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A comprehensive lipid binding and activity validation of a cancer-specific peptide-peptoid hybrid PPS1

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Abstract

We recently identified a peptide-peptoid hybrid, PPS1, which recognizes lipids that have an overall negative charge, such as phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidic acid (PA), and phosphatidylinositol (PI), but that does not bind to neutral lipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and sphingomyelin (SM). The simple dimeric version of PPS1, PPS1D1, displayed strong cytotoxicity to cancer cells over normal cells *in vitro* and tumor burden *in vivo*. In this study, we comprehensively characterized the direct binding and activity of PPS1 on PS, PG, and PA using liposome-based assays and lung cancer cell lines that express these negatively charged lipids. First, the fluorescence polarization (FP) binding studies of fluoresceinated-PPS1 (PPS1-FITC) to PS-, PG-, and PA-containing PC-liposomes showed that the binding of PPS1 to PC-liposomes increased as concentrations of these lipids increased. In terms of activity, PPS1D1 induced the release of calcein from large, unilamellar PC-liposomes containing 15–30% PS, PG, and PA. PPS1D1 had no activity when the liposomes were composed of 100% PC. This effect was higher at 30% lipids than 15%, and the EC₅₀ for PG and PA were higher than that of PS, indicating that PPS1D1 is more specific towards PS. PPS1D1 binds to and induces significant cytotoxicity in lung cancer cell lines H1693, HCC95, and H1395, which express negatively charged lipids, but had no effect on normal HBEC30KT cells, which has mostly PC in the outer layer. In addition, a series of previously developed PPS1D1 derivatives, which retain or lose activity, were tested with these liposome-based assays, and the data were equivalent to previous observations. This study provides comprehensive binding and activity validations of a unique peptide-peptoid hybrid, PPS1, on negatively charged lipids PS, PA, and PG that are elevated on cancer cell surfaces relative to normal human cell surfaces.

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Keywords

Peptoids; phosphatidylserine; phosphatidylglycerol; phosphatidic acid; liposomes; anti-cancer agents

Introduction

The eukaryotic cell membrane is a bilayer and is predominantly composed of neutral and anionic lipids [1, 2]. In human cells, the outer leaflet of the cell membrane is predominantly composed of neutral lipids and the inner leaflet is composed of anionic lipids. Lipids such as phosphatidylcholine (PC), sphingomyelin (SM), and phosphatidylethanolamine (PE) are examples of neutral lipids, and phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidic acid (PA) carry overall negative charges [3–5]. These lipids can be present in various states or phases. The lamellar phase is mostly present in biomembranes, whereas non-bilayer lipid phases are present in transient events, such as fusion, fission, or pore formation in biomembranes [5]. These non-bilayer lipid phases are hexagonal and cubic phases [5, 6]. The distinct composition of these lipids undergoes drastic transformations during various cellular conditions and processes [7, 8].

The charge of the cell membrane plays an important role in cell-cell interactions and many other biological events. While neutral lipids are present on normal, healthy eukaryotic cells, the flipping of negatively charged lipids onto the outer layer occurs during cell death [9, 10]. This signals the beginning of apoptosis and marks cells for engulfment by macrophages [11, 12]. The striking feature of the outer cell membranes of cancer cells and tumor endothelial cells is that they are heavily composed of anionic lipids, most prominently PS, followed by PA and PG [1, 2]. The presence of anionic lipids and other anionic moieties, such as sialic acid and heparan sulfate, provides additional negative charges to these tumorigenic cell membranes [13, 14]. These negative charges that are found on cancer cell surfaces have been shown to help cancer cells escape the immune system and proliferate undetected [1, 3, 15]. The universal presence of these anionic moieties on cells within the tumor microenvironment provides a unique opportunity to develop targeted agents that can act as universal cancer targeting agents. This is an extremely important aspect in oncology, as targets for conventional drug development are exclusively proteins, such as enzymes, hormones, receptors, and signaling kinases, and those proteins are heterogeneously expressed. Due to this extreme heterogeneity, those targeted drugs are effective only with limited cancer sub-groups or even at individual levels.

