Secretory phospholipase A2 as a tumor-specific trigger for targeted delivery of a novel class of liposomal prodrug anticancer etherlipids

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Abstract
The use of many common clinically relevant chemotherapeutics is often limited due to insufficient delivery to the tumor and dose-limiting systemic toxicities. Therefore, therapeutics that specifically target tumor cells and are nontoxic to normal cells are required. Here, we report the development of a novel class of liposomes composed of lipid prodrugs, which use the increased secretory phospholipase A2 type IIA (sPLA2) activity of the tumor microenvironment as a trigger for the release of anticancer etherlipids (AEL). Treatment of sPLA2-secreting tumor cells in vitro with liposomes consisting of proAELs resulted in growth inhibition comparable with addition of the AELs alone. Using a specific sPLA2 inhibitor, we showed the low cytotoxicity of the non-hydrolyzed proAEL liposomes and have proven the sPLA2 dependency of the activation of proAELs to cytotoxic AELs. In addition, we showed that our proAEL liposomes circumvent the inherent hemolytic toxicities associated with the use of etherlipids, thereby allowing i.v. administration of such therapeutics as nontoxic prodrug liposomes. Furthermore, using an sPLA2-secreting human colon cancer xenograft model, we showed that the proAEL liposomes are capable of inducing a tumor growth delay in vivo. Taken together, these data support the validity of this novel tumor-selective liposomal prodrug delivery strategy. This new approach also provides a promising system for tumor-selective delivery and release of conventional chemotherapeutics encapsulated in the sPLA2-degradable prodrug liposomes. [Mol Cancer Ther 2004;3(11):1451–8]

Introduction
One of the major goals of modern medicine is the identification and development of more effective tumor-selective anticancer treatments. Although many chemotherapeutics are highly potent in laboratory-based cell studies, their clinical usage is limited as a result of inadequate delivery of therapeutic doses to the tumor or harmful effects on normal organ function. To circumvent these limitations and lower the systemic side effects, novel targeted microcarrier technologies involving tumor-specific drug delivery and activation are clearly required. Several such approaches for targeting of therapeutics specifically to malignant tissue have been described including antibody-directed prodrug therapy (1), protease-activated prodrug and liposomal drug delivery (2–6), and mild hyperthermia-mediated liposomal drug release (7).

In this article, we report a new tumor-selective prodrug delivery strategy in which tumor enzymology is used as a site-specific trigger (8) for activation of a new generation of liposomes composed of prodrug anticancer lipids. This liposomal prodrug system has the advantage that in addition to tumor-selective prodrug activation and release it can also be used as a microcarrier system for many anticancer therapeutics including radiation sensitizers, cytokines, immunomodulators, or conventional chemotherapeutics such as the anthracyclines, platins, camptothecins, or topoisomerase inhibitors (8, 9).

Enzymes used to activate prodrugs in tumors must fulfil two criteria: (a) significantly higher expression and activity in tumor tissue compared with normal tissues and (b) a substrate specificity amenable to the development of prodrugs. One class of enzyme that satisfies these requirements are the phospholipase A2 (PLA2) family, specifically secretory PLA2 type IIA (sPLA2). Unlike other PLA2 family members, sPLA2 is released into the extracellular matrix where it associates with the cell surface and induces fatty acid release for downstream prostaglandin synthesis (10–13). Elevated expression of sPLA2 has been shown in several human tumor types including breast, gastric, pancreatic, prostate, small bowel, and colon (10, 11, 13–20). In addition, sPLA2 levels have also been shown to be significantly higher during tumor vascularization (21) and in tumors that show distant metastasis (11). With reference to its substrate specificity, sPLA2 is an interfacially activated lipase that has a preference for organized
lipid substrates (such as liposomes) compared with monomeric lipids in solution (22–24), supporting the use of sPLA₂ as a tumor-specific trigger for liposomal prodrug activation.

