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Prostate Specific Membrane Antigen-Targeted Photodynamic Therapy Induces Rapid Cytoskeletal Disruption

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Abstract

Prostate-specific membrane antigen (PSMA), an established enzyme-biomarker for prostate cancer, has attracted considerable attention as a target for imaging and therapeutic applications. We aimed to determine the effects of PSMA-targeted photodynamic therapy (PDT) on cytoskeletal networks in prostate cancer cells. PSMA-targeted PDT resulted in rapid disruption of microtubules (α -/ β -tubulin), microfilaments (actin), and intermediate filaments (cytokeratin 8/18) in the cytoplasm of LNCaP cells. The collapse of cytoplasmic microtubules and the later nuclear translocation of α -/ β -tubulin were the most dramatic alternation. It is likely that these early changes of cytoskeletal networks are partly involved in the initiation of cell death.

Keywords

cytoskeleton; prostate-specific membrane antigen; photodynamic therapy; pyropheophorbide-a; apoptosis

1. Introduction

Photodynamic therapy (PDT) has emerged as a minimally invasive regimen for the treatment of cancers, offers an attractive alternative or complement to conventional therapies [1;2;3]. The therapeutic action of PDT is based on the generation of reactive oxygen species (ROS) that are formed upon specific wavelength-light activation of a photosensitizer (PS) such as a porphyrinic pigment. For clinical PDT applications, light in the red and infrared range is generally employed for enhanced tissue penetration. The resulting excited PS transfers its energy to molecular oxygen in tissue to generate ROS. Singlet oxygen is assumed to be the key cytotoxic ROS responsible for localized oxidative cell damage and initiation of cell death [1;3;4].

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Conflict of interest

Dr. Berkman is the inventor of a patent on the PSMA inhibitor described in this report and presently serves as the CSO of Cancer Targeted Technology.

PDT for prostate cancer has not yet advanced beyond clinical trials and consequently is not yet an integral part of clinical practice [5]. The successful practice of PDT requires both the delivery of a sufficient light flux to the entire prostate and the adequate accumulation of photosensitizer in tumors. While Hahn's group has developed procedures to measure and optimize light source parameters for prostate PDT [6;7;8], it is expected that improved targeting delivery and accumulation of photosensitizer molecules in prostate tumor may be additionally advantageous. Our group recently has developed a method for the selective delivery of PSs by targeting the enzyme-biomarker prostatespecific membrane antigen (PSMA) [9;10]. PSMA is a type II cell-surface glycoprotein predominantly restricted to prostatic tissue and is strongly expressed on prostate tumor cells [11]. Expression levels increase with disease progression, being highest in metastatic disease, hormone refractory cancers, and higher-grade lesions [12;13]. Endothelial-expression of PSMA in the neovasculature of a variety of non-prostatic solid malignancies has also been detected. Therefore, PSMA has attracted considerable attention as a biomarker and target for the delivery of imaging and therapeutic agents.

Microfilaments, microtubules, and intermediate filaments form the cytoskeleton systems of vertebrate cells. These fibrillar networks are composed of distinctly different proteins and exhibit unique structural and functional characteristics. The dynamic cytoskeletal networks that they form as well as their crosstalk play a critical role in the maintenance of cellular morphology and membrane integrity. They are also involved in cellular processes such as cell division, adhesion and migration, intracellular transport, and apoptosis [14;15]. Cytokeratin filaments have been found to aggregate at an early stage of the apoptotic sequence, while at later stages cytochrome c is degraded [16;17]. Reorganization of the microfilament and microtubule cytoskeleton has also been observed during the execution phase of apoptosis [18]. Cleavage of α -tubulin [19], cytochrome c [20;21], and actin [22] by caspases during apoptosis has also been reported. With respect to PDT, there are characteristic changes in the cytoskeletal networks induced when phthalocyanines or 5-aminolevulinic acid are used as the PS [23;24;25;26].

