Photodynamic therapy: discriminating between healthy and cancerous tissue

Charles Spangler, Mikhail Drobizhev, Jean Starkey, and Aleksander Rebane

New two-photon–activated porphyrin designs allow specific, noninvasive targeting of subcutaneous tumors several centimeters below the skin surface.

Photodynamic therapy (PDT) is a noninvasive technique in which specific compounds, termed photosensitizers, are introduced into the bloodstream and subsequently incorporated into tissues throughout the body. Application of a specific wavelength of light can discreetly activate these compounds, leading to cell death in the area of treatment. This approach has considerable potential as an aggressive cancer treatment without the side effects associated with traditional radiation and chemotherapies.

Until recently, however, direct treatment of tumors deeper than a few millimeters below the skin surface has remained problematic with the use of currently FDA-approved PDT photosensitizers. In addition, these therapeutic agents often do not adequately discriminate between healthy tissue and tumor cells and can continue to reside in healthy tissue for several days or weeks following treatment, sometimes leading to severe sunlight sensitivity.

The PDT killing mechanism is derived from the ability of the photo-sensitizers, typically porphyrins or phthalocyanines, to efficiently form excited-state triplets upon light activation. This is followed by the transfer of the triplet state energy to oxygen molecules present in the blood supply, thus forming cytotoxic singlet oxygen species. The limitation of this mechanism is that porphyrins or phthalocyanines normally absorb light below 700nm, thus limiting light penetration through the skin and underlying tissue to 2–4mm. To solve this difficulty, we have designed a new class of porphyrin-based photosensitizers that exhibit greatly enhanced intrinsic two-photon absorption cross sections when activated by femtosecond-pulsed lasers in the near-IR spectrum. This allows activation of these new PDT agents deep in the tissue transparency window (780–900nm) where both absorption and scattering are minimized, and it supports their application in direct treatment of subcutaneous tumors several centimeters below the skin surface.

In order to more efficiently deliver PDT agents to the tumor site and avoid the problem of residual incorporation into healthy tissue and prolonged sun sensitivity, we have coupled small-molecule targeting agents to these new porphyrins to direct them to overexpressed tumor receptor sites. The last synthesis step for our PDT agents introduces a small-peptide targeting agent that allows matching of therapeutic agents to an individual patient’s tumor receptor site. This greatly improves the discrimination between healthy and cancerous tissue. To identify the optimum therapy window, it would also be advantageous to image the tumor’s position, follow the rate of accumulation of the PDT agent within the tumor, and determine its clearance from the surrounding healthy tissue.

Our new two-photon-activated PDT agents incorporate all of the above capabilities into one new synthetic triad nanoensemble. These triads incorporate a two-photon-activated porphyrin, a small-molecule targeting agent that direct the triads to overexpressed tumor receptor sites, and a near-IR imaging agent that allows both 3D modeling of the tumor site and tracking of the rate of accumulation of the PDT agent. Figure 1 depicts a typical triad for targeting and treating cancerous tumors. This molecule incorporates a porphyrin photosensitizer (RA-105), an octreotate SST2 receptor targeting peptide, and a near-IR imaging agent (indodicarbocyanine).

In order to estimate the killing capability of these new triads, we demonstrated in collagen tissue phantoms that human breast cancer cells can be killed efficiently (80–90%) to depths of 4cm, and perhaps even deeper. In similar phantoms, Photofrin®, a prototypical, one-photon, FDA-approved PDT agent, rapidly declines in killing power as the required penetration depth ap-

Continued on next page
Figure 1. RA-301, a Rasiris triad incorporating the central porphyrin PDT agent (RA-105), a pegylated near-IR imaging agent (indodicarbocyanine, left-hand side), and an octreotate cyclic SST2 targeting moiety (right-hand side).

proaches 1cm. We have also described the treatment of breast cancer xenografts implanted in mammary fat pads of mice.\(^2\) Irradiation using a Ti:sapphire laser at 780–800nm (15 minutes, 600–800mW) was carried out with the anesthetized mouse mounted on a moveable stage that allowed precise X-Y positioning in real time so that the focused laser beam could be rastered in a stepwise fashion throughout the tumor volume (typically 1cm in diameter).\(^1\) The mice showed essentially complete tumor regression with complete healing of the irradiation site, including regrowth of hair that had been removed prior to PDT. Internal organs were harvested post-PDT, and no PDT-induced damage could be discerned.\(^1, 3\)

