Biological and Therapeutic Potential of Membrane Lipid Modification in Tumors

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Abstract

The membrane fatty acid composition of cancer cells can be modified either in culture or during growth in animals without disrupting basic membrane cellular integrity. Only fatty acids are affected; no changes occur in membrane cholesterol, phospholipid, or protein content. There are changes in membrane physical properties and certain cellular functions, including carrier-mediated transport, receptor binding, ion channels, and eicosanoid production. Fatty acid modification also can enhance the sensitivity of the cells to hyperthermia and Adriamycin. This technique provides a new approach to understanding the membrane properties of neoplastic cells. Membrane fatty acid modification also may be of potential value as a therapeutic approach designed to augment the cytotoxicity of other antineoplastic therapies.

Introduction

Biological membranes are composed of a lipid bilayer that contains a central core of fatty acyl chains. This hydrophobic core acts as a barrier, preventing the unregulated movement of ions and metabolic products across the membrane. The fatty acyl chains also interact with the proteins that penetrate into the membrane, including enzymes, receptors, and transporters. Many different kinds of fatty acyl chains normally make up the lipid bilayer. Most of them have an even number of carbon atoms, between 16 and 22. About 35 to 40% are saturated; the remainder are unsaturated and contain between 1 and 6 double bonds. Any change in the composition of the fatty acyl chains can alter the structure of the lipid core and thereby has the potential to affect its normal barrier function, as well as the responsiveness of the integral proteins with which the core interacts. Certain properties of the cell may change as a consequence.

By the early 1970s, it became evident that neoplastic cells might be ideal targets for membrane fatty acid modification. Tumors obtain a substantial amount of fatty acid preformed from the host (1, 2). This is supplied primarily from the circulating free fatty acid (3, 4) and to a lesser extent by the triacylglycerols contained in plasma lipoproteins (5, 6). Although tumors can synthesize fatty acid from glucose (7), synthesis is reduced when an adequate supply of circulating fatty acid is available (8). Therefore, the mixture of fatty acids provided by the host, after only minor structural modification, is incorporated into all of the complex lipids formed by the neoplastic cells. This is the rationale for the studies carried out over the last decade to assess the biological and therapeutic potential of membrane fatty acid modification in neoplastic cells.

Membrane Polyunsaturated Fatty Acids

Most efforts to manipulate the fatty acid composition of cells have focused on polyunsaturates. As opposed to saturated and monounsaturated fatty acids, the polyunsaturates normally present in animals cannot be synthesized de novo and therefore are ultimately derived from the environment. Animals obtain them from the diet; cells obtain them from the plasma and extracellular fluid. There are two main classes of polyunsaturates, the n-6 or plant polyunsaturated class and the n-3 or fish oil class. This point is illustrated in Fig. 1, which shows the relationship of the various acids in each series, their metabolic conversions, and the structures of the main components. The n-6 polyunsaturates, also known as the ω-6 class, are often referred to as essential fatty acids because serious illness results if they become deficient. Terrestrial plants synthesize the first member of this series, linoleic acid (18:2n-6), and they are present in the diet primarily in this form. Arachidonic acid (20:4n-6), another member of this series, is the main substrate for prostaglandin and leukotriene synthesis in mammalian tissues.

The n-3 polyunsaturates, also known as the ω-3 class, are contained in the diet primarily in fish oils. Cold water vegetation synthesizes the first member of this series, linolenic acid (18:3n-3), and the fish that feed on these organisms convert the 18:3 to the two most abundant components of this class, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). Although these fatty acids, especially 22:6, accumulate in relatively large amounts in certain excitable membranes, their functional role is presently uncertain, and there is a question as to whether they are necessary for optimum health. As indicated in Fig. 1, mammalian cells can interconvert the fatty acids within each series, but they cannot convert one class into the other.

Fatty Acid Modifications

Tumor Growth in Animals. Substantial differences in the phospholipid fatty acid composition can be produced in Ehrlich ascites carcinoma cells by changing the type of fat fed to the tumor-bearing mice (9). The diets that have been tested for this purpose contained either sunflower seed oil, which has about 70% linoleic acid, or coconut oil, which has 93% saturated fatty acid and only 1 to 2% linoleic acid. These diets contained 16% fat by weight, amounting to 30% of the total caloric intake. While the plasma membrane fatty acid composition of the Ehrlich cells was modified, no changes occurred in the membranes.