We previously reported that phosphoramidate peptidomimetic PSMA inhibitors were capable of both cell-surface labeling of prostate cancer cells and intracellular delivery for targeted PDT applications [9;10]. Apoptosis of prostate cancer cells following PSMA-targeted PDT with Ppa-CTT-54 (Fig. 1) was confirmed by the appearance of early chromatin condensation, poly(ADP-ribose) polymerase (PARP) p85 fragment, and DNA fragmentation [10]. In this present study, we attempted to delineate the molecular mechanism by which PSMA-targeted PDT induces apoptosis and concluded that disruption of three kinds of filamentous networks and degeneration of cytoskeletal components occur at the initiation sequence of cell death or apoptosis.

2. Materials and methods

2.1. Cell line and reagents

The LNCaP cell line was obtained from the American Type Culture Collection (Manassas, VA, USA). The mouse monoclonal anti-cytokeratin 8 antibody, mouse monoclonal anti-cytokeratin 18 antibody, mouse monoclonal anti- α -tubulin antibody, mouse monoclonal anti- β -tubulin antibody, rabbit polyclonal anti-actin antibody and goat anti-rabbit IgG-FITC were obtained from Sigma-Aldrich (St. Louis, MO, USA). The goat anti-mouse secondary antibody-TRIC was obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Protein blocking solution was obtained from BioGenex (San Ramon, CA, USA). Rhodamine conjugated phalloidin, Alexa Fluor 488 conjugated Deoxyribonuclease I (DNase I) and Hoechst 33342 (H342) were obtained from Invitrogen-Molecular Probes (Carlsbad, CA, USA). Pyropheophorbide-a (Ppa) was obtained from Frontier Scientific, Inc (Logan,

UT, USA) and its conjugate (Ppa-CTT-54) was prepared by our lab as described previously [10]. All other chemicals and cell-culture reagents were purchased from Fisher Scientific (Sommerville, NJ, USA), Pierce (Rockford, IL, USA), or Sigma-Aldrich.

2.2. Cell culture

LNCaP (PSMA-positive; PSMA+) cells were grown in T-75 flasks with complete growth medium [RPMI 1640 containing 10% heat-inactivated fetal calf serum (FBS), 100 units of penicillin and 100 µg/mL streptomycin] in a humidified incubator at 37°C with 5% CO₂. Confluent cells were detached with a 0.25% trypsin 0.53 mM EDTA solution, harvested, and plated in 2-well slide chambers at a density of 4×10^4 cells / well. Cells were grown for 3 days before conducting the following experiments [10].

2.3. In vitro PDT with Ppa-CTT-54 or unconjugated Ppa

LNCaP cells grown in 2-well slide chambers for 3 days were washed twice in 37°C pre-warmed *medium A* (phosphate-free RPMI 1640 containing 1% FBS), and then incubated with 1 mL of Ppa-CTT-54 or unconjugated Ppa (5 µM) in pre-warmed *medium A* for 2.5 hrs in a humidified incubator at 37°C and 5% CO₂, which allowed internalization of Ppa-CTT-54 bound to PSMA to occur. Cells treated with Ppa-CTT-54 were washed in 37°C pre-warmed phenol red-free medium RPMI 1640 once, and then irradiated with light (600~800 nm, 7.5 J/cm², with 25 mW/cm² fluence rate) for 10 min in pre-warmed phenol red-free RPMI 1640. The light source was a 100-watt halogen lamp, which was filtered through a 10 cm column of water (absorbing above 800 nm), and then filtered through a Lee Primary Red filter (Vincent Lighting Systems, Cleveland, OH, USA) to remove light with wavelengths below 600 nm.

