

Phosphatidylserine-Targeted Nanotheranostics for Brain Tumor Imaging and Therapeutic Potential

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Abstract

Phosphatidylserine (PS), the most abundant anionic phospholipid in cell membrane, is strictly confined to the inner leaflet in normal cells. However, this PS asymmetry is found disruptive in many tumor vascular endothelial cells. We discuss the underlying mechanisms for PS asymmetry maintenance in normal cells and its loss in tumor cells. The specificity of PS exposure in tumor vasculature but not normal blood vessels may establish it a useful biomarker for cancer molecular imaging. Indeed, utilizing PS-targeting antibodies, multiple imaging probes have been developed and multimodal imaging data have shown their high tumor-selective targeting in various cancers. There is a critical need for improved diagnosis and therapy for brain tumors. We have recently established PS-targeted nanoplateforms, aiming to enhance delivery of imaging contrast agents across the blood–brain barrier to facilitate imaging of brain tumors. Advantages of using the nanodelivery system, in particular, lipid-based nanocarriers, are discussed here. We also describe our recent research interest in developing PS-targeted nanotheranostics for potential image-guided drug delivery to treat brain tumors.

Keywords

phosphatidylserine (PS), molecular imaging, blood–brain barrier (BBB), brain tumor, nanotheranostics

Mechanisms for Maintenance and Loss of Phosphatidylserine Asymmetry

Asymmetric Distribution of Phosphatidylserine in Normal Cell Membrane

Biological cell membrane is composed of phospholipid bilayer, of which the outer leaflet is formed predominantly with the cholinephospholipids while the anionic aminophospholipids such as phosphatidylserine (PS) are restricted to the inner leaflet.^{1,2} The asymmetric distribution of PS is maintained by a group of P-type ATPases, known as aminophospholipid translocases, that catalyzes the transport of aminophospholipids from the outer leaflet to the inner leaflet of the plasma membrane against the concentration gradient.^{2,3} Several Rhesus-associated proteins may also play a role on maintaining the PS asymmetry.^{2,3}

Disruption of PS Asymmetry in Apoptotic or Necrotic Cells

Loss of PS asymmetry, which results in the appearance of PS at the cell surface, occurs often under pathophysiological

conditions, that is, apoptosis and necrosis. Accompanying with the influx of Ca²⁺ into the cytoplasm at the early stage of cell death, the activity of translocase to transport PS inward is inhibited, whereas an adenosine triphosphate (ATP)-independent scramblase is activated to disrupt the PS asymmetry by moving

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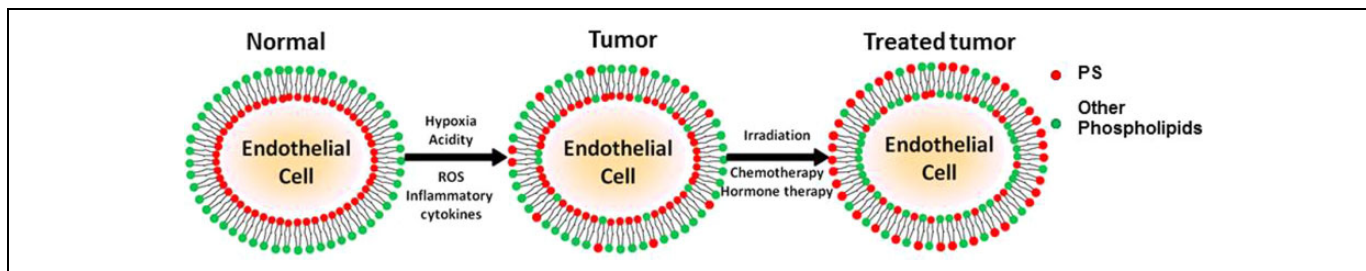


Figure 1. Schematic mechanisms for PS asymmetry in normal cell membrane and its loss in tumor vascular endothelial cells. PS indicates phosphatidylserine.

the PS outward to the cell surface. Consequently, the membrane distribution of PS becomes gradually symmetric with increased number of PS on the outer leaflet.⁴⁻⁶ However, it is noteworthy that the translocase activity is reversible following the Ca^{2+} efflux, which can lead to restoration of lipid asymmetry. As a result of loss of PS asymmetry, the cell surface-exposed PS may serve as an eat-me signal recognized by PS receptor on macrophages such as T-cell immunoglobulin and mucin families and the TYRO3, AXL, and MERTK to initiate the phagocytosis.^{7,8}