The fatty acids are signified as number of carbon atoms: number of double bonds, followed by the class. Thus, linoleic acid contains 18-carbon atoms and two double bonds and is of the n-6 polyunsaturated fatty acid class. The notations n-3 and n-6 signify the number of carbon atoms from the methyl terminus where the first double bond is located.
accounts for about 40% of the membrane fatty acids. However, there was a 53% reduction in monounsaturated fatty acids and a 77% increase in polyunsaturated fatty acids when the L1210 cells were grown in the mice fed the sunflower oil diet. Among the individual fatty acids, the main differences occur in oleic acid (18:1) and 18:2.

Solid tumors also can be modified in this way. For example, large differences in the fatty acid composition of a transplanted mammary adenocarcinoma result from feeding the tumor-bearing mice diets containing corn oil as opposed to hydrogenated cottonseed oil (14). The phospholipid fatty acid composition of hepatoma 7288CTC was modified in the rat by changes in dietary fat content (15). Modifications in the murine HSDM1 fibrosarcoma also have been produced in this way (16). The membranes of the solid tumors are affected; for example, the fatty acid compositions of microsomes prepared from the murine CA755 mammary adenocarcinoma and a rat mammary tumor induced by N-methyl-N-nitrosourea are altered in response to changes in the type of dietary fat (17, 18).

The modifications in phospholipid fatty acid composition produced by diet are fairly stable. At the time of removal from the host, the phospholipids of L1210 cells obtained from mice fed the sunflower oil diet contained 47% polyunsaturated fatty acids, as opposed to 31% when coconut oil was fed. When these cells were subsequently maintained in culture in a standard medium containing 20% fetal bovine serum for up to 96 h, the polyunsaturated fatty acid content of the phospholipids changed by only 2% in each case (19).

The fatty acid compositional changes are not limited to membranes when the modifications are produced in vivo by the dietary approach. Differences in fatty acid composition also occur in the cellular neutral lipid fraction (9), which is composed primarily of triacylglycerol contained in the cytoplasm in the form of lipid inclusions.

Cell Culture. While the dietary studies in animals established that membrane fatty acid compositional changes in tumors can be produced in vivo, the method does not permit a precise evaluation of specific fatty acid substitutions. To examine this question, tumor cells grown in culture have been modified with individual fatty acids as indicated in Table 1 (20-32). Many different types of fatty acid have been incorporated, including plant polyunsaturates, fish oil polyunsaturates, unsaturates having double bonds in the trans-configuration, and fatty acids

Table 1  Fatty acid modification of tumor cells in culture

<table>
<thead>
<tr>
<th>Tumor cell</th>
<th>Fatty acids tested*</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6 glioblastoma</td>
<td>18:2, 18:3n-3</td>
<td>20, 21</td>
</tr>
<tr>
<td>Ehrlich ascites carcinoma</td>
<td>14:0, 15:0, 18:1, 18:2, 18:3, 18:4, 18:5, 18:6</td>
<td>22</td>
</tr>
<tr>
<td>EL-4 T-lymphoma</td>
<td>17:0, 18:1, 18:2, 18:3, 18:4, 19:0</td>
<td>23</td>
</tr>
<tr>
<td>FM3A mammary tumor</td>
<td>18:3n-3, 20:3, 20:3n-3, 20:5n-3</td>
<td>24</td>
</tr>
<tr>
<td>Friend erythroleukemia</td>
<td>18:1, 18:2</td>
<td>25</td>
</tr>
<tr>
<td>HeLa</td>
<td>18:2, 20:4</td>
<td>26</td>
</tr>
<tr>
<td>Hepatoma 7777</td>
<td>18:1, 18:2</td>
<td>27</td>
</tr>
<tr>
<td>L5178Y leukemia</td>
<td>18:3n-3, 20:2, 20:5n-3, 20:5n-3</td>
<td>24</td>
</tr>
<tr>
<td>M1 neuroblastoma</td>
<td>18:2, 18:3n-3, 20:4, 22:6n-3</td>
<td>29</td>
</tr>
<tr>
<td>NG108-15 neuroblastoma × glia</td>
<td>18:1, 18:2, 20:4</td>
<td>30</td>
</tr>
<tr>
<td>PC-12 pheochromocytoma</td>
<td>16:0, 18:0, 18:1, 18:2, 20:4</td>
<td>31</td>
</tr>
<tr>
<td>V-79 retinoblastoma</td>
<td>18:1, 18:2, 18:3n-3, 20:4</td>
<td>32</td>
</tr>
</tbody>
</table>