2.4. Immunofluorescence detection of cytoskeletal changes

The above PDT-treated cells were replaced in pre-warmed complete growth medium RPMI 1640, allowed to recover for increasing periods of time (0, 15, 30 min) in darkness at 37°C in a humidified incubator at 37°C and 5% CO₂, washed twice in ice-cold phosphate buffered saline (PBS), fixed in 4% paraformaldehyde in PBS for 15 min at room temperature (RT), permeabilized in cold-methanol for 5 min at -20°C, then blocked for 2 h in protein blocking solution at room temperature. Cells were then incubated with either mouse primary antibodies against (α-tubulin, 1:2000; β-tubulin, 1:200; cytokeratin 8, 1:200; cytokeratin 18, 1:1000) or rabbit primary antibody against (actin, 1:500) and then incubated with a respective fluorescently labeled second antibody (goat anti-mouse antibody-TRITC, 1:50; or goat anti-rabbit antibody-FITC, 1:40) in 1% BSA, PBS for 1 h at RT. The cellular nuclei were counterstained with H342, then the cells were mounted in VECTASHIELD® Mounting Medium (Vector Laboratories, Inc., Burlingame, CA, USA) for microscopy [10].

2.5. Affinity labeling of F-/G-actin

Cellular actin is generally present in two forms: globular monomer form (G-actin) and filamentous polymer form (F-actin). In order to discriminate G- and F-actin, the selective fluorescent probes (Alexa Fluor 488 conjugated DNase I and Rhodamine conjugated Phalloidin) with high affinity for G- or F-actin were employed in the following experiments. The above PDT- treated cells were replaced in pre-warmed complete growth medium RPMI 1640 to recover for different times (0, 15, 30 min) in darkness at 37°C incubator, then washed twice in ice-cold PBS and fixed in 4% paraformaldehyde in PBS for 15 min at room temperature (RT), permeabilized in 0.1% Triton X-100, PBS for 5 minutes. F-actin was stained with rhodamine conjugated phalloidin (12.5 µL/500 µL PBS +1%BSA) and G-actin was stained with Alexa Fluor 488 conjugated DNase I (1 µL/500 µL PBS) for 20 minutes at room temperature. The cellular nuclei were counterstained with H342, and anti-fade solution

was mounted on cells. Cellular fluorescent image was captured by a Confocal laser scanning microscope.

2.6. Confocal laser scanning microscopy

Cells were visualized under 40X oil immersion objective using a LSM 510 META Laser Scanning Microscope. H342 was excited with a Diode Laser (405 nm), and the emission collected with a BP420-480 nm filter. Fluorescein isothiocyanate (FITC) or Alexa Fluor 488 was excited using 488 nm from an Argon Laser, and the emission collected with a LP505 nm filter. Tetramethyl Rhodamine Isothiocyanate (TRITC) or rhodamine was excited using 543 nm from a HeNe Laser, and the emission collected with a BP560-615 nm filter. To reduce interchannel cross-talk, a multi-tracking technique was used, and images were taken at a resolution of 1024×1024 pixels. Confocal scanning parameters were set up so that the control cells without treatment had no fluorescent signal from background. The pictures were edited by National Institutes of Health (NIH) Image J software (<http://rsb.info.nih.gov/ij>) and Adobe Photoshop CS2.