In our previous study, we unbiasedly identified a peptide-peptoid hybrid called PPS1 (Fig. 1A) that bound to HCC4017 lung cancer cells and that did not bind to HBEC30KT normal bronchial epithelial cells from the same patient, utilizing our unique on-bead two-color combinatorial cell screen [16]. In that study, a 400,000-compound library was given an unbiased chance to recognize any type of a biomolecule present on the cancer cell surface that is not found on normal cells, and PPS1 was identified as one of three compounds that displayed this characteristic. Through a series of ELISA-like, on-bead, lipid dot blots and flow cytometric binding assays, we identified and confirmed PS as the target of PPS1 [17].

In addition, our preliminary data indicated that PPS1 binds to other anionic lipids, such as PA, PG, and PI, but not to neutral lipids, such as PC, SM, and PE [17]. The simple dimeric version of PPS1, PPS1D1 (Fig. 1B), was cytotoxic to a series of lung cancer cells that were tested, including HCC4017 and H460, with *in vitro* IC₅₀'s of 5–7 μ M. PPS1D1 displayed significant tumor burden in mouse models as a single agent and in combination with the chemo-compound docetaxel [16, 17]. We later found the minimum pharmacophore of PPS1D1, which indicated that two positively charged groups at the C-terminus and four hydrophobic residues towards the N-terminus are critical for binding and activity [18].

In this study, we comprehensively characterized the binding of PPS1 as well as the activity of PPS1D1 and many other derivatives directly on PC-liposomes that display various levels of the anionic phospholipids PS, PG, and PA. These studies were then extended to cellular level by targeting three lung cancer cell lines that are known to express more anionic phospholipids over the normal bronchial epithelial cells that lack (or have minimum levels) of those anionic phospholipids.

Materials & Methods

Synthesis of PPS1

NovaSyn TGR resin (150 mg; EMD Millipore, MA) was swelled in dimethylformamide (DMF, Acros Organics, NJ) for 30 min at room temperature in 5 ml reaction vessels (Intavis AG, Germany). The reaction vessels were drained and treated with 2 M Fmoc-Met-OH amino acid (with coupling agents 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), Hydroxybenzotriazole (HOBt) and N,N-diisopropylethylamine (DIPEA) in anhydrous N,N-Dimethylformamide (DMF)). All reagents were purchased from Sigma-Aldrich, MO. The reaction vessels were placed on a shaker overnight, drained, and washed 10 times with 5 ml DMF. The Fmoc groups were removed by twice treating the beads with 20% piperidine (Sigma-Aldrich, MO) for 10 min on the shaker. After washing the reaction vessel, subsequent amino acids, Fmoc-D-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH, were added (for a 2 h reaction time), each time removing the Fmoc group as described above. The 5-mer peptoid region was synthesized using the microwave-assisted peptoid synthesis protocol. Reaction vessels were treated with 1 ml of 2 M bromoacetic acid in anhydrous DMF and 1 ml of 2 M N,N'-Diisopropylcarbodiimide (DIC) in anhydrous DMF, gently shaken for 30 s, and microwaved (1000 W) for 15 s with the power set at 10%. The beads were shaken again for about 15 s and microwaved again as described above. The reaction vessels were drained and washed 10 times with 2 ml DMF. Then the reaction vessel was treated with 2 ml of a 1 M solution of the primary amine and microwaved two times for 15 s after gentle shaking. The primary amines used were Boc-diaminobutane, 4-methoxybenzylamine, (R)-methylbenzylamine, piperonylamine and (R)-methylbenzylamine. At the end of synthesis, the beads were washed 10 times with 2 ml dichloromethane (DCM), the compound was cleaved with 2.5 ml of cleavage cocktail containing 95% trifluoroacetic acid (TFA), 2.5% water, and 2.5% triisopropylsilane (TIS) on the shaker for 2 h, and the compound was purified using High Performance Liquid Chromatography (HPLC). Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

Synthesis of PPS1D1

PPS1D1 was synthesized on NovaSyn TGR resin (EMD Millipore, MA). First, Fmoc-Lys(Fmoc)-OH was coupled overnight as the central linker, and both Fmoc groups were removed simultaneously, allowing two copies of the sequence to be built on two amine groups of this central Lys. Three amino acids, Fmoc-Met-OH, Fmoc-D-Lys(Boc)-OH and Fmoc-Lys(Boc)-OH, were loaded onto the resin after Fmoc removal each time. The 5-mer peptoid region containing Boc-diaminobutane, 4-methoxybenzylamine, (R)-methylbenzylamine, piperonylamine and (R)-methylbenzylamine was synthesized using the microwave-assisted peptoid synthesis protocol. At the end of synthesis, the beads were washed 10 times with 2 ml DCM, the compound was cleaved with 2.5 ml of cleavage cocktail containing 95% TFA, 2.5% water, and 2.5% TIS on the shaker for 2 h, and the compound was purified using HPLC. Synthesis was confirmed using MALDI-TOF MS (Voyager DePro, AB Systems, MA).