PS Exposure in Tumor Vascular Endothelial Cells

It has recently been observed that PS becomes exposed on the outer surface of endothelial cells (ECs) in tumor blood vessels, whereas vascular ECs in normal tissues, even in those highly angiogenic ovarian blood vessels during ovulation, lack exposed PS. Although it is not fully understood how and why tumor vascular ECs expose PS to the outer plasma membrane, several factors of characteristic tumor microenvironment are believed to contribute to this phenomenon.⁹⁻¹¹ Indeed, hypoxia, low pH, and tumor-specific cytokines such as interleukin 1 and tumor necrosis factor α (TNF- α) have been correlated with the PS abnormality (Figure 1).⁹⁻¹¹ In our study, incubation of TNF- α with human vascular umbilical vein cells observed massive PS exposure on the cell surface (unpublished data). These factors may perturb the ATP-dependent translocase activity and/or enhance the ATP-independent scramblase activity to transport PS outward.¹²⁻¹⁴ Importantly, these PS-exposed ECs are viable and not subject to apoptotic process.^{15,16} Unlike the apoptotic cells, they are not costained by anti-active caspase 3 antibody and can resume growth and reestablish phospholipid asymmetry, which enable them to evade immune surveillance.^{11,17,18} Examination of large panels of tumor types has found that PS exposure on luminal surface of tumor vasculature is universal despite the extent of exposure that varies between tumors, ranging from about 15% to 50%.^{9,19,20} In response to cancer treatment such as radiotherapy^{18,21} and/or chemotherapy,²² significantly increased PS exposes on the tumor vascular ECs.

Phosphatidylserine-Targeting Antibody and Its Mode of Action

Phosphatidylserine-targeting antibodies have been developed by the Thorpe laboratory, including murine antibodies 2aG4 and 3G4 and a chimeric monoclonal antibody

bavituximab.^{10,20-23} The antibodies recognize PS complexed with the PS-binding protein, β 2-glycoprotein 1 (β 2GPI).²⁴ The β 2GPI is a 50-kDa glycoprotein that binds weakly to anionic phospholipids under physiological conditions. With the PS antibodies, the binding of β 2GPI to exposed PS is enhanced to form a stable multivalent complex of antibody β 2GPI-PS.²⁵ Since there are abundant β 2GPI in blood, it is unnecessary for in vivo study to have exogenous β 2GPI. The PS antibodies are observed to localize to PS-positive blood vessels in multiple tumor models after systemic infusion. Further studies have shown that the PS-bound antibodies induce monocytes to bind to the tumor vasculature and destroy it by antibody-dependent cellular cytotoxicity, leading to tumor growth inhibition.^{21,22} Antitumor effects of these antibodies are enhanced by chemotherapy,²² radiation,^{18,21} and small molecule tyrosine kinase inhibitor,¹⁹ all of which increase the levels of exposed PS in the tumors and thus amplify the target for attack by the antibodies. More recent studies have suggested that exposed PS suppresses host immunity against tumor cells and PS-targeting antibodies enhance antitumor effect with immune checkpoint cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or Programmed cell death protein 1 (PD-1) blockade by inducing more CD8^+ T cells and fewer immune suppressive myeloid-derived suppressor cells and M2 macrophages.²⁶⁻²⁹ Bavituximab, the chimeric monoclonal PS-targeting antibody (blood half-life \sim 30 hours), is in advanced clinical trials in patients with lung and breast cancer.^{30,31} More recently, a new, fully human PS-targeting antibody, PGN635, is similar in affinity to bavituximab ($K_d \approx 10^{-10}$ M).³²⁻³⁴ In vitro binding assay has demonstrated its high specificity for the cell-exposing PS. The PS antibodies have a more restricted specificity for PS than does annexin V, known as a PS-binding ligand. Annexin V also recognizes Phosphatidylethanolamine (PE) in addition to PS and other anionic phospholipids.^{9,25} Moreover, annexin V has a blood half-life of 3 to 7 minutes, which may limit its use for clinical imaging and measuring peak probe uptake responses to therapy.³⁴ We have exploited $\text{F}(\text{ab}')_2$ fragment PGN635 (blood half-life \sim 16 hours) for development of PS-targeted imaging probes and nanoplatforms, which will be described in details in the following sections.

Phosphatidylserine-Targeted Molecular Cancer Imaging

In vivo molecular imaging enables visualization of cellular and molecular events in living organisms.^{35,36} Unlike the invasive

pathohistological examination, molecular imaging provides a noninvasive means to assess sensitivity and specificity of disease biomarkers. Various imaging modalities have been applied for evaluation of PS as a cancer biomarker.

