Role antitumor lipids in cell-cell adhesion and invasion
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Approximately 400 b.c. it was Hippocrates who compared the swollen blood vessels originating from some breast tumors with the legs of a crab, calling it *Karkinoma* in Greek.

Drawing: sand crab with ET-18-OMe and HePC (Steelant W., 1999)
1. Structure-function relationship of antitumor lipids

Together with proteins, phospholipids constitute the major components of biological membranes [1]. There are two possible pathways in the phospholipid metabolism:

A. **De novo** synthesis of phosphatidylethanolamine, phosphatidylcholine (Figure 1) and phosphatidylserine (Figure 2)[2-4].

B. Incorporation of fatty acid into 2-lysophospholipid via 2-lyso-phosphatidylacyltransferase (Lands pathway)(Figure 3)[3, 4].

![Diagram of De novo synthesis of phosphatidylethanolamine and phosphatidylcholine](image1)

Figure 1: De novo synthesis of phosphatidylethanolamine and phosphatidylcholine.

![Diagram of De novo synthesis of phosphatidylserine](image2)

Figure 2: De novo synthesis of phosphatidylserine.
It was discovered in the 70's that immunological adjuvants activate phospholipase A2 in macrophages, resulting in degradation of cellular phosphatidylcholine into lysophosphatidylcholine (Figure 4) [5-7], presumably to assist in phagocytosis.

In natural systems, lysophosphatidylcholine is quickly reverted to phosphatidylcholine (Figure 4). This probably has a protective function for the organism. It was therefore decided to synthesize lysophosphatidylcholine analogs which were not substrates for the enzymes that participate in the metabolism of natural lysophosphatidylcholine, and to determine whether they
could be used more effectively as immunopotentiators [8]. Of the various possibilities of modifying synthetically the structure of natural lysophosphatidylcholine, the following modifications have been realized (Figure 5).

![Figure 5: Possible synthetic analogs of lysophosphatidylcholine.](image)

1. The ester (ES) linkage was replaced by an ether (ET) linkage.
2. The number of carbon atoms has been varied from 16-20 in 2-LPC to 8-26.
3. The OH group was substituted.
4. The polar head group has been modified, changing thereby the charge pattern of the molecule.

The synthetic analogs with modifications in positions 1, 2 and 3 were symbolized in the following way:

- **Position 1**: ES or ET = ester or ether linkage
- **Position 2**: \((\text{CH}_2)_x = x \) number of C atoms
- **Position 3**: The functional group replacing \(-\text{OH}\) is indicated.

As a result, the alkyletherlipid 1-O-octadecyl-2-O-methylglycero-3-phosphochoiline (ET-18-OMe)(Figure 6) was synthesized.

![Figure 6: The structure of ET-18-OMe.](image)
ET-18-OMe represents a new class of antitumor agents (AELs) and has been shown to inhibit cellular invasion (see point 2). The experimental results obtained with 2-LPC and the synthetic analogs indicate that they increase the production of antibodies. Short-chain ether analogs have a slightly suppressive effect. When the paraffin side-chain is elongated to 20 or more carbon atoms, the adjuvant activity of the analog is lost. The effect of 2-LPC analogs was tested on allograft rejection [8]. In this experiment, NMRI-mice were injected with 250 µg of different analogs, and 4 days later inoculated with $10^4$ Ehrlich ascites tumor cells adapted to grow in NMRI-mice: $0.5 - 1 \times 10^2$ tumor cells will kill 90-100% of the animals [9] after 3-4 weeks. Ether analogs with 18 carbon atoms caused rejection of the inoculated tumor cells, and the mice remained free of tumor. By contrast, ester analogs with 18 or 22 carbon atoms and the ether analogs with a shorter side-chain in position 1 were less effective or ineffective. Meanwhile, numerous analogs of ET-18-OMe have been synthesized and analyzed for their antitumor activity in a variety of \textit{in vitro} and \textit{in vivo} models [10-35]. Most of the synthesized analogs of ET-18-OMe are summarized in table 1.

Table 1: Analogs of ET-18-OMe, modifications at positions C-1, C-2 and C-3 of the glycerol backbone.

<table>
<thead>
<tr>
<th>X</th>
<th>CH$_2$, S, SO, NH, NMe, O$_2$C, OCONH, OCOCH$_3$, Cl', F', OCH$_3$CF$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>H, F, CI, OH, Oet, OCH$_2$CF$_3$, OCONHMe, OCOCH$_2$COMe, OSO$_2$R, CH$_2$OMe, SMe, NHR, NHCOMe, NHCO$_2$Me, NSO$_2$Me, OCOCH$_3$, OPO(OH)$_2$, OPO(O)O(CH$_2$)$_n$NMe$_3$</td>
</tr>
</tbody>
</table>
In addition, the glycerol backbone has been omitted [36] by substitution with an ethylene glycol or ethanolamine backbone. The most prominent representative is hexadecylphosphocholine (HePC)(Figure 7).