* Fatty acids were added individually to the culture medium.
1 Denotes polysaturated of the fish oil series.
2 Double bonds in the trans-configuration.
3 12-Methylmyristic acid.
having an odd number of carbon atoms or a branched hydrocarbon chain. Fatty acid substitutions occur in the plasma membrane (22) and microsomes of these cells (33, 34). All of the phospholipid classes are involved (22, 34). The extent of enrichment with a particular fatty acid depends on the amount of the supplemental fatty acid added to the culture medium (32). Although not tested extensively, there is no indication thus far of any change in the cholesterol or phospholipid content of the membrane, or in the phospholipid head group composition as a result of fatty acid replacements in culture (32).

In addition to allowing for more varied fatty acid substitutions, the cell culture approach also can produce more extensive enrichments than the dietary modification procedure. For example, when L1210 leukemic lymphoblasts are enriched with 22:6, the polyunsaturated fatty acid content of the phospholipids increases almost 3-fold, and there is a corresponding 58% reduction in monounsaturates. These differences are due primarily to an extremely large increase in 22:6 itself and a 46% decrease in 18:1. Although changes of this magnitude may not be strictly applicable to tumors in vivo, they provide useful models for investigating possible functional perturbations associated with specific fatty acid substitutions.

As in the case of the dietary method, the fatty acid compositional changes produced in culture are not confined only to membranes. The neutral lipid fraction, which again is composed almost entirely of triacylglycerol, also is modified (25, 27). When the cells are exposed to relatively high concentrations of a supplemental fatty acid, the amount of triacylglycerol increases (27), so that there is a change in both the content and fatty acid composition of the cytoplasmic lipid inclusions. While these neutral lipid changes are a complicating factor, they probably are not the primary cause of the perturbations in certain membrane-related processes, described in subsequent sections, that occur as a result of modifications in cellular fatty acid composition.

Membrane Fluidity

Electron spin resonance studies with Ehrlich ascites carcinoma and L1210 leukemia cells show that these kinds of fatty acid modifications are sufficient to affect the physical properties of the plasma membrane. Small differences in the packing density of the membrane lipids, as indicated by changes of as much as 0.06 unit in the electron spin resonance order parameter, occur when the cells are modified either by the dietary procedure (13, 35) or in culture (22, 36). Plasma membranes enriched in polyunsaturated fatty acids have the lowest order parameters, indicating greater fluidity; this is not due to localization of the spin probe in cytoplasmic lipid accumulations (37). The development of a membrane fluidity change is consistent with the fact that the fatty acid compositional modifications are not accompanied by any compensatory changes in membrane cholesterol content or phospholipid head group composition.

Another electron spin resonance parameter that is perturbed is the temperature dependence of the rotational correlation time of the spin probe. Two distinct discontinuities normally are observed in plasma membrane preparations, one at 31.5°C and the other at about 22°C. These discontinuities are considered to be a measure of phase transitions in the membrane lipid bilayer. The 31.5°C transition is not appreciably affected by the fatty acid compositional modifications thus far produced. By contrast, changes of more than 5°C have been noted in the lower transition, which can range from 19 to 26°C (13, 22, 35).

As the polyunsaturation of the plasma membrane increases, the temperature of this transition decreases (22). This also is an indication that polyunsaturated fatty acid enrichment causes an increase in plasma membrane fluidity. Actually, the interpretation is more complicated. The two transitions suggest that the spin probe is contained in at least two different membrane microenvironments, e.g., in the inner and outer leaflets of the lipid bilayer or in coexisting liquid and crystalline domains within the bilayer (38, 39). Apparently, the fatty acid compositional modifications perturb the structural organization of the more fluid microenvironment but have little or no effect on the more solid domain of the plasma membrane.