Near-Infrared Optical Imaging

Optical imaging is increasingly being used in preclinical cancer research.^{37,38} It is being used in particular to study cancer-specific markers, drug pharmacokinetics, and to monitor drug effects in small animals.³⁹⁻⁴¹ The attraction of the technique is that it is inexpensive, simple to conduct, and gives real-time results. In the clinic, optical imaging by visualizing fluorescently labeled tumor cells has recently emerged as an attractive approach to facilitate intraoperative identification of tumor margins or sentinel lymph node metastases.^{42,43} We have thus previously labeled the F(ab')₂ fragment of PGN635 with a near-infrared (NIR) dye, IRDye 800CW to image U87 glioma xenografts in a mouse model.³² Near-infrared optical imaging revealed a clear tumor contrast as early as 4 hours post (intravenous) IV injection of PGN-800CW, which became maximal 24 hours later. Pretreatment of gliomas with a single dose of 6 Gy irradiation to induce increased PS exposure resulted in significantly enhanced tumor contrast. Localization of PGN-800CW to tumors was antigen specific, since an 800CW-labeled control probe of irrelevant specificity did not localize to the tumors. Similarly, Gong et al observed significantly higher uptake of PGN-800CW in docetaxel-treated than nontreated PC3 prostate tumors.³⁴ Compared to the visible fluorophores, NIR fluorescence penetrates more deeply into tissues, as evidenced in the above study of imaging orthotopic glioma in mice.³² Clinical applications of optical imaging are currently limited to the detection of tumor margins or deposits during surgery, to the detection of superficial tumors, and to the detection of deep-seated tumors by endoscopy.

Positron Emission Tomography and Single-Photon Emission Computed Tomography Imaging

Both positron emission tomography (PET) and single-photon emission computed tomography (SPECT) are nuclear medicine imaging techniques involving introduction of radioactive tracers into patients and detection of gamma rays emitted directly or indirectly from the tracer. Because of the superb sensitivity and clinical applicability of PET and SPECT imaging, development of radiotracers for cancer imaging has attracted intense interest. Technetium-99m (^{99m}Tc, $t_{1/2} \approx 6$ hours) has been used to label annexin V for SPECT in humans and has shown prognostic value for various cancers.^{44,45} However, to best match the biological half-life of PS-targeting antibody (bavituximab, ~ 30 hours), those radioisotopes with longer half-life of radioactive decay are preferable. Jennewein et al selected arsenic radioisotope, ⁷⁴As (β^+ , $t_{1/2} = 17.8$ days) in their study to radiolabel bavituximab for PET imaging of prostate tumors in rats.⁴⁶ N-succinimidyl S-acetylthioacetate-modified bavituximab was used to react with ⁷⁴As (Arsenic triiodide [AsI₃])

to achieve stable conjugates. The PET imaging data showed that the prostate tumor-to-liver ratio was 22 for bavituximab compared with 1.5 for an isotope-matched control antibody at 72 hours postinjection. To obtain shorter blood residence times than those required for ⁷⁴As-bavituximab imaging, Stafford and colleagues chose to use the F(ab')₂ fragment of PGN635 (blood half-life ≈ 16 hours) conjugated with iodine-124, ¹²⁴I ($t_{1/2} = 4.2$ days).³³ Forty-eight hours after injection, PET imaging detected ¹²⁴I-PGN635 F(ab')₂ uptake in the PC3 prostate tumors in mice that was significantly higher than that of the ¹²⁴I-labeled F(ab')₂ of a control antibody. An SPECT imaging radioisotope, indium-111 (¹¹¹In, $t_{1/2} = 2.8$ days), was also used to radiolabel the full-length bavituximab in a study of non-small cell lung cancer (NSCLC) xenografts.⁴⁷ Similar to the PET findings, the SPECT imaging detected a peak uptake (tumor to muscle ratio = 5.2) by the NSCLC tumors at 72 hours post IV injection of ¹¹¹In-DOTA-bavituximab.