Anticancer ether lipids (AEL) belong to a potent class of drug, which can inhibit tumor cell growth without causing mutagenic or myelosuppressive effects (25–27). Unfortunately, AELs have been shown to be severely hemolytic in vivo, thereby prohibiting their i.v. administration at therapeutic concentrations (25–27) and limiting their clinical use (27, 28). To circumvent the hemolytic effects of AELs, several strategies have been used previously including p.o., i.p., and topical administration as well as incorporation of a minor fraction of the AELs into stable liposomes (29–31). Our strategy involves the design and synthesis of novel prodrug lipids (proAEL), which can be used to make prodrug liposomes creating both a sPLA₂-dependent anticancer prodrug and a drug delivery system (Fig. 1). This integrated concept would allow for transport of high concentrations of the nontoxic proAEL liposomes to the tumor where the proAELs subsequently would undergo a site-specific sPLA₂-mediated activation to AELs and induce tumor cell cytotoxicity.

In this study, we describe the rational design and development of these proAEL liposomes, their specific activation by sPLA₂, their cytotoxicity against sPLA₂-secreting human tumor cell lines, and their potential use as a new promising tumor-activated prodrug delivery system.

Materials and Methods

Synthesis of proAEL and AEL

ProAELs were synthesized using a short enantiopure strategy using (R)-O-benzyl glycidol as the chiral starting material (32, 33). Regioselective opening of the epoxide followed by acylation and deprotection resulted in 1-O-hexadecyl-2-hexadecanoyl-sn-glycerol, which immediately was phosphorylated using POCl₃ and choline tosylate, resulting in 1-O-hexadecyl-2-hexadecanoyl-sn-glycero-3-phosphocholine (proAEL-PC). (S)-1-O-hexadecyl-2-hexadecanoyl-sn-glycero-3-phosphoglycerol (proAEL-PG) was synthesized using methyl dichlorophosphate followed by deprotection. (S)-1-O-hexadecyl-2-hydroxy-sn-glycero-3-phosphoglycerol (AEL-PC) and 1-O-hexadecyl-2-hydroxy-sn-glycero-3-phosphocholine (AEL-PC) were synthesized using the same conditions but with 1-O-hexadecyl-2-O-benzyl-sn-glycerol as the chiral starting material. The synthesis is briefly depicted in Fig. 2.

Preparation of proAEL Liposomes

ProAEL liposomes were prepared by hydration of proAELs in water and sonicated for 14 minutes using a probe sonicator to give small unilamellar proAEL liposomes (<100 nm). Pegylation of liposomes was done using PE-PEG2000 (di-octadecanoyl-sn-glycero-3-phosphoanamine-N-[methoxy-(polyethylene glycol)]2000; Avanti Polar Lipids, Alabaster, AL).

Hydrolysis of proAEL Liposomes by sPLA₂

The concentration and degree of hydrolysis of the proAEL liposomes was quantitated using high-performance liquid chromatography (32). ProAEL liposomes (0.3 mmol/L) were incubated with sPLA₂ (100 ng/mL) derived from human tear fluid as described previously (34). All samples were dissolved in chloroform/ethanol/water/acetic acid (4:8:1:1 v/v) and desalted by extraction with water. High-performance liquid chromatography analysis was done using an eluent mixture of chloroform/methanol/water (73:23.3 v/v), a 5 µm spherical diol column, and an evaporative light scattering detector.