![Figure 7: The structure of hexadecylphosphocholine.](image)

HePC is the first of a series of alkylphosphocholines, lacking the glycerol backbone, exerting antitumor activity against a broad spectrum of established rodent and human tumor cell lines in culture and is also found to inhibit the growth of experimental tumors in rat and to induce antitumor responses in skin nodules from human breast cancer and in patients with cutaneous lymphomas, when applied topically [37-42].

Experiments with a series of HePC analogs have shown that the anticancer activity of this group is restricted to certain structural properties of the molecule. Parallel with the increasing alkylchain length, toxicity and anticancer efficacy of these analogs were stronger [43-45]. Besides the length and configuration of the alkyl chain, the phosphocholine moiety was found to be of similar importance. Extension of the distance between the phosphate and the trimethylammonium group to 3, 4 or 6 carbon atoms to generate hexadecylphospho (N,N,N-trimethyl) propanolamine, -butanolamine and -hexanoline resulted in structures with progressively decreasing anticancer activity [46].

Clinical trials with ET-18-OMe, HEPC and 1-hexadecylmercapto-2-methoxymethyl-rac-glycero-3-phosphocholine, (BM 41.440, ilmofosine) (Figure 8), were started in the 1980’s with stage IV patients.

![Figure 8: The structure of ilmofosine.](image)
An objective response was observed in patients with solid tumors of different histology, including non-small cell bronchiogenic carcinomas and in acute myeloid leukemia [47-53]. Table 2 lists the majority of clinical trials documented to date and their clinical outcome [39-41, 47, 48, 50, 51, 54-68].

Table 2: Clinical studies with antitumor lipids

<table>
<thead>
<tr>
<th>Agent/Route</th>
<th>Target Cancers/Disease</th>
<th>Phase</th>
<th>Patients</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET18/i.v. &amp; oral</td>
<td>NSCLC, leukemias</td>
<td>I</td>
<td>16</td>
<td>2 PR, 2 SD</td>
</tr>
<tr>
<td>ET-18-OMe/i.v.</td>
<td>monomyelocytic leukemia case</td>
<td>I</td>
<td>1</td>
<td>PR</td>
</tr>
<tr>
<td>ET-18-OMe/oral</td>
<td>NSCLC</td>
<td>II</td>
<td>116</td>
<td>2 PR, 68 SD</td>
</tr>
<tr>
<td>ET-18-OMe</td>
<td>purging leukemic bone marrow</td>
<td>I</td>
<td>24</td>
<td>7 CR</td>
</tr>
<tr>
<td>ET-18-OMe</td>
<td>purging leukemic bone marrow</td>
<td>I</td>
<td>29</td>
<td>11 CR</td>
</tr>
<tr>
<td>BM 41.440/oral</td>
<td>various</td>
<td>I</td>
<td>19</td>
<td>3 PR, 6 SD</td>
</tr>
<tr>
<td>BM 41.440/oral</td>
<td>various</td>
<td>I/II</td>
<td>102</td>
<td>0 PR, 0 SD</td>
</tr>
<tr>
<td>BM 41.440/oral</td>
<td>malignant melanoma</td>
<td>II</td>
<td>43</td>
<td>0 PR, 13 SD</td>
</tr>
<tr>
<td>BM 41.440/i.v.</td>
<td>various</td>
<td>I</td>
<td>33</td>
<td>0 PR, 2 SD</td>
</tr>
<tr>
<td>BM 41.440</td>
<td>various</td>
<td>I</td>
<td>22</td>
<td>1 PR, 2 SD</td>
</tr>
<tr>
<td>HePC/oral</td>
<td>various</td>
<td>I</td>
<td>34</td>
<td>no responses</td>
</tr>
<tr>
<td>HePC/oral</td>
<td>NSCLC</td>
<td>II</td>
<td>26</td>
<td>1 PR, 3 SD</td>
</tr>
<tr>
<td>HePC/oral</td>
<td>metastatic head &amp; neck cancer</td>
<td>II</td>
<td>14</td>
<td>0 SD</td>
</tr>
<tr>
<td>HePC/oral</td>
<td>soft tissue sarcoma</td>
<td>II</td>
<td>23</td>
<td>1 SD</td>
</tr>
<tr>
<td>HePC/oral</td>
<td>hematological effects, various</td>
<td>II</td>
<td>59</td>
<td>rise in WBC and Platelet counts</td>
</tr>
<tr>
<td>HePC/topical</td>
<td>breast cancer skin metastasis</td>
<td>I</td>
<td>25</td>
<td>4 CR, 3 PR, 8 SD</td>
</tr>
<tr>
<td>HePC/topical</td>
<td>breast cancer skin metastasis</td>
<td>II</td>
<td>29</td>
<td>8 PR</td>
</tr>
<tr>
<td>HePC/topical</td>
<td>breast cancer skin metastasis</td>
<td>II</td>
<td>14</td>
<td>4 CR, 9 PR</td>
</tr>
<tr>
<td>HePC/topical</td>
<td>breast cancer skin metastasis</td>
<td>II</td>
<td>31</td>
<td>2 CR, 6 PR, 10 SD</td>
</tr>
<tr>
<td>HePC/topical</td>
<td>cutaneous lymphomas</td>
<td>I/II</td>
<td>15</td>
<td>5 CR, 5 PR, 3 SD</td>
</tr>
</tbody>
</table>