2.7. Whole cell lysate extraction and western blotting

The control (inhibitor treatment without light irradiation) and PDT-treated LNCaP cells (at 0, 15 and 30 min post-PDT) were collected by scraping, washed once in ice-cold PBS, resuspended in 3-fold cell pellet volumes of lysis buffer (1% NP-40, 20 mM Tris pH 8.0, 137 mM NaCl, 10% glycerol) [27] supplemented with $1 \times$ anti-protease cocktail (Pierce, Rockford, IL) for 15 min on ice, then transferred to eppendorf tubes for centrifugation at 10,000g for 15 min at 4°C, the supernatant was saved as whole-cell protein extracts. Protein concentrations were determined using BCA protein assay (Pierce, Rockford, IL, USA). Western blotting was performed as described previously with only minor modifications [28]. In brief, detergent soluble proteins (30 μ g) were loaded and separated on a NuPAGE™ 4–12% Bis-Tris Gel (Invitrogen, Carlsbad, CA, USA), electrophoresed for 40 min at a constant 200 Volts under reducing conditions, and then transferred to a 0.45 μ m PVDF Immobilon-P Transfer Membrane (Millipore Corporation, Bedford, MA, USA) at 400 mA for 100 min in a transfer apparatus-Owl Bandit VEP-2 (Owl, Portsmouth, NH, USA) according to the manufacturer's instructions. Membranes were incubated with primary antibody overnight at 4°C and then with horseradish peroxidase conjugated-second antibody for 1 h at room temperature. The immunoreactive bands were visualized using Protein Detector TMB Western Blot Kit (KPL, Gaithersburg, MD, USA) following the manufacturer's instructions.

3. Results

3.1. Effects of targeted PDT on microtubules

Control LNCaP cells exhibited well-organized microtubule networks throughout the cytoplasm. In contrast, targeted PDT-treated cells displayed a rearrangement of microtubule networks to ring-like perinuclear structures (Fig. 2A). With increasing time after PDT treatment, the collapse of microtubular networks in the cytoplasm was followed by the nuclear translocation of α -tubulin at 1 h post-PDT or β -tubulin at 2 h post-PDT (Fig. 2B). In contrast, unconjugated Ppa just induced the aggregation of microtubules with enlarging nuclei following PDT (1, 2, 4 h). There are no loss of cytoplasmic microtubules and no nuclear translocation of α - and β -tubulin to be detected at 4 h post-PDT (Fig. S1).

3.2. Effects of Targeted PDT on Actin Microfilaments

In control cells, the F-actin is found mainly beneath the membrane, and G-actin localizes in both the cytoplasm and nucleus (Fig. 3A). In contrast, targeted PDT induced rapid reduction of cytoplasmic G-actin. F-actin was minimally affected immediately (0 min) and slowly

disrupted over time (15 and 30 min) following PDT (Fig. 3A). Immunofluorescent analysis further supported the observation that cytoplasmic actin but not membrane-localized actin is mainly affected by targeted PDT (Fig. 3B). Western blot analysis confirmed that the total amount of actin decreased over time following PDT treatment and provided evidence that actin is cleaved in the process (Fig. 3C).

3.3. Effects of Targeted PDT on Intermediate Filaments

Because cytokeratin 8 and 18 are confirmed to be main protein components of intermediate filaments in prostate cancer cell lines including LNCaP cells [29], we sought to observe the reorganization or re-distribution of these intermediate filaments after targeted PDT. In control cells, the cytokeratin 8 and 18 intermediate filaments are found throughout the cytoplasm and cell periphery (Fig. 4A). Loss of cytoplasmic localization and increase of membrane-localized intermediate filaments were induced immediately following PDT (Fig. 4A). Cleaved or aggregated forms (irreversible) of cytokeratin 8 were immediately induced by targeted PDT treatment (0 min post-PDT), and remained relatively stable with increasing time (15 or 30 min). In contrast, only a minimal increase of aggregated forms (irreversible) of cytokeratin 18 was detected by Western blotting (Fig. 4B).