Cell Culture and Measurement of sPLA₂ Secretion

The KATO III human gastric carcinoma cell line was purchased from the Japan Health Sciences Foundation (Tokyo, Japan). The COLO 205 human colon carcinoma cell line and human umbilical vein endothelial cells (HUVEC) were purchased from European Collection of Animal Cell Cultures (Salisbury, United Kingdom) and American Type Culture Collection (Manassas, VA), respectively. KATO III
and COLO 205 cells were grown in RPMI 1640 supplemented with 10% heat-inactivated FCS, l-glutamine (2 mmol/L), and sodium pyruvate (1 mmol/L) in a humidified 5% CO₂ atmosphere at 37°C. HUVECs were grown in Ham’s F-12 medium supplemented with 10% FCS, l-glutamine (2 mmol/L), heparin (0.1 mg/mL), and endothelial cell growth supplement (50 ng/mL, Sigma, Poole, United Kingdom). Following 72 hours of growth, cell growth medium and cell pellets were collected. Quantitation of sPLA₂ protein was determined by a human-specific sPLA₂ ELISA assay according to manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI). All studies were done in triplicate.

In vitro Determination of Cytotoxicity

Cells (KATO III and COLO 205) were plated in 96-well plates at a density of 1 × 10⁴ cells per well in RPMI 1640. After overnight incubation to allow for cell attachment, the medium was replaced and refreshed with fresh medium containing varying concentrations of AELs (10–200 μmol/L) and proAEL liposomes (50–200 μmol/L) and the cells were further incubated for 72 hours at 37°C. To study sPLA₂ specificity, the specific sPLA₂ inhibitor LY311727 (kindly provided by Eli Lilly & Co., Indianapolis, IN) was added 10 minutes prior to the proAEL liposomes at a concentration of 25 μmol/L. To verify efficient proAEL hydrolysis, sPLA₂ from human tear fluid was added to the cells at a concentration of 100 ng/mL. Chemosensitivity was assessed using a standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (35). Values for cell survival are expressed as the percentage reduction in cell numbers relative to the solvent controls. All studies were done in triplicate.

Expression of sPLA₂ in COLO 205 Xenograft

Localization of sPLA₂ was assessed by immunohistochemistry in paraffin-embedded 5 μm sections of COLO 205 human colon tumor xenograft. Briefly, sections were dewaxed in xylene and rehydrated to water through graded alcohols. Heat-mediated antigen retrieval was done by microwaving the slides in citric acid buffer (0.01 mol/L, pH 6.0). Endogenous peroxidase activity was quenched with freshly prepared 1% hydrogen peroxide. Nonspecific antibody binding was inhibited using 5% normal horse serum. Sections were then incubated overnight at 4°C in a humidified atmosphere with the primary monoclonal antibody raised against sPLA₂ (Cayman Chemical) diluted 1:20 in PBS. Negative staining controls were done using normal mouse IgG (DAKO, Cambridge, United Kingdom) in place of the primary antibody. After washing in PBS, sections were incubated for 30 minutes at room temperature with an anti-mouse biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) followed by amplification and detection using a Vectastain ABC kit according to the manufacturer’s instructions (Vector Laboratories). Immunocomplex visualization was done using 3,3’-diaminobenzidine according to the manufacturer’s instructions (Vector Laboratories). Sections were counterstained with Harris’ hematoxylin and mounted in DPX mountant (Sigma).

Hemolysis Assay

Human RBC (4% in PBS) were incubated with AEL or proAEL liposomes (0.5 mL, 1 mmol/L) for 1 hour at 37°C with constant rotation. The suspension was centrifuged (2,000 × g) and the level of free hemoglobin was measured spectrophotometrically at 550 nm. RBC (4%) in PBS served as a negative control. Total hemolysis was defined as that obtained after incubation of RBC with 1% Triton X-100. The ET-18-OCH₃ ether lipid, edelfosine (Avanti Polar Lipids), was used as a control.