Fluorescence polarization (FP)

Fluorescein-labeled PPS1 was prepared by modifying purified, cysteine-tagged PPS1 with fluorescein isothiocyanate (FITC), as per the manufacturer's protocol (ThermoFisher, MA). An FP assay was performed on the Spectramax i3 (Molecular probes, CA). Excitation and emission wavelengths of 485 and 535 nm, respectively, were used. Experiments were carried out in black non-binding 96-well plates. FP was determined by measuring parallel ($F_{\parallel}$) and perpendicular fluorescence ($F_{\perp}$) intensity with reference to the linearly polarized light. The following equation was used to calculate FP:

$$FP = \frac{F_{\parallel} - F_{\perp}}{F_{\parallel} + F_{\perp}}$$

Liposome preparation

1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1, 2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), 1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (DOPG) and 1,2-dioleoyl-sn-glycero-3-phosphate (DOPA) were purchased from Avanti Polar Lipids (Alabaster, AL). For anionic, lipid-containing liposomes, 40, 35, 30, 25, 20, 15, 10, and 5 mol% DOPS, DOPG, or DOPA was used, along with POPC. For PC-containing liposomes, POPC alone was used. Dried lipids were re-suspended in Tris buffer solution (10 mM, pH 7.4) to 1 mg/ml. This stock was fully mixed and exposed to more than five freeze-thaw cycles. A mini-extruder from Avanti Polar Lipids and Whatman Nuclepore Track-Etch 1.0 μ m filters from GE Healthcare were used to produce the liposomes.

Calcein leakage assay

For making calcein-encapsulated, unilamellar liposomes, a lipid film containing POPC/DOPS (85:15 or 70:30) was rehydrated with Tris buffer (10 mM, pH 7.4) containing 70 mM calcein. The liposome suspension was freeze-thawed for ten cycles and extruded ten times through polycarbonate filter (100-nm pore size). The free calcein was removed by dialyzing calcein-containing liposomes through a Slide-A-Lyzer 10K dialysis cassette (10,000 MWCO, 0.1–0.5 mL, ThermoFisher, MA).

To measure calcein leakage from the liposomes, a method in the Spectramax i3 (Molecular probes, CA) program was used with an excitation setting of 490 nm and an emission setting of 520 nm. Calcein leakage due to peptoid addition was monitored for 60 min. Maximal vesicle leakage was then measured by addition of the detergent Triton X-100 from a 10% stock solution to achieve a final concentration of 0.1% v/v concentration and by monitoring for a further 1.5 min period on the program.

Calculation of calcein leakage, % leakage, was done as follows:

$$\% \text{ leakage} = 100 \times (I - I_0) / (I_t - I_0)$$

In this equation, I is the fluorescence intensity 60 min after peptoid addition.

I_0 is the intensity of intact vesicle baseline acquisition, and is measured after the addition of Triton X-100.

Magnetic bead binding assay

In each of two separate vials, nearly 9×10^6 magnetic Dynabeads M-280 Streptavidin (Invitrogen) were re-suspended in PBS with 0.1% BSA, and biotinylated PPS1 and control PC462 were added to each vial and incubated for 30 minutes at RT. The beads were washed 3 times, and 1 million each of H1693, HCC95, H1395 and HBEC30KT cells were added to each vial and incubated for 30 minutes at RT with gentle shaking. The bead-bound cells were isolated by placing the vial on the magnet; and, after removing supernatant, cells were counted with a hemocytometer.

Cell viability assay

H1693, HCC95, H1395, and HBEC30KT cells were grown in clear bottom 96-well plates. On the second day, H1693, HCC95, H1395 cells were treated with PPS1D1 and control PC462D1 in RPMI medium with 5% FBS containing 3% BSA. For HBEC30KT cells, Keratinocyte-SFM with 3% BSA media was used. On day 4, the treatment was repeated. On day 5, 20 μ l of CellTiter 96[®] Aqueous One Solution (Promega, WI) were added to each well, and absorbance was measured at 490 nm.

