Anionic Phospholipid-Targeting Agent for Brain Tumor Imaging

Xiaoyang Qi[1], Zhengtao Chu[1], Balveen Kaur[2], Antonio Chiocca[2], Brian D. Gray[3], Bradley D. Smith[4], Koon Yan Pak[3]

[1]Division of Human Genetics, Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, OH; [2]Department of Neurological Surgery, The Ohio State University, Columbus, OH; [3]Molecular Targeting Technologies, Inc, West Chester, PA. [4]University of Notre Dame, Notre Dame, IN.

Abstract

Synthetic bis-zinc(II)-dipicolylamine (Zn-DPA) containing compounds have been shown to have a strong binding affinity for biological membranes enriched with surface anionic phospholipids, and a growing body of evidence has demonstrated that anionic phosphatidylserine (PS) is highly exposed on the surfaces of cancerous cells and tumor blood vessels. Based upon these observations, we set out to determine whether Zn-DPA bearing molecules could be used for selective targeting to brain tumor cells and vessels through specific association with inside-out PS. Zn-DPA complexes conjugated to visible and near-infrared emitting fluorophores were used. *In vitro* studies using a FITC conjugated Zn-DPA molecule (PSVue™ 480) showed that the conjugate bound to human glioblastoma multiforme (GBM) cells. While *in vivo* studies using the near-infrared analog (PSVue™ 794) demonstrated that this conjugate selectively stained and accumulated in orthotopically implanted human GBM tumors in live nude mice in a dose-dependent manner using an IVIS fluorescence imaging system. These intriguing observations support the use of the Zn-DPA motif as the targeting component for new imaging agents to improve early diagnosis of brain tumors as well as to assist imaging-guided surgery.

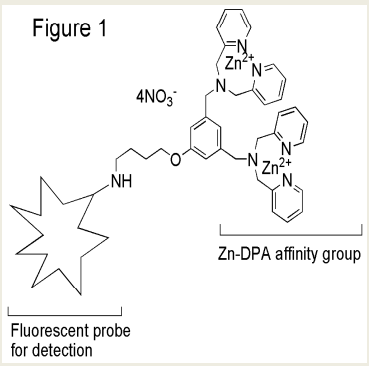
Introduction

PSVue™ reagents are a family of fluorescent probes containing a bis(zinc2+dipicolylamine) group (Zn-DPA), a motif that has been found to bind with high affinity to surfaces enriched with anionic phospholipids, especially phosphatidylserine (PS) exposed on cell membranes. The fluorescent part of the probe is a reporter element that provides a means of detecting the probe once it is bound to the membrane of interest. Key Features of PSVue™ Probes are following: (I) Bind to a tumor cells which have negatively charged phospholipids exposed on their membranes. (II) Available in a range of detection wavelengths from long-UV to near infrared. (III) Suitable for *in vitro* and *in vivo* use. (IV) Suitable for high-throughput screening assays. PSVue™ binds to the same PS site as annexin-V. However, there are several advantages of PSVue™ over Fluorescent Annexin-V: (1) Binding kinetics are fast; annexin-V binding is slow. (2) Binding is Ca^{2+} independent; means no artifacts due to activation of nonspecific membrane scramblases by Ca2+. (3) Cheap compared to most annexin-V fluorescent analogs. (4) Apoptosis can be detected under a wide variety of conditions (e.g. in presence of 10% serum, temperatures from 4 to 37°C). (5) Can provide more intense labeling due to their much smaller size (i.e. >10 PSVue™ molecules can bind to the same area as 1 annexin V molecule).

Materials and Methods

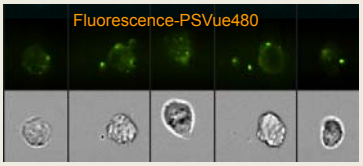
Animal studies were conducted using procedures approved by the Cincinnati Children's Hospital Medical Center Institutional Animal Care and Use Committee. The tumors are derived from human U87-ΔEGFR-luc and EMT-6 cancer cells in nude mice. Tumor luminescence and fluorescence (including uptake of fluorophore) were measured using an imaging system developed by Caliper Life Sciences (Mountain View, CA), which is comprised of a highly sensitive, cooled CCD camera mounted in a light-tight specimen box. Images and fluorescent signals were measured for PSVue™ (Molecular Targeting Technologies, West Chester, PA) fluorescence. Cell imaging was acquired by Amnis Imagestream and/or FACSCanto and analyzed using IDEAS or FACSDiva software, respectively .