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is a commonly used imaging tool for clinical disease diagnosis. Despite its inherently low sensitivity, MRI has high spatial resolution and excellent soft tissue contrast. In comparison to paramagnetic gadolinium-based T₁ contrast agents, superparamagnetic iron oxide nanoparticle (SPIO) has much higher molar relaxivity and is thus widely used for molecular MRI applications.⁴⁸⁻⁵⁰ We have previously conjugated polyethylene glycol PEGylated SPIO with PGN635 F(ab')₂ to evaluate the feasibility of MRI to study its tumor vascular-targeting specificity in 4T1 mouse mammary tumors.⁵¹ Consistent with other imaging modalities, MRI clearly visualized intratumoral signal loss due to specific binding of PGN635 F(ab')-SPIO to tumor vascular ECs. Importantly, MRI provided more detailed information about intratumoral distribution of the nanoprobe, which was determined to colocalize with histological staining of tumor vascular ECs. Unlike the T₁-positive contrast, SPIO, the T₂ contrast agent SPIO generates negative contrast on T₂- or T₂*-weighted images. It is noticeable that signal loss due to SPIO shortening of T₂ relaxation time is often difficult to differentiate from those low signals induced by B₀ inhomogeneity or susceptibility artifacts. To overcome it, we applied the "hot spot" analysis approach in our study, which was found useful to distinguish SPIO-induced signal voids from initial baseline level of dark signals (Figure 2).⁵¹

Development of PS-Targeted Lipid-Based Nanoplatfoms

Rationale for Developing PS-Targeted Liposomal Nanocarrier

Nanoparticles are emerging as promising carriers of drugs or imaging agents because of their advantageous properties such as large payload capacity, prolonged blood circulation time, and protection of the enclosed molecules from interacting with

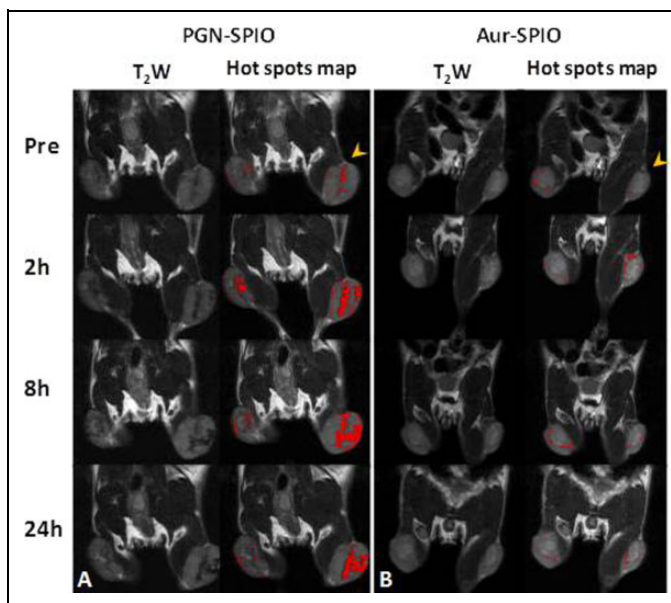


Figure 2. Quantitative “hot spot” analysis of heterogeneous intratumoral distribution of PGN635 F(ab')₂-SPIO. Hot spots maps were created by identifying hypointense regions in tumor on T₂-weighted images and then overlapping them on the corresponding T₂-weighted images. A, Baseline level of hot spots prior to injection of PGN-SPIO was presented in a representative nonirradiated and irradiated tumor (arrowhead). Increased hot spots were observed in both of the tumors after injection of PGN-SPIO. Compared to the nonirradiated tumor, the irradiated tumor appeared to have more hot spots. B, In contrast, there was essentially no change in hot spots before and after injection of the control antibody conjugates, Aur-SPIO in either a nonirradiated or irradiated tumor (arrowhead). Adapted from Zhou et al.⁵¹ SPIO indicates superparamagnetic iron oxide nanoparticle.

blood components.⁵²⁻⁵⁵ Among various types of nanoparticles, lipid-based liposomes and micelles are the most investigated for biomedical applications. Several Federal Drug Administration (FDA)-approved drug preparations utilizing liposomal delivery systems have shown promises in the treatment of various cancer types in clinic. Liposomes are composed of either natural or synthetic amphiphilic lipids. Amphiphilic or hydrophobic molecules can be solubilized in the bilayer, whereas the aqueous core can be loaded with water-soluble biomaterials. We have previously fabricated PS-targeted liposomes (PS-L) by conjugating PGN635 F(ab')₂ to distal termini of Polyethylene glycol (PEG) chains.^{56,57} To prove that the enhanced tumor-targeted imaging can be achieved by the liposomal delivery system, we encapsulated IRDye 800CW to the core of PS-L to conduct optical imaging of gliomas.⁵⁶ In comparison to the nonliposomal probe, PGN-800CW, the liposomal PS-L-800CW achieved >10-fold increase in tumor contrast by *in vivo* optical NIR imaging (tumor to normal ratio = 20).⁵⁶ Accompanied with increased tumor contrast, there was significantly less accumulated PS-L-800CW in the liver and spleen. The large payload of 800CW dye and increased binding affinity owing to multiple surface antibodies per liposome likely contribute to the enhancement.