Effect of proAEL Liposomes in the COLO 205 Xenograft Model

Female mice (nu/nu from an inbred colony, B&K Universal, Hull, United Kingdom) 6 to 8 weeks old were implanted s.c. with 2 to 3 mm³ fragments of COLO 205 tumor. Once tumors could be accurately measured, mice were allocated into groups of six by restricted randomization to keep group mean tumor size variation to a minimum. Mice were given i.p. injections of vehicle or proAEL liposomes [300 mg/kg; proAEL-PC/proAEL-PG/PE-PEG2000 (20:70:10 mol %)] on days 0, 1, 2, 3, 4, 7, 8, 9, 10, and 11 (the initial day of therapy being designated day 0). Tumor size and body weight of each mouse were monitored daily thereafter. Tumor volume was determined using the formula: (a² × b) / 2, where a is the smaller and b the larger dimension of the tumor. Tumor volume was then normalized to the respective volume on day 0, and semilog plots of relative tumor volume versus time were made. Maximum tumor inhibition (% treated versus control) was calculated by the formula: [100 × (Relative tumor volume (control animals)) / (Relative tumor volume (control animals))]. Mann-Whitney U tests were done to determine the statistical significance of any differences in growth rate (based on tumor volume doubling time: 8.5 days for control and 12.0 days for proAEL liposomes) between control and treated groups. All procedures in this phase of the study were carried out under a project license issued by the UK Home Office, and UK Coordinating Committee on Cancer Research guidelines were followed.

Results

Specificity of sPLA₂ toward proAEL Liposomes

Hydrolysis of the proAEL liposomes by sPLA₂ was monitored over time using high-performance liquid chromatography. The level of proAEL hydrolysis was calculated on basis of the integrated area before and after addition of sPLA₂. Following 24 hours of incubation of proAEL liposomes composed of proAEL-PC or proAEL-PE (with sPLA₂), ~60% of the proAEL-PC lipids were hydrolyzed and <10% of the proAEL-PE liposomes were hydrolyzed (Fig. 3A and B). This is in agreement with previous studies suggesting a preference of sPLA₂ for anionic lipid substrates (PG) over zwitterionic lipids (PC; refs. 24, 34). Incorporation of small amounts of polymer lipids (5 mol % PE-PEG2000) into the anionic proAEL-PC liposomes, which is necessary to avoid the clearance by the reticuloendothelial system in the bloodstream (36), did not...
inhibit the sPLA₂-catalyzed hydrolysis of the proAEL-PG lipids (Fig. 3C). This is consistent with earlier hydrolysis studies showing that the steric barrier induced by the polymer coverage does not prevent the small lipolytic sPLA₂ enzyme (14 kDa) from reaching the liposomal surface, where the interfacial lipid hydrolysis occurs (8, 32).

**Secretion of sPLA₂ by Human Tumor Cell Lines**

Levels of sPLA₂ were evaluated and measured in the growth medium of human KATO III and COLO 205 epithelial tumor cell lines and normal endothelial cells (HUVEC). The amount of sPLA₂ secreted from the KATO III and COLO 205 tumor cell lines into the growth medium following 72 hours of *in vitro* culture was 3 ± 1.7 and 75 ± 20 ng/mL sPLA₂, respectively. In contrast, sPLA₂ was undetectable (<15 pg/mL) in medium from HUVECs.

**In vitro Toxicity of AELs and proAELs**

The cytotoxic effect of AEL-PG and AEL-PC and their corresponding prodrg liposomal formulations (proAEL-PG and proAEL-PC) were assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and the concentration capable of inhibiting cell growth by 50% (IC₅₀) was determined for each compound in each cell line. The AELs showed cytotoxic activity against both KATO III and COLO 205, with IC₅₀ values in the 50 to 200 μmol/L range. Exposure of COLO 205 and KATO III cells to proAEL-PG/PE-PEG2000 liposomes (95:5 mol %) for 72 hours resulted in a dose-dependent cytotoxic activity, with IC₅₀ values similar to treatment with the free AELs (Fig. 4A). In accordance with the substrate specificity of sPLA₂ reported in Fig. 3, proAEL-PC liposomes did not induce growth inhibition in any cell type tested (data not shown). Addition of sPLA₂ (∼100 ng/mL) to KATO III or COLO 205 cells treated with the proAEL-PG/PE-PEG2000