General Structure of PSVue™ Probes



Results

Cancer Cells Staining with PSVue™



Brightfield-Cell Images

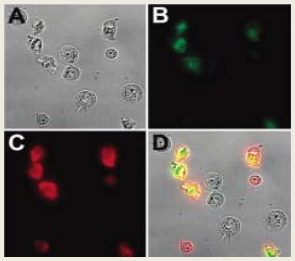


Figure 2. Top panel: Amnis imaging of human glioma cells (U87ΔEGFR-luc) staining with PSVue480) using FTIC channel. **Bottom panel**: Micrographs (60X magnification) of Jurkat cells, treated with cytotoxic camptothecin (10 μM) for 3.5 h and stained simultaneously with Annexin V-Alexa Fluor 488, and PSVue794 (10 μM). Brightfield image (A); cells stained with Annexin V-Alexa Fluor 488 (B); cells stained with PSVue794 (C); overlay of images A, B, and C (D).

Proposed Model of Membrane Binding

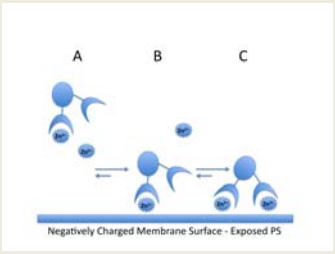


Figure 3. Illustration of the 3 of PSVue with PS-rich membranes. Under physiologic concentrations of Zn2+ the predominant coordination complex is the mono-zinc species (species A). The binding of species A to the anionic PS exposed membrane (species B) would promote the binding of the second Zn2+ with subsequent binding to the membrane forming a bivalently-bound species C. PSVue reagents are selective for membrane phosphates and do not stain the cytosol. component assembly process that results in high affinity association

Brian-Tumor Imaging using PSVue794

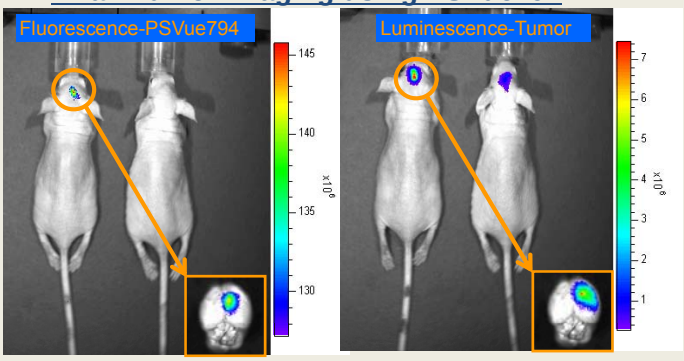


Figure 4. Location of PSVue794 in tumor-bearing mice revealed by fluorescence imaging. Luciferase-expressing glioma cells (Human U87ΔEGFR-luc, 10^5 cells per animal) were implanted in 2 mouse brains. After 4 days, to reveal tumor location, luciferin was injected followed by whole-animal imaging using the IVIS-200 (30 sec imaging time). Next, PSVue794 or PBS were injected via tail vein. The in vivo distribution of fluorescence was again recorded using an IVIS-200 live-animal imaging device (1 sec imaging time, 745/840 filter set). Photon emission from tumors or tissues was detected, digitized and electronically displayed as a pseudocolor overlay onto a gray scale animal image. Luminescence and fluorescence imaging of excised brains was also performed using the IVIS-200 system.

Dose-Dependent Brain Tumor Imaging

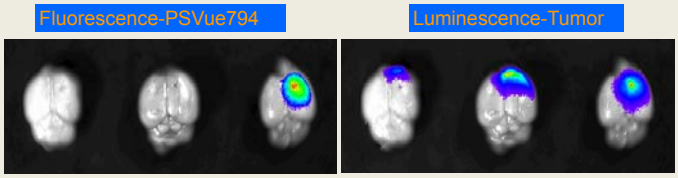


Figure 5. **Dose-dependent imaging of brain tumor by tail vein injection of PSVue794 for 24 h.** Left panel: fluorescence imaging with 1 sec imaging time, 745/840 filter set. Right panel Luminescnece imaging for tumors with 10 sec imaging time.

Conclusion

PSVue™794 targets to human gliomas orthotopically implanted in mouse brains in a dose-dependent manner.

Acknowledgment

This work was financially supported in part by Validation Research Grants from Cincinnati Children's Hospital Medical Center (to X.Qi).

M · T · T · I