Abbreviations: NSCLC = non-small cell lung cancer; CR = complete response; PR = partial response; SD = stable disease; WBC = white blood cells.

The good response of leukemic cells in in vitro experiments, combined with the substantial resistance of normal bone marrow progenitor cells, suggested that ET-18-OMe might be clinically useful for purging bone marrows [69-71]. Preclinical trials using autologous bone marrow transplantation in mice were also encouraging [56, 72, 73]. A number of clinical trials using ET-18-OMe-purging in concert with cryopreservation have been concluded to date and show uniformly good results [56-61]. The combination of purging with ET-18-OMe and the photosensitizing dye, Merocyanine 540, used in photodynamic therapy, may provide a simple, versatile, and effective means of eliminating large numbers of leukemia cells from autologous bone marrow grafts without causing excessive damage to normal hematopoietic stem cells [74]. As AEL’s are very promising anticancer agents, combinations of AEL’s with radiotherapy or
several chemotherapeutic drugs were tested in vitro on human cell lines and showed a positive interaction between ether phospholipids and radiotherapy [42, 75] and/or chemotherapeutic drugs [76]. Due to its amphiphilic behavior, HePC, was expected to possess favorable penetration characteristics. Moreover, a pronounced in vivo anticancer activity has been demonstrated in chemically induced rat mammary carcinomas [39, 77]. Topical application of HePC has not only been shown to induce complete tumor regression of subcutaneously inoculated tumor cells in nude mice [78] but also has shown significant activity against subcutaneous breast metastases and lymphomas in human patients. This suggests a therapeutic advantage where a high and persistent local concentration can be achieved. HePC is now marketed in Germany for the topical treatment of subcutaneous breast cancer metastases [41, 79].

Given the potent and selective nature of the antiproliferative effects observed in vitro, [13, 80-82], clinical trials to date have been relatively unsuccessful. The most notable exceptions so far are the topical applications of HePC and the autologues transplantation of ET-18-OMe-purged bone marrow.

1.1. Cellular uptake of antitumor lipids

When added in tissue culture, the antitumor ether lipids will bind to serum proteins. Under these conditions, the antitumor lipids are forming lipo-protein complexes [86-90]. After the exchange from the lipo-protein complexes of the culture medium to the cell membrane, these compounds enter the cellular phospholipid pool [11, 49, 88-94]. The most likely mechanism of uptake into the intracellular compartments is insertion into the outer leaflet of the plasma membrane followed by membrane renewal [94].

The uptake of ET-18-OMe by HL-60 human as well as L1210 murine leukemia cells could be suppressed by inhibitors of endocytosis such as chloroquine, monensin and vinblastine, suggesting that the uptake does not happen by a receptor-mediated pathway, but is mainly due to endocytosis [87, 95]. The ET-18-OMe sensitive HL60 human leukemia cell line was compared with the less ET-18-OMe sensitive K562 human leukemia cell line and it was suggested that a greater sensitivity to ET-18-OMe is correlated with a more rapid endocytic uptake and a higher content of ether-containing phospholipids [96].

Sterically stabilized liposomes

Alkylether lipids were put into sterically stabilized liposomes before they were added to cells in vitro or to animals in vivo and so reduced the side effects in the mice which received liposomal alkylphosphocholines. Especially gastrointestinal toxicity and hemolysis, the major side effects of alkylphosphocholines, were reduced by the use of liposomal preparations [97, 98]. However, a major drawback for the use of liposomes as a drug release system is the high affinity of these vesicles for the mononuclear phagocyte system.
Especially in the human breast carcinomas, MaTu, MT-1 and MT-3, the sterically stabilized HePC liposomes resulted in significantly reduced tumor growth in comparison to conventional HePC liposomes or free HePC which is probably related to the extended circulation and its accumulation in tumors [99].