![Figure 3. sPLA₂-catalyzed hydrolysis at 37°C of proAEL liposomes composed of (A) proAEL-PC (100 mol %), (B) proAEL-PG (100 mol %), and (C) proAEL-PG/PE-PEG2000 (95:5 mol %). The high-performance liquid chromatography chromatograms show the amount of proAEL lipids before sPLA₂ was added (solid lines) and the amount of unhydrolyzed lipids following 30-minute incubation (dashed lines) and 24-hour incubation (fine dashed lines) with sPLA₂. The concentration of the proAEL liposomes was 300 nmol/L and sPLA₂ was 100 ng/mL in each case.](image)

![Figure 4. Cytotoxicity of proAEL liposomes composed of proAEL-PG/PE-PEG2000 (95:5 mol %) against (A) sPLA₂-secreting COLO 205 (hatched columns) and KATO III (solid columns). The inhibition of cytotoxicity by coaddition of the sPLA₂ inhibitor, LY311727, shows the dependence of the effect on sPLA₂ activity. Efficient proAEL liposome hydrolysis is shown by the lack of further activity following administration of exogenous sPLA₂. B, lack of proAEL liposome-mediated cytotoxicity against the non-sPLA₂-secreting HUVEC cells further reinforced the specificity of the proAEL concept toward sPLA₂. All studies were done in triplicate.](image)
liposomes only marginally increased growth inhibition, indicating rapid and almost complete proAEL-PG hydrolysis by sPLA2 secreted by the cell lines (Fig. 4A). Addition of the specific sPLA2 inhibitor, LY311727, 10 minutes prior to addition of the proAEL liposomes resulted in a lack of cytotoxic activity, supporting a dependence of the proAEL hydrolysis and cytotoxic etherlipid generation on sPLA2 activity (Fig. 4A). In contrast, no growth inhibition was observed in the non-sPLA2-secreting HUVEC cells following exposure to proAEL-PG liposomes for 72 hours (Fig. 4B).

**Lack of Hemolytic Effects of proAEL Liposomes Compared with Free AELs**

Hemolysis is the major limiting factor to the use of AELs in vivo. In the in vitro assay, both AEL-PC and AEL-PG showed hemolytic activity comparable with that observed by the potent edelfosine etherlipid (Fig. 5). In contrast, no significant hemolysis was observed in the presence of any of the proAEL liposomes (Fig. 5). These data are further supported by a lack of systemic toxicity following i.v. administration of >300 mg/kg of the proAEL liposomes in the mouse studies.

**Lack of Systemic In vivo Toxicity by proAEL Liposomes**

Because AELs have been shown previously to cause severe toxicity in vivo, we attempted to determine the maximum tolerated dose for the proAEL liposomes prior to commencing antitumor studies. No systemic toxicity was observed in non-tumor-bearing mice even at i.p. doses of 1,000 mg/kg proAEL liposomes (proAEL-PC/proAEL-PG/PE-PEG2000, 20:70:10 mol %). No significant body weight loss was observed in any of these mice (data not shown).

**Growth Inhibition by proAEL Liposomes in the COLO 205 Xenograft Model**

Because the proAEL liposomes showed promise in vitro, we examined the potential antitumor activity in vivo against s.c. implanted sPLA2-secreting COLO 205 human colon adenocarcinoma xenografts in nude mice. Immunohistochemical analyses of COLO 205 xenograft tumors confirmed the continued expression of sPLA2 in vivo, with highest sPLA2 expression being observed at the tumor periphery (Fig. 6). I.p. injection of proAEL liposomes resulted in a statistically significant tumor growth delay (3.4 days) with a maximum tumor inhibition on day 4 at 67.9% compared with controls. Although a slight reduction in mouse body weight 1 day post-commencement of treatment was observed in both control group (2.8% weight loss) and treated group (5.1% weight loss), all mice returned to their starting weight within a few days and continued to gain weight ($P < 0.01$), suggesting minimal toxic effects.

