 ± 0.06 P < 0.010
18:3	cis 6-9-12 (n-6)	1.31 ± 0.04 P < 0.001
18:3	cis 9-12-15 (n-3)	1.39 ± 0.08 P < 0.001
20:3	cis 8-11-14 (n-6)	1.59 ± 0.12 NS
20:3	cis 11-14-17 (n-3)	1.60 ± 0.07 NS
20:4	cis 5-8-11-14 (n-6)	1.37 ± 0.05 P < 0.001
20:5	cis 5-8-11-14-17 (n-3)	1.39 ± 0.06 P < 0.001
20:6	cis 4-7-10-13-16-19 (n-3)	1.49 ± 0.02 P < 0.001

[a] Control group.
[b] Mean ± SD.
[c] Student's *t* test, versus control value.
[e] Not significant.

were as follows: (1) When cells were incubated with exogenous fatty acid ester for 10 h, the constituent fatty acids of phospholipids were easily substituted with the exogenous fatty acids, resulting in the modulation of lipid fluidity of the membrane without affecting its viability. (2) Exogenous unsaturated fatty acid esters produced a significant increase in the membrane fluidity as shown in Table 5, whereas the saturated fatty acid esters did not cause any significant change. (3) In vitro passage experiment of the modified cells through the pulmonary vessel showed that the modified cells with increased membrane fluidity were able to pass through the peripheral lung vessels more efficiently than the control cells, and the metastatic potential in vivo was also reduced in proportion to the increased level of membrane fluidity. This is the first demonstration suggesting that fatty acid modification might control the passage potential of circulating tumor cells through periferal vessels. Since fatty acid modification of the cells can be done easily in vivo, and since cellular modification with an unsaturated fatty acids, such as 18:3, produces high sensitivity to X-irradiation in mammary tumor cells (unpublished data), the results described here might suggest a new method of cancer therapy. Further studies along this line would be desirable.

In connection with the cell rigidity, cellular deformability to pass through the narrow space of the capillary is also an important factor in tumor cell emboli. The deformability seems to be dependent upon the cytoskeletal organisation, which is necessary to maintain the cell shape. Indeed, many reports have revealed that the treatment of tumor cells with cytoskeletal agents reduces the number of blood-borne metastatic foci.[92,119]

The glycocalyx of the cell surface plays an important role in cellular stickiness to the vessel wall of the post-capillary venules after passage of the cell through the capillary. There are reports that the treatment of the cell surface with various enzymes in vitro modifies the clinging property of the cell to the matrix surface or the cultured endothelial surface, and also alters the cell's ability to form metastatic nodules.[21,117] Recently, it has been reported that CD_{44},[53] a polymorphic integral membrane glycoprotein with a postulated role in matrix adhesion, lymphocyte activation, and lymphnode homing, is the principal cell-surface receptor for hyaluronate, and that its family protein(s) on the cell surface has a close relation with tumor metastasis.[122] Other carbohydrate-recognition glycoproteins on the cell surface have also been reported, being designated as the selectins or LEC-CAMS.[123] These carbohydrate-recognition proteins on the cell surface may promote the lodgement of the tumor cell in the endothelial cell of the vessel wall. In cell-to-cell interaction, not only protein-to-sugar recognition but also sugar-to-sugar recognition seems to be important for cell lodgement.[124–126] Although sugar-to-sugar interaction is weak in its binding stability, it may act as the initial recognition of tumor cell to endothelial cell. Thus, the mechanisms for final cell lodgement into the vessel wall involve complex molecular events.

Final remarks

To understand the nature of the cancer cell, studies on the plasma membrane as well as on the regulation mechanism of DNA synthesis are essential, since tumor cells are distinguishable from normal ancestral cells only in their abnormal cell-to-cell interaction. However, details of these molecular events are as yet insufficient. Since the plasma membrane is a complex molecular assembly consisting of lipid, protein, and carbohydrate, the situation of the functional molecules inserted into the lipid bilayers may be far different from in vitro conditions composed of water-soluble molecules. Development of a new assay system for the functional molecules, therefore, is desired to understand in vivo membrane function more clearly. In this connection, analytical and functional studies using artificially reconstituted membrane inserted with functional molecules will give us important information to understand the molecular pathogenesis of the plasma membrane in cancer cell biology.

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