ET-18-OMe has an anticancer activity, but a systemic toxicity restricted its therapeutic use. Ahmad et al. investigated the effects of ET-18-OMe in stable liposomes on P388 leukemia cells, Lewis lung metastasis and B16/F10 melanomas (lung cancer nodules) in mice. The entrapment of ET-18-OMe in liposomes decreased the acute toxicity of ET-18-OMe after i.v. administration and so transforms ET-18-OMe into an effective anticancer agent [100].

Motility and membrane ruffling

There is considerable evidence indicating that increased motility and particularly ruffling and other types of fast membrane movements might be a characteristic of the malignant phenotype [101-107]. It was suggested that the concentration of ET-18-OMe inducing resistance towards invasion of heart tissue in organ culture stimulated the motility of heart cells cultured on solid substrate [108]. Human HL-60 leukemia cells grown in the presence of ET-18-OMe accumulated this ether lipid in their membranes, which resulted in a decrease in plasma membrane fluidity and inhibition of tumor cell invasiveness in embryonic chick heart fragments [23, 92, 108]. Scanning electron microscopy demonstrated that ET-18-OMe induced morphological alterations of the membrane of leukemic cells consisting of formation of blebs and pores which lead to loss of cell viability [109].

It was also suggested that local accumulation of antitumor lipids within the cell membrane could increase the permeability for ions like Ca$^{2+}$ and thereby cause tumor growth inhibition [31, 110].

1.2. Influence of antitumor lipids on phospholipid metabolism

Influence of alkylether lipids on the de novo synthesis of phospholipids

Alkylether lipids inhibit the synthesis of phosphatidylethanolamine at three different levels [111]. ET-18-OMe inhibits the CDP-choline pathway of the phosphatidylethanolamine synthesis at the CTP:phosphocholine cytidylyltransferase step [112]. ET-18-OMe and HePC inhibited CTP:phosphocholine cytidylyltransferase in MDCK cells due to translocation from the membrane to the cytosol of CTP:phosphocholine cytidylyltransferase [113].

Secondly, the uptake of choline to form phosphocholine was inhibited with HePC [114]. Dodecylphosphocholine, octadecylphosphocholine and HePC inhibited the uptake of choline into rat synaptic neurons [115].

Phosphatidylserine is a component of all prokaryotic and eukaryotic cell membranes with minor quantities when compared to phosphatidylethanolamine and phosphatidylcholine and is particularly important as an activator of different enzymes, including protein kinase C [116-118]. Bleivik et al. observed that human leukocytes treated with ET-18-OMe had a 5 fold increase of serine incorporation into phospholipids to form phosphatidylserine and suggested
that ET-18-OMe could regulate in that way protein kinase activity [119].
Thirdly, alkyletherlipids apparently interfere with phospholipase activity and ET-18-OMe and BM41.440 inhibit the hydrolysis of phosphatidylethanolamine and phosphatidylcholine by phospholipase D in NIH3T3 fibroblasts [120].
HePC stimulates phospholipase D activity in human breast fibroblasts CCD-986-SK cells [121] and stimulates the degradation of phosphatidylcholine by phospholipase C [122]. However, Berkovic et al. investigated the influence of HePC on phospholipase A2 in the human leukemia cell line U937 and found that alteration in phospholipase A2 activity by HePC is most probably not the mechanism by which HePC mediates its antiproliferative effects [123].