**Discussion**

The clinical use of many chemotherapeutics is often limited by a combination of poor delivery to the tumor and dose-limiting side effects on normal tissue function. To overcome these limitations, increased tumor selectivity and efficacy can be achieved by using nontoxic prodrugs that are converted to the active parent drugs at the target tumor site. ProAEL liposomes are a new generation of prodrug liposomal carriers that can be used for targeted transport and delivery of etherlipids to malignant tissue. Activation of the proAEL liposomes to AELs is triggered by sPLA2,
which is highly expressed and active in tumor tissue. As such, proAEL liposomes avoid the systemic toxicity problems related to many chemotherapeutics by allowing delivery of high doses of proAEL liposomes to tumors where the proAEls subsequently become activated to AELs by overexpressed sPLA2.

Although both AEL-PG and AEL-PC showed similar levels of cellular toxicity, differential toxicities were observed when the proAEls of these etherlipids were investigated as prodrg liposomes. Whereas the negatively charged proAEL-PG liposomes inhibited in vitro growth of sPLA2-secreting cells comparable with AEL-PG, the zwitterionic proAEL-PC liposomes did not inhibit growth of these cells. This differential substrate specificity of sPLA2, as reported previously (24, 34), is of particular importance to our approach, as it shows that sPLA2 present at the tumor site acts preferentially on the anionic prodrg liposomes. Furthermore, it offers a rational way to tailor the degradation profile of the prodrg liposomes by sPLA2 at the tumor target site. The relevant degradation time refers to a dynamic process that is determined by the accumulation and residence time of the prodrg liposomes in the tumor and the amount of secreted and active sPLA2 in the extracellular environment of the tumor tissue. These variables are presently unknown but need to be explored in future biodistribution and pharmacokinetic studies to control and optimize the degradability of the proAEL liposomes by sPLA2 in the tumor tissue. It should however be pointed out that a high degree of proAEL-PC hydrolysis is achievable (data not shown) if the anionic proAEL-PC lipids compose a major part (> 60 mol %) of the liposomal membrane. By adjusting the proAEL-PG/proAEL-PC composition and the surface properties of the liposomal prodrg substrate, it is therefore possible in future studies to fine-tune and optimize the liposomal sPLA2 lability at the tumor target site to regulate the tumor-specific drug release, suggested recently by Allen and Cullis (36) as a crucial variable for future liposome-based drug delivery. Pegylation of the liposomes, which is required to avoid reticuloendothelial clearance and promote blood circulation and passive accumulation in the tumor (37), did not inhibit the interfacial sPLA2-mediated hydrolysis of the proAEL liposomes, thereby supporting the pharmacokinetic and pharmacodynamic profile of our approach.

Tumor specificity of the new prodrg concept is provided by the dependence of proAEL liposomal cleavage on sPLA2. This is supported by cytotoxicity against sPLA2-secreting cell lines, COLO 205 and KATO III, compared with a lack of significant response in non-sPLA2-secreting HUVEC cells. Furthermore, inclusion of a sPLA2-specific inhibitor prevented proAEL-PG-mediated cytotoxicity of sPLA2-secreting cells in vitro, reinforcing the requirement for sPLA2. Addition of exogenous sPLA2 to the culture system did not significantly increase growth inhibition, suggesting complete and rapid AEL generation by sPLA2 secreted from the cancer cells.