To determine whether these fatty acid replacement effects might be localized to only certain depths within the lipid bilayer, the L1210 plasma membrane preparations were tested with stearic acid probes containing the spin group either 5 or 12 carbon atoms distant from the carboxyl end. Under all conditions, the lipid bilayer was found to be more ordered in the region closer to the polar head groups. However, fatty acid substitutions produced the same kinds of perturbations with both spin probes, indicating that changes in the electron spin resonance parameters occur at both the superficial and deeper regions of the lipid bilayer (13).

The electron spin resonance changes resulting from fatty acid replacements in the L1210 and Ehrlich ascites cell plasma membranes are similar to effects seen in other systems (40). By contrast, no plasma membrane fluidity effects were observed when EL-4 T-lymphoma cells were enriched with polyunsaturated fatty acids (23) or when Friend erythroleukemia cells were enriched with linoleic acid (41). In the Friend cell, however, the fluidity measurements were made in microsomes rather than purified plasma membranes. It is possible that the crude microsomal fraction, which is composed largely of endoplasmic reticulum, may be less structurally organized than the plasma membrane and therefore not perturbed by these types of fatty acid substitutions.

In summary, small changes in fluidity occur in certain plasma membrane microenvironments when the fatty acid composition of at least some tumor cells is modified. This may be responsible for several of the functional perturbations that result from these modifications.

Effects on Membrane Proteins

Transport Proteins. Transporters are integral membrane proteins that span the lipid bilayer. It is reasonable to assume that changes in membrane fatty acid composition sufficient to perturb the physical properties of certain regions of the lipid bilayer may affect the function of integral proteins that happen to be located in these regions. Such effects might have important consequences for neoplastic cells, possibly influencing the uptake of nutrients or chemotherapeutic agents.

The effects of fatty acid modification on the transport of nutrients and chemotherapeutic agents in tumor cells are summarized in Table 2. Positive effects on carrier-mediated transport have been observed in a number of cases, and enrichment with polyunsaturated fatty acids lowers the \( K_{\text{m}} \) for several high-affinity transport systems (12, 13, 33, 34). However, the response is not uniform; in other cases, polyunsaturated fatty acid enrichment causes an increase in \( K_{\text{m}} \) (32, 42). In these cases, the \( V_{\text{max}} \) for high-affinity transport also is increased (32, 42), whereas no change in \( V_{\text{max}} \) occurs for any of the other transport systems (12, 13, 33, 34). There are also a number of transport systems with kinetic properties that at 37°C are unaffected by changes in fatty acid composition (43, 44). How-
even, in two of these cases where no kinetic changes are observed at 37°C, the phenylalanine and melphalan uptake systems, the transporters still must be influenced by the membrane fatty acid modifications because the temperature-dependent transitions of the transport rates are affected (43, 44).

To complicate matters further, different responses for the uptake of a single compound have been reported in two different neoplastic cells. For example, polyunsaturated fatty acid enrichment decreases glutamate uptake in M1 neuroblastoma cells but has no effect on glutamate uptake in Y79 retinoblastoma cells (34, 42). Even in a single neoplastic cell, the Y79 retinoblastoma, only some of the transport systems are sensitive, and even the systems that are responsive are not all affected in the same way (32–34).

Where positive responses occur, the effect does not appear to be specific for a single type of polyunsaturated fatty acid. Similar effects have been obtained in many of these cases with n-3 and n-6 polyunsaturates (32–34). The response is clearly to polyunsaturation, however, because no effects were observed with 18:1 enrichment in any of these cases. Adriamycin uptake was also increased as a result of enrichment with polyunsaturates; studies with six different fatty acids indicate that the magnitude of the increase directly correlates with number of double bonds that they contain (28).

No effect of fatty acid modification has as yet been observed on the efflux of any compound from a neoplastic cell. The compounds thus far tested are Adriamycin (28), glycine (45), and phenylalanine (43).