**Influence of alkyletherlipids on Lands pathway**

Naturally occurring 2-lysophosphatidylcholine or its analogs, which have an acyl bond in the sn-1 position of the glycerol, can be degraded to glycero-3-phosphocholine by lysophospholipase [124] or reacylated in the sn-2 position by 2-lysophosphatidylcholine transferase to 3-sn -phosphatidylcholine. Of the analogs that are substituted in the sn-2 position, this transfer reaction will be blocked, but the deacylating reaction at the sn-1 position is still sufficient to metabolize these lysophospholipids and to prevent cellular damage [11]. However, the synthetic anti-tumor phospholipids can't enter the deacylation-acylation cycle of cellular phospholipids [125] because a lysophospholipase can't split the alkyl bond, and acylation is blocked in most of these compounds by a stable substitution of position sn-2 of the glycerol backbone [125]. Since alkyl-lysophospholipids are antimitobolites in the synthesis of 3-sn-phosphatidylcholine, it was suggested that destruction of Meth A sarcomas grown in BALB/C mice can be correlated with the disturbance of this metabolism. A decreased synthesis of 3-sn-phosphatidylcholine is accompanied by an increased degradation of cellular 3-sn-phosphatidylcholine in the presence of alkyl-lysophospholipids [14]. As a consequence, endogenously formed lysophospholipid accumulates in the presence of alkyllysophospholipids and might be an additional factor in the tumor cell destruction [126, 127]. It has been postulated by Hermann et al. that the inhibitory effects of ET-18-OMe are directly related to the degree of interference in the acylation of lysophosphatidylcholine to phosphocholine [124]. Lu et al. observed that the antiproliferative effect of ET-18-OMe is not dependent on inhibition of acylation processes and a strict correlation between the quantity of ET-18-OMe accumulated and the sensitivity to the compound does not exist [128, 129].
On the other hand, it has been reported that the metabolism of antitumor phospholipids depends partially on the activity of a specific oxygen requiring O-alkyl cleavage enzyym [130-134]. Morris hepatoma cells lack these alkyl cleavage enzymes and have high levels of various alkyl-lysophospholipids [14, 133-135].

**1.3. Other action mechanisms of antitumor lipids**

Antitumor lipids are active against cancer cells by a number of different mechanisms. Both AELs and APCs are taken up spontaneously and localize preferentially in tumor tissues in vivo,
where they can act as direct cytotoxic agents, causing detergent lysis and possibly as inducers of controlled cell death or apoptosis [31].

In some cell types AELs successfully induce differentiation. Moreover, AELs are potent inducers of macrophage activation which may also contribute to their in vivo antitumor activity. APCs do not activate the immune system, but are potent co-stimulators of bone-marrow proliferation. Other activities such as inhibition of invasion and reduced metastasis have also been reported and will be discussed in section 2 in this chapter.

AELs have also attracted scientific and clinical interest in the treatment of conditions other than cancer. Experimental allergic encephalomyelitis in rats is believed to closely mirror the effects of multiple sclerosis in humans [136]. ET-18-OMe delayed the onset, reduced the duration and limited the severity of allergic encephalomyelitis in the Lewis rat model [136, 137]. Similar studies [138] demonstrated that SRI 62-834, a cyclic ether analogue of ET-18-OMe, could attenuate clinical disease and, after withdrawal, resulted in long-lasting curative effects.

Some activity against HIV-virus propagation in vitro has also been reported [139-142]. ET-18-OMe, HePC, BM 41.440 and SRI 62-834 are reported to be effective against diseases of tropical and sub-tropical regions, which are caused by closely related haemoflagellate protozoa belonging to the genera Leishmania and Trypanosoma, e.g. Leishmaniasis, South American trypanosomiasis (Chagas’ disease) and human African trypanosomiasis (sleeping sickness)[143-145].

Taken together, other therapeutic applications for these versatile agents may yet await discovery. Inhibition of cellular transport systems has also been demonstrated. The molecular mechanisms for some of these activities are beginning to be unraveled, but others are still highly speculative. It is clear, however, that in marked contrast to today’s conventional chemotherapeutic armamentarium, antitumor lipids do not interact with DNA and are not mutagenic [31].

2. Adhesion, invasion and metastasis

Approximately 400 b.c. it was Hippocrates who compared the swollen blood vessels originating from some breast tumors with the legs of a crab, calling it Karkinoma in Greek and Cancer in the Latin equivalent [146].

Cancer is a disease generated by disturbance of the most fundamental rules of behaviour of the cells in a multicellular organism. To understand cancer we have to understand both the cells and their social interactions in the tissues of the body. The body of an animal can be considered as a society or ecosystem whose individual members are cells, reproducing by cell division and organized into collaborative assemblies or tissues [1].

Cancer cells have three properties: (i) they reproduce in defiance of the normal restraints giving rise to a tumor or neoplasm, (ii) they have loss of differentiation and (iii) they invade and colonize territories normally reserved for other cells giving rise to secondary tumors or metastasis [146].
Cancer is the result of a series of genetic mutations leading to tumor progression in which oncogenes and tumor-suppressor genes are involved [147-149](Figure 9).