Results

Direct binding analysis of fluoresceinated-PPS1 (FITC-PPS1) to PS-, PG-, and PA-containing PC-liposomes by FP

First, we wanted to confirm the binding of PPS1 to PS using the liposome platform. A constant amount of 50 nM FITC-PPS1 (Supplementary Figure S1) was treated with PC-liposomes that displayed increasing concentrations of PS up to 40% using FP. The 0% PS condition represented 100% PC-liposomes. As shown in Fig. 1C, binding of FITC-PPS1 with PS was enhanced with increasing concentration of PS present on the liposomes. Next, the same assay was used to test FITC-PPS1 binding to PC-liposomes containing PG and PA, as described above. FITC-PPS1 binding to PG and PA was also enhanced when the PG and PA concentrations were increased on the PC-liposomes (Fig. 1D & E). Overall, these results

indicate that FITC-PPS1 has no binding to PC, but binding increases with higher concentration of PS, PG, and PA present on the PC-liposomes. In addition, the increase in FP was higher for PS than PG and PA, indicating stronger binding of PPS1 to PS than PG and PA.

PPS1D1 causes calcein leakage on PS-, PG- and PA-containing PC-liposomes

Next, we wanted to identify whether PPS1D1 (Supplementary Figure S2) causes any changes to the membrane bilayer. The membrane perturbation effect of PPS1D1 was investigated with calcein leakage experiments. Calcein leakage is a standard method for investigating membrane perturbations. Calcein is a water soluble dye that is impermeable to lipid bilayers [19]. It self-quenches at concentrations of 60–100 μ M and is pH sensitive [20]. For membrane permeabilization experiments, self-quenched calcein is encapsulated in lipid vesicles. If calcein leaks out, it is no-longer quenched by other calcein molecules and its emission can be measured at 515 nm [20]. As PPS1D1 displayed cell cytotoxicity [16,17], and we confirmed its direct binding to PS-, PG-, and PA-containing PC-liposomes in this study, we hypothesized that membrane perturbation by PPS1D1 may be a part of this activity. Therefore, we used calcein-loaded PC-liposomes composed of 15 mol% PS (DOPS), PG (DOPG), and PA (DOPA). 100 μ M of these calcein-containing liposomes were treated with 10 nM to 5 μ M PPS1D1. We observed an increase in calcein leakage with increasing concentration of PPS1D1 across all three anionic phospholipids (Fig. 2A). The non-binding control compound, PC462D1 (Supplementary Figure S3) had no effect on any of these liposomes, confirming that the observed calcein leakages were due to PPS1D1 specific binding. Calcein leakage occurred at a much lower concentration of PPS1D1 with PS-containing PC-liposomes compared to PG- and PA-containing PC-liposomes. The effective concentration for 50% leakage (EC_{50}) for PS-containing liposomes was 225 nM compared to 363 nM for PG- and 915 nM for PA-containing PC-liposomes. The maximum calcein leakage at the highest concentration (5 μ M) was approximately 80% for PS-containing PC-liposomes, whereas calcein leakage was approximately 50% and 38% for PG- and PA-containing PC-liposomes (Fig. 1B). These results suggest that PPS1D1 disrupts the lipid bilayer of PC-liposomes composed of 15% PS, PA, and PG. The perturbation of PS-containing bilayers is stronger and occurred at a lower concentration of PPS1D1 compared to PA- and PG-containing bilayers.

Leakage of calcein at different lipid concentrations

We previously found that PPS1D1 has higher binding to PS compared to PG and PA using ELISA-like and lipid-blot assays [17], and above observations further confirm it. Here, we wanted to test if the calcein leakage observed with PS, PG, and PA was proportional to lipid concentrations on the PC-liposomes. Liposomes composed of only PC (POPC), 15 mol% and 30 mol% of PS (DOPS), PG (DOPG), and PA (DOPA) were studied in this experiment. Upon PPS1D1 treatment, we observed higher leakage with 30 mol% anionic phospholipids than with 15 mol% anionic phospholipids (Fig. 2C–D). The calcein leakage with both 15 and 30 mol% was higher in PS-containing PC-liposomes than in PG- and PA-containing PC-liposomes. We observed only a small difference in calcein leakage between 15 to 30 mol% for PS-containing PC-liposomes, as calcein leakage happened at very low concentrations of PPS1D1 and already showing stronger effect (Fig. 2C). However, the increase in calcein