Another reason behind developing lipid-based nanoparticles for PS-targeted nanodelivery systems is due to their ability to deliver the cargos into cytoplasm. As mentioned above, the mode of action of PS-targeting antibodies is that they bind to β 2GPI-PS to form complexes that remain on external cell membrane without entry into the cell, which is evidenced by visualizing the cell membrane-localized complexes by immunofluorescence microscopy. The cell membrane localization was also observed for the conjugates of PS antibodies with fluorescent dyes or metallic SPIO nanoparticles in our studies (Figure 3). Intriguingly, when PS-L loaded with the dye and/or SPIO were incubated with the PS-exposed cells, intracellular localization of the dye or SPIO was clearly seen (Figure 3). Although exact mechanisms accounting for this disparity are not fully clear, it is believed that the cell surface bound PS-L leads to close apposition and subsequent fusion or hemifusion between the lipid layers of liposome and cell membrane and then the release of the cargos into the cytosol. Indeed, others have reported similar findings of other types of lipid-based nanoparticles, targeting cell surface molecules.⁵⁸⁻⁶¹

PS-Targeted Liposomal Nanoprobes for Brain Tumor Imaging

The most common types of malignant brain tumors in adults are brain metastasis and primary glioblastoma multiforme (GBM), both of which are highly lethal, with a median survival of less than a year.^{62,63} Existence of blood–brain barrier (BBB) constitutes a critical challenge for accurate diagnosis and effective treatment.⁶⁴⁻⁶⁷ It is well recognized that disruption of the BBB occurs with tumor growth; however, the tumor BBB disruption is incomplete even at the late stage of these malignant brain tumors, which prevents sufficient therapeutic or diagnostic agents from entry in the tumor in brain parenchyma.^{68,69}

Much effort has been made to improve delivery of therapeutic or imaging agents to brain tumors by penetrating the BBB.⁷⁰⁻⁷³ Although various strategies have been explored to improve drug permeation into brain tumors via physical or chemical means to manipulate the tumor BBB, limited success has been achieved. Clearly, the discovery of a glioma-specific biomarker will be critical for development of a glioma-targeted nanodelivery system. Ideally, this biomarker needs to be accessible to its ligands or antibodies. Thus, vascular luminal surface-exposed molecules have attracted intense interests.⁷⁴ A number of endogenous transporters on the surface of the blood vessels are well known for their roles on receptor-mediated transport of large molecules across the BBB. This process is recognized as the receptor-mediated transport. Such receptors as transferrin receptor, low-density lipoprotein receptor, insulin-like growth factor receptor, and nicotinic acetylcholine receptor have been well investigated in functionalizing various nanocarriers, aiming to transport their cargos into brain tumor parenchyma.⁷⁵⁻⁷⁷ Tumor angiogenic factors such as vascular endothelial growth factor and its transmembrane receptor and various integrins have also been extensively explored for developing brain tumor-targeted