<table>
<thead>
<tr>
<th>GROWTH</th>
<th>DEDIFFERENTIATION</th>
<th>INVASION</th>
<th>METASTASIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDKs</td>
<td>erbB2</td>
<td>MMPs</td>
<td>mts-1</td>
</tr>
<tr>
<td>Ki-ras</td>
<td>TGFβ</td>
<td>Integrins</td>
<td>67LR</td>
</tr>
<tr>
<td>β-CTN</td>
<td></td>
<td>TF</td>
<td>MUC-1</td>
</tr>
</tbody>
</table>

Legend: CDKs, cyclin dependent kinases; Ki-ras, oncogene from Kirsten murine sarcoma virus encoding a 21 kDa guanine nucleotide binding protein; β-CTN, β-catenin; erbB2, oncogenic tyrosine kinase belonging to the EGFR family; TGFβ, transforming growth factor β; MMP’s, matrix metalloproteinases; TF, tissue factor, invasion promotor expressed in tumor associated myofibroblasts; Tiam-1, exchange factor for Rac, invasion promotor for lymphoma cells and invasion-suppressor for epithelial cells; mts-1, gene encoding a small calcium binding protein; LR67, 67 kDa high affinity laminin receptor, MUC-1, mucin-1 or episialin, a cancer associated mucin, mediating adhesion of cancer cells to endothelial cells; APC, adenomatous polyposis coli, IGF-IIR, insulin like growth factor II receptor; DCC, deleted in colorectal cancer, encoding a transmembrane glycoprotein of the immunoglobulin superfamily; p53, nuclear protein that arrests replication of damaged DNA, also called ‘guardian of the genome’; Rb, retinoblastoma protein encoding a phosphoprotein implicated in the regulation of the cell cycle; TGFβR-II, type II receptor for TGFβ; E-CAD/CTN, E-cadherin/catenin complex; TIMPs, tissue inhibitors of metalloproteinases. With permission from [150].

Figure 9: Schematic representation of cancer development.
We consider invasion within a micro-ecosystem in which there is continuous molecular cross-talk between cancer cells and host elements such as inflammatory cells, immunocytes, endothelial cells, fibroblasts and extracellular matrix, produced by them [148]. Metastasis is a multistep-process of invasion in which cancer cells detach from the tissue in which they originate when the balance between invasion-suppressor and invasion-promotor genes is in favor of the invasion-promotor genes, they enter into the circulation (intravasation), are transported into the circulation, leave the circulation (extravasation) and invade into the host tissue, forming a secondary tumor or metastasis. From this tumor, a new invasive process can start and give rise to more metastasis at other locations [151](Figure 10).

Figure 10: The metastatic spread of colon cancer to the liver, lungs and brain. En, endothelium; Ep, epithelium; BM, basement membrane; IS, interstitial stroma; C, cancer cell; L, leukocyte; F, fibroblast; H, hepatocyte; K, kupffer cell; SD, Disse's space; RE, respiratory epithelium; RL, respiratory lumen; A, astrocyte; N, neural cell; O, oligodendrocyte; M, macrophage; PP, pia perivascularis. From [150] with permission.
Several cellular activities are implicated in invasion: cell-cell adhesion, cell-matrix interaction, motility and proteolysis. In this work we were mainly interested in the effect of AELs on the two elements implicated in cell-cell adhesion and invasion; namely the E-cadherin/catenin complex and episialin.

Cadherin is a homophillic Ca\(^{2+}\)-dependent cell-cell adhesion molecule with four different glycosylation sites and many sialic acid moieties. Cadherins with an HAV-sequence in the first extracellular domain are E-(epithelial), P-(placental), N-(neural), R-(chicken retina), B-(brain of early chicken embryo's) and EP-(C-cadherin in unfertilized eggs and early Xenopus embryos). E-cadherin linked by its cytoplasmic part to \(\beta\)-catenin, plakoglobin, \(\alpha\)-catenin and the actin cytoskeleton, acts as an invasion-suppressor molecule (Figure 11)[152]. E-cadherin can be regulated at various levels, namely gene mutations, protein phosphorylation of the promotor and protein-protein associations [153-158]. Such disregulation leads to increased invasiveness as documented in experimental as well as in clinical cancers [154].

![Figure 11](image-url)

**Figure 11**: PM, plasmamembrane; \(\alpha\)-CTN, \(\alpha\)-catenin; \(\beta\)-CTN, \(\beta\)-catenin; PLAKO, plakogorbin; \(p120/^\text{CTN}\), \(p120/^\text{catenin}\); HAV, histidine alanine valine sequence; P, phosphorylation site; MUC1, mucin1 or episialin. From [159] with permission.