In summary, the effects of fatty acid modification on transport in neoplastic cells are diverse and complex. Why only some systems are affected and how these positive effects are brought about are not known. With respect to mechanism, one possibility is that the structural changes in the surrounding lipid matrix affect the conformation of the transporter, thereby altering the accessibility of its binding site, the tightness of binding of the ligand, the size of the transmembrane channel, or the extent of electrochemical interactions. Alternatively, the mobility of the transporter in the lipid bilayer may be affected. In either case, it is clear that only certain transporters are sensitive to lipid modulation, and even those that respond are not all affected in the same way. This may be due to differences in the lipid domain surrounding each type of transporter, with different lipid microenvironments being influenced to varying extents by a change in membrane polyunsaturated fattyacyl chain content. Another possibility is that the heterogeneous response is due to structural differences in the various kinds of transporters, with only some structures responding to changes in the surrounding fatty acyl chains.

Only in the case of Adriamycin has the change in uptake been shown to affect the amount of the compound actually present in the neoplastic cell (28). Most of the other kinetic changes that have been observed are small and possibly may have little influence on the capacity of the cell to accumulate the compound. Therefore, more work is needed to assess how many of these types of transport changes actually have any functional significance.

Ion Channels. Enrichment of NG108-15 neuroblastoma × glialoma cells in culture with 18:2, 18:3n-3, or 20:4 lowers the rate and amplitude of the sodium action potential due to a reduction in the number of sodium channels (31). Saturated and trans-unsaturated fatty acids have the opposite effect, whereas 18:1 does not produce any change. The polyunsaturates have no effect on resting membrane potential, calcium action potential, or membrane capacitance. Therefore, as noted for the transporters, the effect of increased polyunsaturation is selective and thus far appears limited to only one of the proteins that influences membrane excitability.

Receptor Binding. Like transporters, receptors contain a membrane-spanning domain that is in contact with the fatty acyl chains of the lipid bilayer. In addition, receptors often undergo association reactions that involve lateral mobility within the plane of the membrane (46). Therefore, changes in membrane fatty acid composition may possibly influence their binding or signal transduction properties.

Effects on binding have been demonstrated in neoplastic cells for several receptors. The binding of drugs to the opiate receptor is reduced when neuroblastoma × glialoma cells are grown in a medium supplemented with unsaturated fatty acids (47). Likewise, the binding affinity of the insulin receptor is reduced and the number of accessible binding sites increases when the plasma membrane of Friend erythroblastemia or Ehrlich ascites cells is enriched in polyunsaturates (25, 48). This is attributed to the increase in membrane fluidity that results from polyunsaturated fatty acid enrichment, which facilitates the insulin-mediated dissociation of the receptor from an aggregated to a monomolecular form (49).

The depolarization-dependent exocytosis of norepinephrine from PC12 pheochromocytoma cells also is reduced when the cells are enriched with unsaturated fatty acids (50). This occurs when exocytosis is triggered by carbamylcholine binding to the nicotinic cholinergic receptor, as well as when the sodium channels are activated by veratridine. None of the steps in the secretion process are affected by fatty acid modification, suggesting that the decrease is due to changes in carbamylcholine binding or in coupling of the nicotinic cholinergic receptors to ion channel activation.

These results suggest that membrane fatty acid modifications can perturb some receptor-mediated processes. Because of the importance of growth factors and their receptors in tumor development (51), this should be a particularly worthwhile area for further study.

Effects on Growth

Certain fatty acids in high concentrations can lower the growth rate without killing cells, and there are even reports that some fatty acids may exert selective toxicity against neoplastic cells (52). However, we find no evidence that the growth of the L1210 leukemia is affected when the fatty acid compo-
position is modified. There was no difference in the number of tumor cells present in the peritoneal cavity of the mice fed different diets, or in the incorporation of [3H]thymidine into these cells during subsequent culture (53). Negative results also were obtained when the L1210 cells were extensively enriched with 22:6 in culture; the cloning efficiency in soft agar and doubling time were unchanged as compared with unsupplemented cells (54). Dietary studies in animals with fibrosarcomas (16) and mammary tumors (55, 56) also indicate that changes in fatty acid composition do not affect tumor growth. By contrast, a growth-promoting effect of polyunsaturated fat has been reported in several adenocarcinomas (14, 57, 58). Furthermore, n-3 polyunsaturates have been observed to decrease the growth of a mammary carcinoma (59). Additional studies are needed to resolve these apparent differences.

Diet rich in polyunsaturated fats also are reported to affect chemical carcinogenesis (60–64). However, chemical carcinogenesis is a complex, multistep process, and it is likely that the effect occurs at an early stage, such as promotion, and not on the growth rate of the established tumor (65–67).