Etherlipids have been shown previously to inhibit tumor cell growth without causing mutagenic or myelosuppressive effects (25–27). Their epigenetic mode of action involves incorporation into cellular membranes, resulting in perturbation of key membrane-associated processes and disruption of normal cell function (25–27). With reference to their use as anticancer agents, these effects include cell cycle arrest, inhibition of invasion and differentiation, induction of apoptosis, and ultimately cell death (27, 28). The major disadvantage to the use of AELs therapeutically is their inherent toxicity against RBC in vivo and subsequent limited use as antitumor therapeutics (27, 28). Consequently, these effects prohibit i.v. administration of AELs at therapeutic concentrations (25–27) and limit their clinical use. In our study, both AEL-PC and AEL-PG showed hemolytic activity comparable with that produced by the potent etherlipid, edelfosine. In contrast, no significant hemolysis was observed by the proAEL liposomes, suggesting that the liposomal prodrg strategy could circumvent the traditional side effects of AELs. Indeed, no systemic toxicity was observed when the proAEL liposomes were given to mice even at doses of 1,000 mg/kg mouse body weight. Taken together, these data support the liposomal prodrg delivery concept as a viable strategy for avoiding the hemolytic effects of AELs.

In the sPLA2-secreting COLO 205 xenograft model, administration of proAEL liposomes resulted in a statistically significant tumor growth delay compared with controls and thus a significant therapeutic effect. No significant body weight loss or systemic toxicities were observed in any of the mice. Although preliminary, these in vivo data strongly support the potential of our anticancer strategy and the evasion of hemolysis by the proAEL liposomes. Further studies are required with both non-sPLA2-secreting xenografts and labeled liposomes to confirm the role of sPLA2 in the in vivo situation and clarify the pharmacokinetics of proAEL liposomal degradation at the tumor target site.

One area of anticancer liposomal technology currently gaining attention is the mode of liposome administration (38). When liposomes are given parenterally via the s.c., i.m., or i.p. routes, they do not access the bloodstream directly but are taken up by the lymphatic capillaries draining the site of administration (e.g., the abdominal and mediastinal lymph nodes) following i.p. administration (38, 39). It has been suggested that minimal lymph node retention occurs of these non-PLA2-degradable stealth liposomes (8) and that the majority pass into the systemic circulation from the lymph nodes. These observations suggest that our sPLA2-degradable prodrg liposome strategy has potential for i.p. administration and treatment of lymph node metastases, which are poorly accessible by antitumor agents given i.v. (38).

The sPLA2 trigger mechanism underlying our strategy suggests that the methodology may also prove successful in the development of several new anticancer lipid-based prodrg drugs that selectively target the tumor and are released specifically in the tumor tissue, both issues being essential for future liposome-based drug delivery. For example, antitumor agents such as docosahexanoic acid
In these cases, targeting using our sPLA\(_2\)-triggered liposomal tumor target sites. Improvement in therapeutic index. With the technology and increase tumor-specific drug delivery resulting in an prodrug delivery system is likely to both decrease toxicity and increase drug delivery in an improvement in therapeutic index. With the technology presented in this work, it would presumably be possible without any prior knowledge of the position, size, and metastatic spread of the tumor to release the chemotherapeutics specifically at the primary and metastatic tumor target sites.

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References

10. Ate T, Sakamoto K, Kamohara H, Hirono Y, Kuwahara N, Ogawa M.

33. Guivisalsky P, Bittman R. An efficient stereocontrolled route to both enantiomers of platelet activating factor and analogs with long-chain

esters at C-2: saturated and unsaturated ether glycerolipids by opening of
34. Buckland AG, Heeley EL, Wilton DC. Bacterial cell membrane
hydrolysis by secreted phospholipases A(2): a major physiological role of
human group IIA sPLA(2) involving both bacterial cell wall penetration and
35. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB.
Evaluation of a tetrazolium-based semiautomated colorimetric assay:
36. Allen TM, Cullis PR. Drug delivery systems: entering the mainstream.
38. Oussoren C, Storm G. Liposomes to target the lymphatics by
39. Phillips WT, Medina LA, Klipper R, Goins B. A novel approach for the
increased delivery of pharmaceutical agents to peritoneum and associated
40. Siddiqui RA, Jenski LJ, Neff K, Harvey K, Kovacs RJ, Stillwell W.
Docosahexaenoic acid induces apoptosis in Jurkat cells by a protein phos-
41. Altucci L, Gronemeyer H. The promise of retinoids to fight against
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