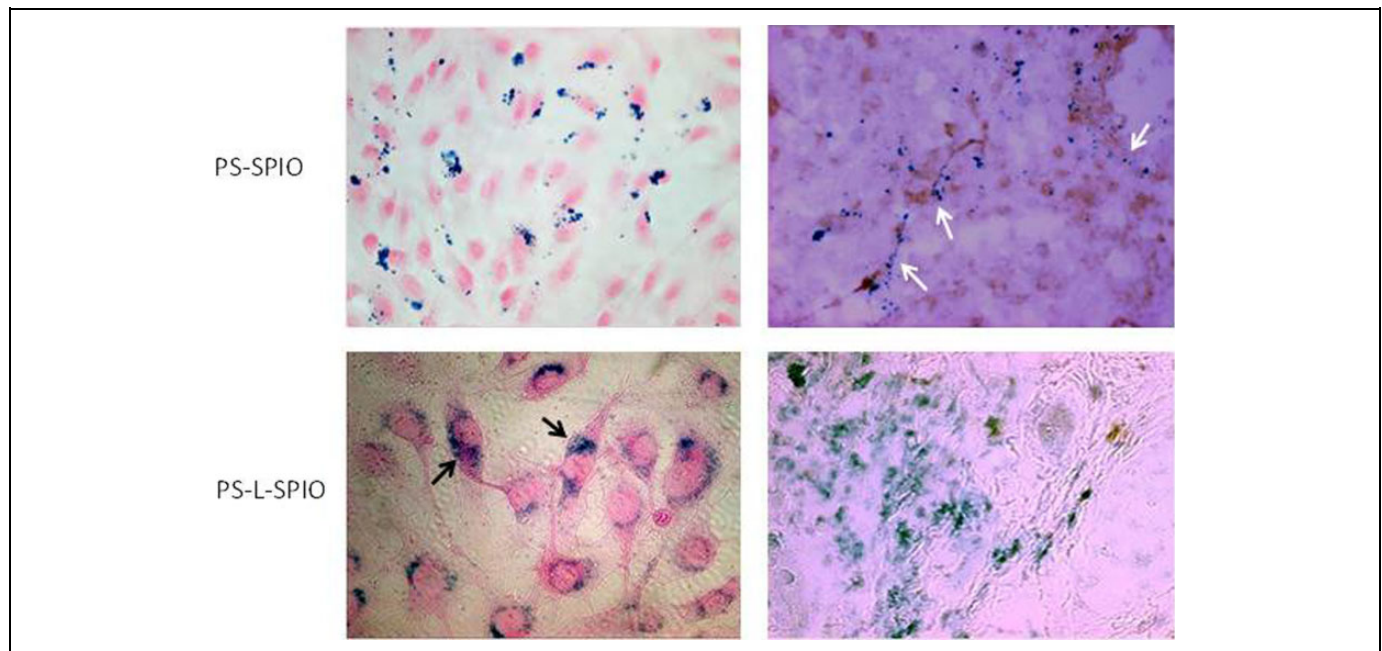


Figure 3. Differential behavior of PS-targeted SPIO and PS-targeted liposomal SPIO. Left: adult bovine aortic endothelial cells (ABAE) were pretreated with a single dose of 6 Gy radiation to induce PS exposure. The cells were incubated with conjugates of PGN635 F(ab')₂-SPIO (PS-SPIO) or PS-targeted liposomal SPIO (PS-L-SPIO) for 1 hour. Prussian blue staining detected PS-SPIO primarily localizing externally on the cell membrane (top), while abundant PS-L-SPIO were detected in the cytosol (bottom). Right: 24 hours after IV injection of PS-SPIO or PS-L-SPIO, double staining of iron (Prussian blue; blue) and vascular endothelial cells (anti-CD31; brown) in tumor tissue specimens revealed punctate iron of PS-SPIO colocalizing with tumor vessels (arrows; top); by contrast, clustering, widespread iron signals of PS-L-SPIO observed in a breast tumor (bottom). Adapted from Zhou et al⁵¹ and Zhang et al.⁵⁷ IV indicates intravenous; PS, phosphatidylserine; PS-L, PS-targeted liposomes; SPIO, superparamagnetic iron oxide nanoparticle.

nanoplatfroms. In particular, a large number of nanocarriers utilizing antibodies or cyclic Arg-Gly-Asp peptides targeting $\alpha_v\beta_3$ integrin have been convincingly shown to deliver therapeutic or imaging agents to brain tumor tissues.^{78,79} The abovementioned receptors are constitutively expressed on the BBB of normal brain; however, their overexpression on the proliferating tumor ECs could lead to preferential delivery to the brain tumor.

Our published and unpublished studies have investigated PS exposure on tumor vascular ECs of various brain tumor xenografts of the established brain tumor cell lines or patient-derived xenografts (PDX) cells.^{56,80} Unlike the tumor angiogenic markers such as $\alpha_v\beta_3$ integrin, immunohistological examinations have shown that extensive PS exposure is not only present in the angiogenic tumor vessels but also in those of the infiltrative tumors, but not in the normal brain.^{21,32} This is important for developing brain tumor-targeted therapeutics because the infiltrative tumor cells that often co-opt with non-disruptive, preexisting brain blood vessels account ultimately for cancer recurrence. Thus, PS exposure on the luminal surface of tumor, but not normal blood vessels in the brain, establishes itself a highly specific biomarker for brain tumors. We thus hypothesize that the systemically administered PS-L binds specifically to tumor vascular ECs, becomes subsequently internalized into the cells, and then enables its cargos to be efficiently delivered to brain tumor parenchyma by penetrating the BBB. To test this, we exploited the PS-L loaded with dual imaging