leakage with 30 mol% PG-and PA-containing PC-liposomes over 15 mol% PG- and PA-containing PC-liposomes was significant (Fig. 2D and E), and calcein leakage happened at higher concentrations of PPS1D1 than those used for PS-containing PC-liposomes (Fig. 2D and E). The maximum leakage was approximately 80% for 30 mol% PG-containing PC-liposomes compared to approximately 50% for 15 mol% PG (Fig. 2D). Similarly, the maximum leakage for PA-containing PC-liposomes was approximately 62% for 30 mol% PA compared to approximately 38% for 15 mol% PA (Fig. 2E). These observations suggest that lipid bilayer perturbation by PPS1D1 is proportional to the amount of anionic lipid present in the bilayer.

PPS1 preferably binds to lung cancer cells expressing negatively charged phospholipids

In our previous study, we showed that H1693, HCC95, and H1395 lung cancer cells predominantly express negatively charged lipids, such as PS, and HBEC30KT normal bronchial epithelial cells do not [17]. We applied our previously published magnetic bead pull-down assay [16] on these four cell groups to further validate the capability of PPS1 in recognizing these negatively charged lipids on human cancer cells. Here, PPS1 and non-binding control compound PC462 were synthesized with a biotin tag at the C-terminus (Supplementary Figure S4). Biotin-PPS1 and biotin-PC462 were linked to streptavidin-magnetic beads and then equilibrated with 1 million cells each of H1693, HCC95, H1395 lung cancer, and normal HBEC30KT cells. The magnetic bead-bound cells were precipitated with a magnet and counted. PPS1-coated magnetic beads readily pulled down H1693, HCC95, and H1395 lung cancer cells but not normal HBEC30KT cells. PC462-coated magnetic beads were unable to pull down H1693 cells. This data indicates that PPS1 preferentially recognizes cell surfaces with predominantly negatively charged phospholipids.

PPSD1 induces cytotoxicity on lung cancer cells expressing negatively charged phospholipids

We also performed standard MTS cell cytotoxicity assays on the H1693, HCC95, and H1395 lung cancer and HBEC30KT normal bronchial epithelial cells. Upon treatment with PPS1D1, significant cytotoxicity was observed with H1693, HCC95 and H1395 cells with IC₅₀ values of 7, 15, and 13 μ M, respectively. The control compound PC462D1 had no effect on the H1693 cells, while PPS1D1 did not induce cytotoxicity on normal HBEC30KT cells, as previously observed [16, 17]. This data suggests that PPS1D1 preferably induces cytotoxicity on cell surfaces with predominantly negatively charged phospholipids, while it has no effect on cell surfaces lacking these negatively charged phospholipids.

Modifications to the PPS1D1 sequence impaired leakage

We have previously identified the minimum pharmacophore of PPS1D1 through sarcosine scans [18]. The two C-terminal, positively charged and four N-terminal, hydrophobic residues were essential for binding and activity, while the positively charged residue (Nlys) at the 4th position and the C-terminal Met seemed to not be important. Therefore, we synthesized three PPS1D1 derivatives by replacing these positions with sarcosine: (1) C-terminal Met (PPS1D1-1) (Supplementary Figure S5), (2) positively charged Nlys at the 4th position (PPS1D1-4) (Supplementary Figure S6), and (3) hydrophobic residues at the 6th position (PPS1D1-6) (Supplementary Figure S7). We evaluated the effects of these sarcosine

mutants on PS-containing PC-liposomes loaded with calcein. As shown in Fig. 4A, PPS1D1-1 and PPS1D1-4 displayed calcein leakage similar to PPS1D1, indicating both these residues are not that important as previously found. In a more rigorous analysis, calcein leakage by PPS1D1-4, showed a moderate reduction in leakage as compared to PPS1D1-1 and the parent PPS1D1 at lower concentrations (below 1 μ M), but all three were similar. Calcein leakage by PPS1D1-6, the sarcosine mutant at the important hydrophobic residue at the 6th position, showed a significant reduction in calcein leakage (Fig. 4A). The control PC462D1 did not affect calcein leakage, as consistently observed. We also previously observed that truncations in hydrophobic residues at the N-terminus of PPS1D1 impaired binding and cytotoxicity towards the lung cancer cell line HCC4017 [18]. Here, we tested those truncated derivatives in our calcein leakage assay (Fig. 4C). The 7-mer derivative (Supplementary Figure S8), which lacked a hydrophobic residue at the 8th position, showed a moderate reduction in calcein leakage. Derivatives with two or more residues truncated from the N-terminus (6-mer, 5-mer & 4-mer) (Supplementary Figures S9, 10 & 11) failed to cause significant leakage and were comparable to the control compound PC462D1. These results further confirm that the 8-mer sequence with all four N-terminus hydrophobic residues is needed for PPS1D1 activity.