Although the available evidence is not conclusive, our interpretation is that in most cases, fatty acid modification of established tumors is unlikely to influence the rate of growth. Therefore, any therapeutic benefit that may possibly result from this approach is likely to be indirect, such as through increasing the sensitivity of the neoplastic cells to other treatment modalities, rather than from a direct action on tumor growth.

Effects on Therapeutic Modalities

Chemotherapy. Adriamycin was selected to test the possibility that fatty acid modification might make the neoplastic cell more sensitive to chemotherapy because membranes appear to be a target for its action (68). P388 leukemia cells that are sensitive to Adriamycin have a different phospholipid composition and membrane fluidity than resistant sublines (69, 70). Likewise, in a series of Sarcoma 180 sublines, a correlation exists between membrane fluidity and resistance to Adriamycin (71). In addition, Adriamycin reportedly decreases membrane fluidity in a dose-dependent manner (72).

In agreement with this idea, we find that enrichment of L1210 cells with polyunsaturated fatty acids in culture considerably increases their sensitivity to Adriamycin (28, 54). The effect of 22:6 enrichment on Adriamycin cytotoxicity is illustrated in Fig. 3. A significant reduction in the surviving cell fraction, as determined by a clonogenic assay, occurred at all times of exposure to the drug longer than 1 h; at 5 h, there was a 10-fold decrease. No reduction resulted from 18:1 enrichment, indicating that this is an effect of polyunsaturation and not exposure to fatty acids per se. Greater sensitivity occurred at all Adriamycin concentrations between 0.1 and 0.6 μM, and sensitivity increased as the cellular enrichment with 22:6 increased (54). Studies with different polyunsaturated fatty acids indicate that the extent of the increase in sensitivity to Adriamycin depends on the increase in average number of double bonds per phospholipid fatty acyl chain (28).

Adriamycin accumulation increased by 30% in the cells enriched with polyunsaturates (28), probably explaining the enhanced sensitivity to the drug. There was no change in the rate of efflux of Adriamycin. Furthermore, lipid partitioning studies indicate that there is no change in the binding of Adriamycin to the polyunsaturated fatty acid-enriched membranes. Therefore, the larger accumulation probably is due to a greater rate of influx.

Enrichment with 22:6 did not confer Adriamycin sensitivity on a resistant murine leukemia-lymphoma, P388/ADR. This may be due to the inability of fatty acid modification to affect Adriamycin efflux, since it is known that resistance in P388/ADR is caused by a more active extrusion of the drug (73).

These findings suggest that polyunsaturated fatty acid enrichment might be useful in increasing the fractional cell kill produced by Adriamycin in tumors that are inherently sensitive to this drug. This most probably occurs by enhanced cellular accumulation of drug. However, one other possibility must be considered. The metabolism of Adriamycin and other anthracyclines yields activated oxygen species (74). These can lead to degradation of unsaturated fatty acids incorporated into phospholipid micelles (75). Thus, polyunsaturated fatty acid enrichment may sensitize the cell to the cytotoxicity of Adriamycin in this manner. Regardless of its mechanism, this approach should be extended to different kinds of neoplastic cells, as well as to in vivo tumor models, because of its potential therapeutic importance.

Hyperthermia. Thermal radiation can reduce the growth of tumors, both in culture and in vivo (76), and there is evidence that membranes are an important target of heat cytotoxicity (77). For example, membrane-perturbing agents such as local anesthetics and amphotericin B potentiate the effect of hyperthermia (78–80). In addition, changes in membrane lipid composition affect the heat sensitivity of prokaryotic cells (81). There are conflicting reports as to whether cholesterol, a major membrane lipid constituent, plays a role in this response (82, 83). On the other hand, the fact that poikilotherms adapt protectively to changes in environmental temperature by altering their membrane fatty acid composition (84) suggests that this lipid component may be involved in the cellular response to heating.