Release of an 80 kDa sE-CAD (soluble E-cadherin) inhibits the function of E-cadherin and mediates a signaling pathway by tyrosine phosphorylation of GSK3\(\beta\) (glycogen synthase kinase 3\(\beta\)) and \(\beta\)-catenin. Episialin is a high molecular weight transmembrane glycoprotein with a short cytoplasmic tail (69 amino acids) and a large extracellular domain (1000-2200 amino acids).
acids) containing a large number of carbohydrate side chains, attached by O-glycosidic linkages [160]. The carbohydrate side chains carry many sialic acid residues conveying a high negative charge on the molecule [161]. Episialin can be released from the cell surface by an unknown mechanism. In patients with breast and other carcinomas, episialin is shed from the cancer cells into the circulation and can be detected by the CA 15-3 assay, which is presently used for monitoring the course of the disease and for early detection of recurrent breast cancer [162]. Episialin is located at the apical surface of most glandular epithelial cells and is present at increased levels in carcinomas, where the molecule is often distributed over the entire cell surface [163]. Overexpression of episialin inhibits integrin-mediated cell adhesion to extracellular matrix components [163], suppresses cellular aggregation [164], and increases invasiveness of human gastric cancer MKN74 cells [165].

**Role of antitumor lipids on adhesion and invasion**

In the 1970's it was already suggested by independent groups that lysophosphatides can activate certain membrane bound glycosyltransferases up to 20 fold [166-171] and that this interaction could play a role in immunological phenomena like cellular cooperation, cellular contact or antigenic recognition [172]. The last decade, protein glycosylation has become an important factor in cancer biology [173-176]. Storme et al. suggested that changes in invasion caused by lipid derivatives depended upon cell surface alterations of N-glycosylpeptides in both the invasive malignant mouse MO4, Lewis lung carcinoma LLC-H61, rat kidney adenovirus type 12 transfected 12R1C-RK cells and the normal PHF tissue [29]. It was demonstrated that pretreatment of the host tissue PHF with ET-18-OMe [177] or HePC [37] inhibits the invasion of MO4 cells when tested in the absence of the drug. The invasion resistance of PHF is still present 11 days after treatment. The surface N-glycosylpeptide profiles from PHF treated with ET-18-OMe or HePC shifted towards apparently higher molecular weight, when compared with those of untreated PHF. This shift in the profiles is abolished after desialylation of the isolated glycopeptides [178]. It was also reported that the antitumor phospholipid ET-18-OMe induces an increase in glycoprotein sialylation in normal cells (HSU rat kidney) to the level observed in malignant cells such as MO4 and NIH 3T3 [179, 180], causing diminished cell-cell adhesion. Increased sialylation of cell adhesion molecules has been observed to decrease intercellular adhesion [181-187]. Also a high level of sialylation of cell adhesion proteins [186-188] and a high content of sialic acid containing gangliosides [189, 190] in the plasma membrane are positively correlated with increased cell migration during embryogenesis or neovascularization of the cornea, whereas an increment in plasma membrane sialic acid containing gangliosides enhances cell migration in vitro [189, 191]. It was also suggested that ET-18-OMe induced reduction of cell surface area leading to inhibition of cell attachment of the basement membrane [192]. ET-18-OMe also downregulates the expression of cell-cell adhesion molecules [193, 194]. Referring to the differential adhesion hypothesis formulated by Steinberg [191], diminished cell-cell adhesion in the host tissue was due to increased sialylation of cell surface
glycoproteins [178-180, 195]. The effect of antitumor lipids on episialin had till now not been studied at all. Tables 3 and 4 give an overview on the effects of antitumor lipids on \textit{in vitro} invasion and Table 5 an update on cell-cell adhesion molecules.
<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Invasion test system</th>
<th>ET-18-OMe</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MO4 virally transformed C3H mouse fibrosarcoma</td>
<td>invasive in PHF</td>
<td>noninvasive in PHF</td>
<td>Storme et al., [23, 29]; van Blitterswijk et al., [92]; Bolscher et al., [186]; Schallier et al., [178]</td>
</tr>
<tr>
<td></td>
<td>invasive in ELF</td>
<td>noninvasive in ELF</td>
<td>Bolscher et al., [179]</td>
</tr>
<tr>
<td>BW-O-Li1 mouse T-lymphoma</td>
<td>invasive in PHF</td>
<td>noninvasive in PHF</td>
<td>Storme et al., [23]</td>
</tr>
<tr>
<td>GaMg human glioblastoma</td>
<td>invasive in RBA</td>
<td>noninvasive in RBA</td>
<td>Engebraaten et al., [196]</td>
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<td>LLC-H61 subclone of Lewis Lung carcinoma</td>
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<td>noninvasive in PHF</td>
<td>Schallier et al., [178]</td>
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<td></td>
<td>noninvasive in PHF</td>
<td>Schallier et al., [178]</td>
</tr>
<tr>
<td>TCC Type</td>
<td>TCC Type</td>
<td>TCC Type</td>
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<tr>
<td>R-TCC</td>
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<td>noninvasive in matrigel</td>
<td>Slaton et al., [197]</td>
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<tr>
<td>M-TCC</td>
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<td>noninvasive in matrigel</td>
<td>Slaton et al., [197]</td>
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<td>T13</td>
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<td>12R1-CK</td>
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<td>Bolscher et al., [180], Storme et al., [29]</td>
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<td>MCF-7</td>
<td>noninvasive in PHF</td>
<td>noninvasive in PHF</td>
<td>Bruyneel et al., [177]</td>
</tr>
<tr>
<td>MCF-7/6</td>
<td>noninvasive in collagen type I</td>
<td>noninvasive in collagen type I</td>
<td>Steelant et al., 1999, in press</td>
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<tr>
<td>MCF-7/AZ</td>
<td>noninvasive in collagen type I</td>
<td>invasive in collagen type I</td>
<td>Steelant et al., 1999, in press</td>
</tr>
<tr>
<td>Cell Line</td>
<td>Invasion Type in PHF</td>
<td>Invasion Type in RBA</td>
<td>Reference</td>
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<tr>
<td>HSU</td>
<td>noninvasive</td>
<td>invasive in PHF</td>
<td>Bolscher et al., [180]</td>
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<tr>
<td>nonmalignant rat kidney cells</td>
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<td>Bruyneel et al., [177]</td>
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<td>NIH/3T3</td>
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<td>nonmalignant mouse cells</td>
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<tr>
<td>NMuMG</td>
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<td>Bruyneel et al., [177]</td>
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<tr>
<td>mouse epithelial cells</td>
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<tr>
<td>MDCK</td>
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<td>invasive in PHF</td>
<td>Bruyneel et al., [177]</td>
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<td>Madin-Darby kidney cells</td>
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<tr>
<td>D37MG</td>
<td>invasive in RBA</td>
<td>noninvasive in RBA</td>
<td>Haugland et al., <a href="a">198</a></td>
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<td>glioma cells</td>
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<tr>
<td>GaMG</td>
<td>invasive in RBA</td>
<td>noninvasive in RBA</td>
<td>Haugland et al., <a href="a">198</a></td>
</tr>
<tr>
<td>glioma cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PHF: embryonal precultured chick heart fragments  
ELF: embryonal chick lung fragments; RBA: rat brain aggregates  
(a) ET-18-OMe was used in combination with vincristine
Table 4: Summary of *in vitro* invasion assays with HePC.