Discussion

In this study, we characterized the interaction of PPS1 and the activity of PPS1D1 with anionic phospholipids using liposome and cellular platforms. To the best of our knowledge, PPS1D1 is the first peptide-peptoid hybrid compound reported to bind to anionic phospholipids, such as PS, PG, and PA, inducing cytotoxicity towards cancer cells over normal cells. The use of a liposome platform enabled us to validate the PPS1 binding and PPS1D1 activity on each of the phospholipids, PS, PG, and PA directly, while it is extremely hard to do so on actual cells, as we cannot find or even create a cell line which only expressing these individual lipids one at a time. Furthermore, we were able to control the percentage, or the concentration, of these individual lipids on PC-liposomes; and observations, such as an increase of PPS1 binding with increment concentrations of PS, PG, and PA, indicated specific recognition of PPS1 by these phospholipids. We found that PPS1D1 had a greater and rapid membrane disruption effect when targeting PS- than PA- and PG-containing bilayers. When compared the PPS1D1 activity on PC-liposomes with constant percentage of PS, PG and PA lipids (e.g. 15%, Fig. 2A), the maximum effect for PS (~75%) was much higher than that of PG (40%) and PA (30%). Our calcein release data also suggests that lipid bilayer perturbation by PPS1D1 is also proportional to the amount of anionic lipid present in the bilayer. Even though we previously published that PPS1D1 treatment induced cytotoxicity [17], the current study revealed the direct connection between PPS1D1 and targeted PS, PG, and PA on a membrane environment. Typically, the release of dyes, such as calcein, indicates pore formation on liposomes or direct disintegration of liposomes. These observations provided us with some indication of how PPS1D1 may induce activity on cancer cells, but more studies are needed to narrow down the exact mechanism. Since conventional protein drug targets are highly variable, even between cells within a single tumor, the hope of developing universal cancer drugs is currently not practical. However, these anionic phospholipids are consistently found on malignant cell

surfaces as compared to normal healthy cell surfaces. This indicates the applicability of PPS1D1 as a universal cancer targeting compound.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Direct binding of a peptide-peptoid hybrid PPS1 to individual lipids is performed.
- PPS1 binds to PS, PG and PA in descending order and not binds to PC.
- Higher the PS, PG and PA found on PC-liposomes, higher the PPS1 binding.
- Dimeric PPS1D1 disturbed the integrity of PS, PG and PA containing PC-liposomes.
- PPS1D1 was cytotoxic to cancer cells that express these negatively charged lipids.

Figure 1.

Binding analysis of FITC-PPS1 by fluorescence polarization: (A) Chemical structure of PPS1 and, (B) Homodimer PPS1D1. (C) – (E) Measurements of fluorescence polarization at fixed concentration of 50nM of FITC PPS1 on liposomes containing 0–40% of: (C) PS, (D) PG, and (E) PA.

Figure 2.

Calcein release from liposomes after addition of PPS1D1: (A) Leakage of calcein from PC-liposomes with 15% PS, PG and PA. (B) Maximum calcein leakage at 5 μM PPS1D1. (C) – (E) Leakage of Calcein from PC-liposomes containing 15% and 30% of: (C) PS, (D) PG, and (E) PA.

Figure 3.

PPS1 binding and PPS1D1 activity on negatively charged lipid expressing lung cancer cells: (A) PPS1-coated magnetic beads pulled down of lung cancer cells H1693, HCC95 and H1395, but not normal HBEC30KT. Control compound PC462-coated magnetic beads did not pulldown H1693 cancer cells. (B) Standard MTS cell viability assay indicates PPS1D1 induce cytotoxicity on H1693, HCC95 and H1395, but no effect on normal HBEC30KT cells. Control compound PC462D1 did induce cytotoxicity on H1693 cancer cells.

Figure 4. Modifications to PPS1D1 sequence impairs leakage: (A) Calcein leakage with sarcosine derivatives of PPS1D1. (B) Calcein leakage with truncated derivatives of PPS1D1 (4mer – 7mer).