4. Discussion

In our previous work, the photosensitizer-PSMA inhibitor conjugate Ppa-CTT-54 was used to selectively induce apoptosis in PSMA+ cells [10]. In contrast to unconjugate Ppa, this selectivity was correlated with the affinity of Ppa-CTT-54 to the cell surface enzyme-biomarker marker PSMA and subsequent internalization through the PSMA enzyme-inhibitor complex. Evidence for the activation of different apoptotic pathways was initially based on differences in the nuclei morphology after PDT with Ppa-CTT-54 and unconjugated Ppa (Fig. 2, S1). We concluded that these differences were likely due to their different sub-cellular localization; late endosomes and lysosomes for Ppa-CTT-54 and mitochondria for unconjugated Ppa [9;10;30;31;32]. Although NPe6 and similar peptide-modified photosensitizers are capable of targeting lysosomes [33], they are generally not specific for tumor cells. The lysosomal photodamage from antibiotics shown to cause changes in the actin filament network [34] and the increasing evidence that cytoskeletal changes are linked to initiation of apoptosis [35;36] provided clues for us to identify the molecular mechanism of apoptosis induced by targeted PDT with Ppa-CTT-54 in the PSMA + LNCaP cell line. Herein we examined and confirmed changes in all three major cytoskeletal filaments types following targeted PDT with Ppa-CTT-54.

Microtubules are the major cytoskeletal components. They consist of polymers of α - and β -tubulin heterodimers and exist in a dynamic polymerization/depolymerization equilibrium dependent upon cellular processes. The observation that targeted PDT with Ppa-CTT-54 induced rapid disruption of cytoplasmic microtubules and subsequent nuclear translocation of α - and β -tubulin was largely unexpected (Fig. 2). Although the functional significance of this remains conjectural, it has been correlated to nuclear events such as chromatin condensation during apoptosis [37]. In stark contrast, PDT with unconjugated Ppa was observed to induce reorganization of microtubules in the cytoplasm, with no nuclear translocation of α - or β -tubulin even at later time points following irradiation (Fig. S1). The relevance of the rapid disruption of microtubules and subsequent nuclear translocation of α - and β -tubulin following targeted PDT with Ppa-CTT-54 may initiate unknown apoptotic pathways in prostate cancer.

In the case of actin microfilaments, a dramatic loss of cytoplasmic G-actin was observed immediately following targeted PDT (Fig. 3). In contrast, F-actin microfilaments largely remained beneath the cell membrane and diminished slowly. These observations may reflect

an imbalance in the equilibrium between monomeric G-actin and polymerized F-actin forms as a result of the rapid degradation of G-actin. Although actin is a substrate for caspase-3 and is known to be cleaved during the later execution phase of apoptosis [22;38], our experiments suggest that the degradation of actin was clearly induced at an early stage as a result of targeted PDT (Fig. 3).

Unlike the few numbers of proteins that represent the classes of actins and tubulins, intermediate filament proteins consist of sixty-five homologous proteins, which can be classified into five types [39;40]. Of them, cytokeratin 8 (Type II) and cytokeratin 18 (Type I) are expressed in simple or predominately single layered, internal epithelia, and have a persistent expression in metastatic cancer cells [39]. In prostate tumor cells including LNCaP cells, high expression of cytokeratins 8 and 18 have been confirmed [29;41]. In a 1:1 ratio, cytokeratins 8 and 18 non-covalently polymerize as heteropolymers to form intermediate filaments. These intermediate filaments are highly dynamic and are reorganized during various cellular events such as differentiation, mitosis and apoptosis [42]. *In vitro* targeted PDT with Ppa-CTT-54 induced the immediate reorganization of the cytoplasmic cytokeratin 8/18 intermediate filament networks beneath the membrane with a concomitant increase of aggregated forms of these proteins (Fig. 4). The aggregation of cytokeratins 8 and 18 likely interferes with dynamic transition (polymerization/depolymerization) and leading to dysfunction of these intermediate filaments.

A question now remains as to the mechanism by which targeted PDT with Ppa-CTT-54, especially in contrast to non-targeted photosensitizers, induces the immediate changes of cytoskeletal networks at early stage of apoptosis. A possible clue arises from recent work in our lab in which LysoTracker Red DND-99 (Invitrogen, Carlsbad, CA) staining in LNCaP cells revealed that photodamage of lysosomes occurs quickly following targeted PDT with Ppa-CTT-54 (data not shown). Therefore, it is reasonable to assume that photodamage of lysosomes, as a result of subcellular accumulation of Ppa-CTT-54, may lead to an increase in lysosomal membrane permeability. Subsequent release of lysosomal enzymes into the cytoplasm would be consistent with the observed and rapid degradation of cytoskeletal proteins.