To examine this possibility, the heat cytotoxicity of L1210 leukemic lymphoblasts enriched in culture with various fatty acids was compared (36). Following enrichment, the cells were transferred to a standard medium, heated, and then tested for cytotoxicity by a clonogenic assay. Cells enriched in 22:6 were more heat sensitive. The effect was greatest at 42°C, but it also was evident at 41°C. The degree of cytotoxicity enhancement was dependent on the degree of enrichment with 22:6 (36). Cell survival was significantly different at 42°C at all heating times after 30 min; the D50 values (min of heat treatment required to reduce cell survival by 63%) on the exponential part of the
cytotoxicity an attractive area for additional study. A mammary carcinoma grown in the mouse leg also is more sensitive to local hyperthermia when the diet fed the host is rich in polyunsaturated fat (17, 56). Therefore, the response is not limited to ascites cells in culture. It also is not limited to neoplastic cells; the heat sensitivity of mouse fibroblasts is greater when the cells are enriched in polyunsaturated fatty acids (83). As opposed to heat cytotoxicity, thermostolerance is not caused or affected by changes in membrane fatty acid unsaturation (85–87).

In summary, changes in membrane fatty acid composition can influence the sensitivity of neoplastic cells to thermal radiation. The molecular mechanism responsible for this is unknown, and more information about the process could lead to new concepts about tissue responses to injury. This, together with the possibility of eventual therapeutic application, makes the relationship between membrane fatty acid composition and heat cytotoxicity an attractive area for additional study.

Radiation. Membranes have been suggested as targets for radiation-induced cytotoxicity (88). This is based primarily on data obtained with prokaryotes, indicating that under certain conditions of temperature and aeration, radiosensitivity can be influenced by the physical state and biochemical properties of the membrane lipids (89–91). We find negative results, however, with neoplastic mammalian cells. No difference in cell survival occurred when human Y79 retinoblastoma cells extensively enriched in culture with 22:6 were treated with X-rays (92). A similar negative result was obtained with L1210 cells modified in vivo by the dietary approach. While more extensive studies might uncover conditions in which additive or synergistic responses between changes in membrane fatty acid composition and sensitivity to ionizing radiation might occur, these negative findings suggest that this is not a promising area for further pursuit.

Immune Cytotoxicity. There are conflicting data as to whether membrane fatty acid modification can make a neoplastic cell more susceptible to immune injury. No effect on cytolysis mediated either by antibody and complement or by effector cells was observed in EL-4 T-lymphoma cells, even though the physical state of the plasma membrane was altered (93). By contrast, fatty acid compositional changes make guinea pig hepatoma cells more susceptible to humoral immune cytotoxicity (94). Likewise, fatty acid enrichment of hepatoma 7777 cells increases complement-mediated cytolysis and susceptibility to natural killer cells (27, 95). The greater sensitivity is thought to be mediated by an increase in either membrane fluidity or the tendency of the cell lipids to undergo peroxidation (27). Immune-mediated cytotoxicity is a potentially important therapeutic application of membrane lipid modification. Therefore, the differences in the responses of the T-lymphoma and hepatoma cells must be resolved in order to determine whether this approach is worth pursuing.

Eicosanoid Production

Eicosanoid production is another area where fatty acid modification might have an influence on the properties and function of neoplasms. Arachidonic acid is the substrate for most of the prostaglandins and lipooxygenase products formed by animal tissues. The arachidonic acid is stored intracellularly in membrane phospholipids and released when the cell is activated by appropriate stimuli. Changes in membrane phospholipid fatty acid composition can alter the amount of arachidonic acid contained in these storage pools or cause the incorporation of structurally similar analogues like 20:5n-3 into these pools (96). This can affect the amount of prostaglandins formed by a number of nonmalignant cells (96). Similarly, the output of prostaglandins E2 and F2α by MC5-5 methylcholanthrene-transformed fibroblasts, the HSDM1 fibrosarcoma, and the J-111 monocytic leukemia is reduced when the cells are exposed in culture to 20:5 (97). Although cellular fatty acid composition was not measured in this study, it is likely that exposure in culture to 20:5 modified the fatty acid composition and reduced the arachidonic acid content of membrane phospholipid substrate storage pools in these neoplastic cells.