<table>
<thead>
<tr>
<th>Cell lines</th>
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<td>BW-O-Li1 mouse T-lymphoma</td>
<td>invasive in collagen type I</td>
<td>noninvasive in collagen type I</td>
</tr>
<tr>
<td>MCF-7/6 variant of human mammary carcinoma</td>
<td>invasive in PHF</td>
<td>noninvasive in PHF</td>
</tr>
<tr>
<td>NMpneoT24T10 murine mammary gland cells</td>
<td>invasive in PHF</td>
<td>noninvasive in PHF</td>
</tr>
</tbody>
</table>

PHF: embryonal precultured chick heart fragments
Table 5: Summary of experiments with ET-18-OMe on cell-cell adhesion.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>cell-cell adhesion molecules (complexes)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMEC human microvascular endothelial cell line</td>
<td>untreated CAM expression cell-junction integrity</td>
<td>Candal et al., [194]</td>
</tr>
<tr>
<td>HUVEC human umbilical vein</td>
<td>CAM expression CD31 CAM-1</td>
<td>Bosse et al., [193]</td>
</tr>
<tr>
<td>HMEC microvascular endothelium</td>
<td>CAM expression downregulated CD31 downregulated CAM-1 not affected</td>
<td></td>
</tr>
<tr>
<td>MCF-7/AZ variant of human mammary 0carcinoma</td>
<td>E-CAD/CTN episialin TER occludin</td>
<td>Steelant et al., 1999, in press</td>
</tr>
</tbody>
</table>

CAM: cell adhesion molecules  
CD-31: 'cluster of differentiation' n°31 surface adhesion molecule  
CAM-1: tumor necrosis factor alpha induced vascular cell adhesion molecule-1  
E-CAD/CTN: E-cadherin/catenin complex  
EPISIALIN: mucin-1  
TER: transepithelial resistance  
Occludin: cell-cell adhesion molecule in the tight junctions
References