We cannot exclude the possibility of apoptosis initiation via activation of the caspase pathway following targeted PDT. In fact, in our latest published paper [43], we noted that targeted PDT with another Ppa-PSMA inhibitor conjugate induced a delayed activation of the caspase-8/-3 apoptotic cascade. In that study, the onset of active caspases 3, 8, and 9 were first detected at 1h-post PDT with the greatest activities observed at 4h-post PDT. The use of caspase inhibitors at high concentration to protect cells from apoptosis via the caspase cascade still resulted in apoptosis of approximately 40% of those treated cells. These results suggested that caspase-independent apoptotic pathways may be activated following targeted PDT such as the immediate cytoskeleton changes observed in the current study.

We also noticed that in this *in vitro* study, a broad spectrum of light (600~800 nm) was used to irradiate Ppa-CTT-54. As Ppa has long wavelength absorption at 667 nm [44], a specific wavelength-laser light will be required and perform for *in vivo* study, and other longer wavelength-absorbed photosensitizer conjugates will be explored in the future.

In conclusion, the rapid reorganization and disruption of microtubules, microfilaments and intermediate filaments after targeted PDT with Ppa-CTT-54, suggests that these changes may be partly related to the initiation of cell death, not unlike the collapse of cytoskeletal networks during the execution phase of apoptosis as a result of caspase-mediated cleavage of cytoskeletal proteins. Considering that the critical role of cytoskeletal networks in cell proliferation, invasion and metastasis of cancer cells, the selective targeting their

components remains an important objective in the development of cancer therapies. Therefore, PSMA-targeted PDT may provide a unique augmentation to the therapeutic arsenal for prostate cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Allison RR, Downie GH, Cuenca R, Hu XH, Childs CJ, Sibata CH. Photosensitizers in clinical PDT. Photodiagnosis and Photodynamic Therapy. 2004; 1:27–42.
2. Sharman WM, van Lier JE, Allen CM. Targeted photodynamic therapy via receptor mediated delivery systems. Adv Drug Deliv Rev. 2004; 56:53–76. [PubMed: 14706445]
3. Detty MR, Gibson SL, Wagner SJ. Current clinical and preclinical photosensitizers for use in photodynamic therapy. Journal of medicinal chemistry. 2004; 47:3897–3915. [PubMed: 15267226]
4. Berg K, Selbo PK, Weyergang A, Dietze A, Prasmickaite L, Bonsted A, Engesaeter BO, Angell-Petersen E, Warloe T, Frandsen N, Hogset A. Porphyrin-related photosensitizers for cancer imaging and therapeutic applications. J Microsc. 2005; 218:133–147. [PubMed: 15857375]
5. Moore CM, Pendse D, Emberton M. Photodynamic therapy for prostate cancer--a review of current status and future promise. Nature clinical practice. Urology. 2009; 6:18–30.
6. Patel H, Mick R, Finlay J, Zhu TC, Rickter E, Cengel KA, Malkowicz SB, Hahn SM, Busch TM. Motexafin lutetium-photodynamic therapy of prostate cancer: short- and long-term effects on prostate-specific antigen. Clin Cancer Res. 2008; 14:4869–4876. [PubMed: 18676760]
7. Li J, Altschuler MD, Hahn SM, Zhu TC. Optimization of light source parameters in the photodynamic therapy of heterogeneous prostate. Phys Med Biol. 2008; 53:4107–4121. [PubMed: 18612172]
8. Zhu TC, Finlay JC, Hahn SM. Determination of the distribution of light, optical properties, drug concentration, and tissue oxygenation in-vivo in human prostate during motexafin lutetium-mediated photodynamic therapy. J Photochem Photobiol B. 2005; 79:231–241. [PubMed: 15896650]
9. Liu T, Wu LY, Kazak M, Berkman CE. Cell-Surface labeling and internalization by a fluorescent inhibitor of prostate-specific membrane antigen. Prostate. 2008; 68:955–964. [PubMed: 18361407]
10. Liu T, Wu LY, Choi JK, Berkman CE. In vitro targeted photodynamic therapy with a pyropheophorbide--a conjugated inhibitor of prostate-specific membrane antigen. Prostate. 2009; 69:585–594. [PubMed: 19142895]
11. Murphy GP, Su S, Jarisch J, Kenny GM. Serum levels of PSMA. Prostate. 2000; 42:318–319. [PubMed: 10679762]
12. Bacich DJ, Pinto JT, Tong WP, Heston WD. Cloning, expression, genomic localization, and enzymatic activities of the mouse homolog of prostate-specific membrane antigen/NAALADase/folate hydrolase. Mammalian genome : official journal of the International Mammalian Genome Society. 2001; 12(2):117–123. [PubMed: 11210180]
13. Fair WR, Israeli RS, Heston WD. Prostate-specific membrane antigen. Prostate. 1997; 32:140–148. [PubMed: 9215402]