contrast agents: SPIO in the core and NIR dye in the lipid layers for multimodal imaging of human U87 gliomas growing orthotopically in mice.⁸¹ Both in vivo optical imaging and MRI depicted clear tumor contrast, distinct from the surrounding normal brain. Intriguingly, longitudinal MRI revealed temporal and spatial intratumoral distribution of the PS-L by following MRI contrast changes, which appeared punctate in tumor periphery at an earlier time point (4 hours) and became clustering and disseminated throughout the tumor at 24 hours postinjection (Figure 4). The noticeable pattern of punctate MRI signal changes at 4 hours post IV injection may result from the vascular phase of PS-L-IO/DiR when significant numbers of the circulating PS-L-IO/DiR bound to the PS exposed on tumor vascular ECs but not yet penetrated the vessels. The timing of the vascular phase actually coincided well with our previous studies of using PGN635 to localize to exposed PS in tumors, in which our histopathological analysis determined massive PGN635 binding to tumor vascular ECs at 4 hours. Following the vascular phase, the tissue phase was occurring over time after subsequent internalization by ECs and then followed by extravasation through the tumor BBB and extravascular tissue distribution of the PS-L-IO/DiR, which was reflected as the widespread clustering dark signals in both tumor center and periphery on T₂-weighted MRI at 24 hours. It is well recognized that the physical features of nanocarriers have significant effects on their intratumoral diffusibility. Nance et al have recently shown that with appropriate PEG coating and a neutral surface charge, the actual size of

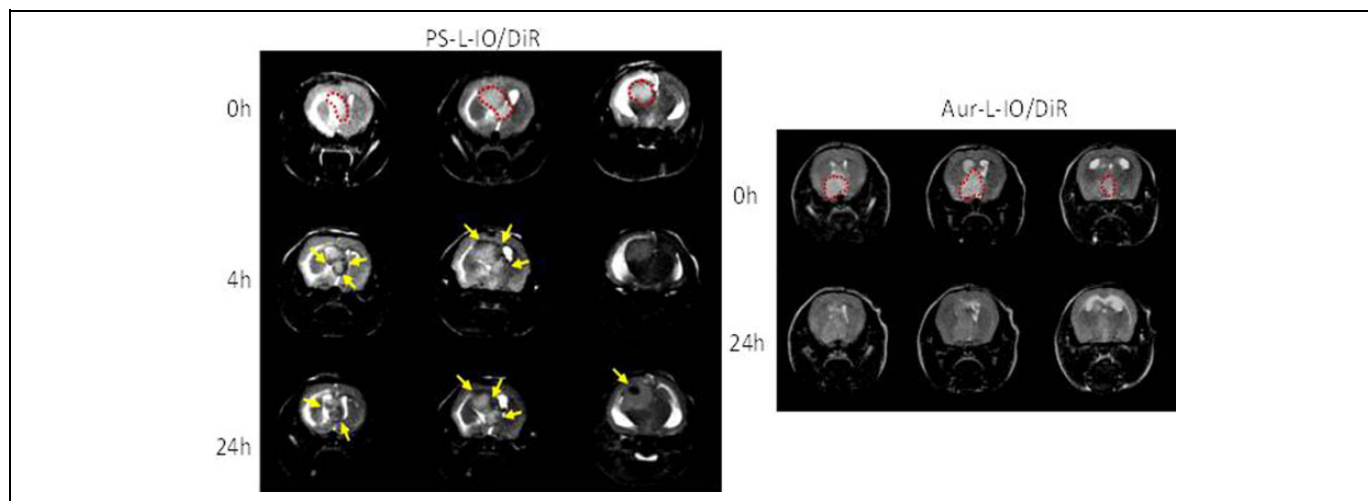


Figure 4. Longitudinal MRI of intratumoral biodistribution of the PS-L nanoprobe in orthotopic U87 glioma. Left: T₂-weighted MR images were acquired before and at different time points postinjection of PS-L-IO/DiR (2.5 mg Fe/kg) via a tail vein of a mouse bearing orthotopic U87 glioma. The hyperintense tumor was depicted (outlined) on 3 consecutive sections before injection. At 4 hours postinjection, signal voids (arrows) started to be seen primarily in tumor periphery (arrows), which became more apparent in both the peripheral and central tumor (arrows) at 24 hours. Right: the targeting specificity of PS-L-IO/DiR was also confirmed with a control nonirrelevant antibody-labeled nanoprobe, Aur-L-IO/DiR, showing no obvious signal change over time. Adapted from Zhang et al.⁸¹ MRI indicates magnetic resonance imaging; PS, phosphatidylserine; PS-L, PS-targeted liposomes.

nanoparticles can go up to 114 nm without significantly affecting their movement within brain tissues.⁸² In our study, the PEGylated PS-L-IO/DiR had a mean size of 110 nm and a surface charge near to 0, which may also contribute to the wide distribution of the PS-L-IO/DiR in glioma.