In our most recent studies,\(^4\) we tested the maximum possible treatment depth in mice by irradiating tumors through the mouse’s body from the side opposite the tumor. These experiments indicated an effective treatment depth of approximately 2cm and provided further evidence that two-photon PDT has little effect on the surrounding healthy tissue. The experimental design for these mouse tumor treatments is illustrated in Figure 2. Two lung cancer tumor types, small cell (SC) and non-small cell (NSC), were implanted into the mice. All 10 of the PDT-treated SC xenografts exhibited excellent regression over the first week to 10 days (Figure 3). Two ‘cures’ were noted where the tumor completely disappeared and did not regrow during the following two months of observation. However, the majority of SC xenografts did not completely regress and after a few weeks started to regrow. Similar regrowth behavior was noted for the NSC tumors. This was most likely due to the difficulty of accurately rastering the laser beam through the body of the mouse from the side opposite the tumor without image guidance, and therefore we missed some of the peripheral tumor tissue. However, the observed tumor regression was robust. When tumor regrowth occurred, in the majority of cases it started about 20 days post-treatment. It should be noted that the mice were still in good physical condition at this point and could easily have withstood another PDT treatment. Very little change was noted in the histology of either the untreated normal tissue or the normal tissue through which the laser beam had passed.

PTD continues to evolve into a mature clinical treatment for a variety of cancer types and offers the tantalizing possibility of an eventual noninvasive outpatient treatment for a wide variety of cancers deep within human tissues. Our results are extremely promising for the future of two-photon PDT for the treatment of aggressive, drug-resistant, and radiation-resistant tumors. Targeted two-photon PDT promises much greater tissue-depth penetration, and more accurate aiming can be achieved using image guidance. We have demonstrated that lung and breast tumors can be effectively targeted using tumor-specific targeting peptides and have successfully treated tumors at significant depths with no damage to healthy tissues. Furthermore, we expect that

Continued on next page
Figure 2. Schematic of the laser irradiation instrumentation used to treat tumor grafts in mice.

Regression of human lung tumors after PDT treatment through the body of the mouse

Figure 3. PDT-induced regressions of NCI-H69 (SCLC, small-cell lung cancer) and A 549 (NSCLC, non-small-cell lung cancer) human lung cancer xenografts. All tumors were irradiated through the body of the host mouse.

tumor regression and cure rates will dramatically improve as we begin to use fluorescence-based image guidance to aim and raster the laser throughout the tumor mass.

This research was partially funded through grants from the Montana Board of Research Commercialization and Technology.

Author Information

Charles Spangler
Rasiris, Inc.
Bozeman, MT

Charles Spangler is the chief science officer of Rasiris, Inc., an early-stage pharmaceutical company focused on new photodynamic therapy agents. He previously was a distinguished research professor at Northern Illinois University and Montana State University for more than 40 years and has published more than 200 papers in the areas of organic nonlinear, electrooptic, and photonic materials.
Mikhail Drobizhev and Aleksander Rebane
Physics Department
Montana State University
Bozeman, MT

After obtaining his doctoral degree in Russia, Mikhail Drobizhev joined the Physics Department at Montana State University and currently is an assistant research professor working in the Rebane lab. He has worked in the areas of two-photon PDT, ultrafast optical memory, and most recently in the design of new fluorescent proteins.

Aleksander Rebane heads an ultrafast laser laboratory at Montana State University, and his focus in recent years has been on the physics of two-photon organic materials, including both design paradigms for enhancing two-photon absorption and the mechanisms responsible for enhanced absorption. Besides photodynamic therapy, he has worked extensively in the area of ultrafast optical memory.

Jean Starkey
Microbiology Department
Montana State University
Bozeman, MT

Jean Starkey has been a research professor first at Washington State University and then at Montana State University for more than 30 years and has been an active cancer researcher for most of that time. Using her expertise as a doctor of veterinary medicine, she has designed protocols for the use of animal models in cancer studies.

References