14. Etienne-Manneville S. Actin and microtubules in cell motility: which one is in control? *Traffic*. 2004; 5:470–477. [PubMed: 15180824]
15. Mollinedo F, Gajate C. Microtubules, microtubule-interfering agents and apoptosis. *Apoptosis*. 2003; 8:413–450. [PubMed: 12975575]
16. Schutte B, Henfling M, Kolgen W, Bouman M, Meex S, Leers MP, Nap M, Bjorklund V, Bjorklund P, Bjorklund B, Lane EB, Omary MB, Jornvall H, Ramaekers FC. Keratin 8/18 breakdown and reorganization during apoptosis. *Exp Cell Res*. 2004; 297:11–26. [PubMed: 15194421]
17. Tinnemans MM, Lenders MH, ten Velde GP, Ramaekers FC, Schutte B. Alterations in cytoskeletal and nuclear matrix-associated proteins during apoptosis. *Eur J Cell Biol*. 1995; 68:35–46. [PubMed: 8549588]
18. Ndozangue-Touriguine O, Hamelin J, Breard J. Cytoskeleton and apoptosis. *Biochem Pharmacol*. 2008; 76:11–18. [PubMed: 18462707]
19. Klaiman G, Petzke TL, Hammond J, Leblanc AC. Targets of caspase-6 activity in human neurons and Alzheimer disease. *Mol Cell Proteomics*. 2008; 7:1541–1555. [PubMed: 18487604]
20. Caulin C, Salvesen GS, Oshima RG. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. *J Cell Biol*. 1997; 138:1379–1394. [PubMed: 9298992]
21. Gilbert S, Ruel A, Loranger A, Marceau N. Switch in Fas-activated death signaling pathway as result of keratin 8/18-intermediate filament loss. *Apoptosis*. 2008; 13:1479–1493. [PubMed: 19002587]
22. Mashima T, Naito M, Tsuruo T. Caspase-mediated cleavage of cytoskeletal actin plays a positive role in the process of morphological apoptosis. *Oncogene*. 1999; 18:2423–2430. [PubMed: 10229193]
23. Juarranz A, Espada J, Stockert JC, Villanueva A, Polo S, Dominguez V, Canete M. Photodamage induced by Zinc(II)-phthalocyanine to microtubules, actin, alpha-actinin and keratin of HeLa cells. *Photochem Photobiol*. 2001; 73:283–289. [PubMed: 11281025]
24. Tsai JC, Wu CL, Chien HF, Chen CT. Reorganization of cytoskeleton induced by 5-aminolevulinic acid-mediated photodynamic therapy and its correlation with mitochondrial dysfunction. *Lasers Surg Med*. 2005; 36:398–408. [PubMed: 15856508]
25. Maftoun-Costa M, Naves KT, Oliveira AL, Tedesco AC, da Silva NS, Pacheco-Soares C. Mitochondria, endoplasmic reticulum and actin filament behavior after PDT with chloroaluminum phthalocyanine liposomal in HeLa cells. *Cell Biol Int*. 2008; 32:1024–1028. [PubMed: 18485750]
26. Uzdensky A, Kolpakova E, Juzeniene A, Juzenas P, Moan J. The effect of sub-lethal ALA-PDT on the cytoskeleton and adhesion of cultured human cancer cells. *Biochim Biophys Acta*. 2005; 1722:43–50. [PubMed: 15716135]
27. Matroule JY, Carthy CM, Granville DJ, Jolois O, Hunt DW, Piette J. Mechanism of colon cancer cell apoptosis mediated by pyropheophorbide-a methylester photosensitization. *Oncogene*. 2001; 20:4070–4084. [PubMed: 11494135]
28. Liu T, Toriyabe Y, Berkman CE. Purification of prostate-specific membrane antigen using conformational epitope-specific antibody-affinity chromatography. *Protein Expr Purif*. 2006; 49:251–255. [PubMed: 16815035]
29. van Bokhoven A, Varella-Garcia M, Korch C, Johannes WU, Smith EE, Miller HL, Nordeen SK, Miller GJ, Lucia MS. Molecular characterization of human prostate carcinoma cell lines. *Prostate*. 2003; 57:205–225. [PubMed: 14518029]
30. Liu T, Toriyabe Y, Kazak M, Berkman CE. Pseudoirreversible inhibition of prostate-specific membrane antigen by phosphoramidate peptidomimetics. *Biochemistry*. 2008; 47:12658–12660. [PubMed: 18983168]
31. Savellano MD, Pogue BW, Hoopes PJ, Vitetta ES, Paulsen KD. Multiepitope HER2 targeting enhances photoimmunotherapy of HER2-overexpressing cancer cells with pyropheophorbide-a immunoconjugates. *Cancer Res*. 2005; 65:6371–6379. [PubMed: 16024640]
32. Sun X, Leung WN. Photodynamic therapy with pyropheophorbide-a methyl ester in human lung carcinoma cancer cell: efficacy, localization and apoptosis. *Photochem Photobiol*. 2002; 75:644–651. [PubMed: 12081327]