Eicosanoids can influence the carcinogenic process in many different ways. They can stimulate tumor growth (98), act as tumor promoters (99), inhibit tumor growth (100, 101), or influence tumor migration and the metastatic potential (102, 103). The eicosanoids that influence the tumor may be produced either by the neoplastic cells themselves (104) or by host tissues (105). Therefore, the relationship between fatty acid modification and eicosanoid production is likely to be complicated and vary for different tumors. Fairly detailed studies will be needed to sort out these possibilities. A related aspect that deserves further study is whether the tumor-promoting actions of the n-6 polyunsaturates (14, 57, 61, 62) and the apparent protection against this afforded by the fish oil polyunsaturates (59, 67) are due primarily to effects on the capacity of the host tissues to produce eicosanoids.

Future Directions

Membranes are a plausible target for antineoplastic therapy, a possibility that has been generally overlooked. The present findings provide a rationale for directing such an approach specifically to membrane fatty acyl groups. The membrane fatty acid composition in a neoplastic cell is not stringently regulated, and fairly substantial modifications can be produced, even in vivo. Fig. 4 is a conjectural model of what occurs within the membrane when the fatty acid composition is modified. Some of the saturated and monounsaturated fatty acyl groups are

![Image](https://example.com/image.png)

Fig. 4. Schematic diagram for a model of membrane fatty acid alteration. The type of fatty acid chain is designated by the shape of the line. Straight lines, saturated chains; single bend, monounsaturated chains; two bends, polyunsaturated chains. When the membrane contains more polyunsaturated fatty acyl chains, the conformation and functional properties of certain enzymes, receptors, and transport carriers or channels are modified. E, representative enzyme such as phospholipase; L, ligand; R, receptor; D, drug; C, transport carrier or channel.
replaced by polyunsaturated groups without disrupting the basic architecture of the membrane. The resulting structural changes within the hydrophobic core of the lipid bilayer may influence the conformation and properties of some of the transporters, ion channels, receptors, and enzymes contained within the membrane, thereby modulating their function or affecting their localization within specific lipid domains. This modulation, in turn, may influence the response of the neoplastic cell to certain stimuli or perturbations.

Many fundamental questions regarding the relationship of membrane fatty acid composition to cellular properties and function remain unanswered. For example, since binding to certain receptors is affected, is signal transduction also affected? Are the fatty acid compositional changes in the inositol phospholipids sufficient to affect the operation of the phosphatidylinositol cycle? Is the effectiveness of the n-3 polyunsaturates due simply to the greater number of double bonds, as suggested by the available data (28), or is there a unique effect due to the unsaturated bond in proximity to the methyl terminus of the fatty acyl chain? Like certain carrier-mediated transport processes, is passive diffusion through the membrane also affected? The application of sophisticated biophysical and biochemical techniques to the fatty acid modification model should provide novel approaches to these and other fundamental questions in cell biology.

Extension of this approach to other membrane lipid components, such as cholesterol or phospholipid head groups, undoubtedly will increase our basic understanding of tumor biology. From a practical standpoint, however, it will be difficult to bring about such changes in vivo. For example, changes in the plasma cholesterol concentration may alter the amount of cholesteryl ester stored within cells, but they appear to have little or no effect on membrane cholesterol content (106). The main advantage of focusing on fatty acid replacements is the potential for clinical application. Although the dietary modifications that have been used thus far are extreme and cannot be achieved with ordinary foods, liquid formulas containing saturated or polyunsaturated fat have been taken by humans for long periods without adverse effects (107). Dietary supplements, such as fish oil capsules, also have been administered successfully to humans (108). For specialized needs, i.e., solutions containing stable polyunsaturated triacylglycerol emulsions can be prepared. This makes it possible to consider future clinical application without first having to develop new technologies.

Since fatty acid replacements do not appear to consistently influence tumor growth, any usefulness that this approach ultimately may have in antineoplastic therapy almost certainly will be indirect, as a potential adjunct to chemotherapy or hyperthermia, not something that is likely to be effective by itself. Furthermore, many tissues of the host, including plasma membranes, are affected when fatty acid modification is attempted in vivo (109). Therefore, fatty acid replacements that augment therapeutic effectiveness also are likely to increase toxicity. Different tissues are modified to varying extents (109), however, and by considering proliferative kinetics, it may be possible to develop protocols that lead to relatively greater enrichment of the neoplastic cells with a particular type of fatty acid. Such selectivity probably will be needed in order to ultimately derive any real therapeutic benefit from this approach.

References


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