Phosphatidylserine-Targeted Nanotheranostics for Image-Guided Drug Delivery

It is well recognized that effectiveness of a nanodrug is often governed by the kinetics of drug release and distribution after systemic administration.^{83,84} There is an increasing interest in developing nanotheranostics, an integrated system of imaging agents with anticancer drugs.⁸⁵⁻⁹² Ideally, the nanotheranostic system enables the noninvasive *in vivo* imaging to monitor the nanodrug delivery to not only their targets but also off-target sites. In particular, some potent chemotherapeutic agents are known for their severe toxicity to normal tissues as well. Arsenic trioxide (ATO) is such an exemplary anticancer drug. Arsenic trioxide is approved by the FDA for the treatment of acute promyelocytic leukemia (PML). Arsenic trioxide has also demonstrated significant activity in treating solid tumors in preclinical studies. Several recent studies have shown that ATO is able to reverse glioblastoma resistance to mechanistic target of rapamycin (mTOR)-targeted therapies by inhibiting PML protein signaling⁹³ and deplete the cancer stem-like cell population by inhibiting Hedgehog and Notch pathways.⁹⁴⁻⁹⁶ However, clinical use of ATO on solid tumors has generally been limited by its systemic cytotoxicity.

Nanoencapsulated ATO within a cancer-targeted nanodelivery system may have a potential to alleviate its damage to

healthy tissues. Swindell et al. and Chen et al. have recently shown a successful strategy to utilize transition metals such as nickel or copper to actively load ATO into liposome. Nickel or copper and ATO form a complex in the core of liposome, preventing the leakage of ATO from liposomes.^{97,98} The stability of the complex depends on pH: It is stable at a neutral pH, while it releases the therapeutic As³⁺ at a low pH, that is, pH < 6.⁹⁸ Utilizing the transition metal approach, we have recently developed a novel nanohybrid of ATO complex with manganese.⁸⁰ We chose to use manganese (Mn²⁺) to entrap ATO into liposome because Mn provides paramagnetic MRI contrast. With 5 unpaired electrons, Mn²⁺ is among the best T₁ contrast agents.^{85,99-101} However, the formation of As-Mn precipitates in the core of liposomes possesses magnetic susceptibility effects, resulting in a dark MRI signal. However, after the cell uptake and exposure to the low pH in the endosome-lysosome system, the As-Mn complex decomposes to release ionic As³⁺, the active form of ATO and Mn²⁺, which gives a bright signal on T₁-weighted images. Thus, the convertible MRI contrast of Mn can serve as a surrogate of delivery and release of free ATO from its inactive nanoformulation. As for other dual functional nanoformulations, of which imaging probes and drugs are often loaded separately into a different compartment of a nanocarrier, that is, core and shell or surface conjugation via a linker, inconsistent release or leakage of the imaging agents with the drugs could be encountered after administration, which will hinder the imaging-based accurate monitoring of drug distribution. Built into the previously established PS-targeted nanoplatform, the nanoencapsulated ATO enables GBM-targeted delivery while minimizing off-target effects. Our *in vitro* studies have demonstrated that PS-L-As-Mn effectively kills temozolomide-resistant GBM PDX cells.⁸⁰

Extensive studies are needed to investigate its *in vivo* specific binding, pH-respondered release kinetics, and ultimately the ability of PS-L-As-Mn to serve as a useful nanotheranostic agent for image-guided delivery of ATO in the GBM PDX models.

Conclusions

In summary, we have demonstrated the utility of PS-targeted liposomal nanoplatform for molecular cancer imaging, in particular, brain tumors imaging via its ability to penetrate the tumor BBB. By encapsulating extremely toxic chemotherapeutic agents, that is, ATO, the PS-targeted delivery of ATO specifically to brain tumors may be useful to treat those malignant brain tumors resistant to the current standard of care, while minimizing its systemic side effects. Furthermore, development of PS-targeted nanotheranostics by incorporating imaging contrast agents with anticancer drugs into the same nanostructure may enable image-guided drug delivery to treat brain tumors. Because PS is the same molecule and has the same distribution and regulation in all mammalian species, it is likely that the mouse data will extrapolate to humans. Along with its favorable safety profile, the PS-targeted lipid-based nanoplatform may be expedited for clinical evaluation of its applicability as a nanocarrier of diagnostic and therapeutic agents for patients with brain tumor.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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