33. Caruso JA, Mathieu PA, Joiakim A, Leeson B, Kessel D, Sloane BF, Reiners JJ Jr. Differential susceptibilities of murine hepatoma 1c1c7 and Tao cells to the lysosomal photosensitizer NPe6: influence of aryl hydrocarbon receptor on lysosomal fragility and protease contents. *Mol Pharmacol.* 2004; 65:1016–1028. [PubMed: 15044632]
34. Ouedraogo G, Morliere P, Maziere C, Maziere JC, Santus R. Alteration of the endocytotic pathway by photosensitization with fluoroquinolones. *Photochem Photobiol.* 2000; 72:458–463. [PubMed: 11045714]
35. White SR, Williams P, Wojcik KR, Sun S, Hiemstra PS, Rabe KF, Dorscheid DR. Initiation of apoptosis by actin cytoskeletal derangement in human airway epithelial cells. *Am J Respir Cell Mol Biol.* 2001; 24:282–294. [PubMed: 11245627]
36. Cabado AG, Leira F, Vieytes MR, Vieites JM, Botana LM. Cytoskeletal disruption is the key factor that triggers apoptosis in okadaic acid-treated neuroblastoma cells. *Arch Toxicol.* 2004; 78:74–85. [PubMed: 14652712]
37. Krajci D, Mares V, Lisa V, Bottone MG, Pellicciari C. Intranuclear microtubules are hallmarks of an unusual form of cell death in cisplatin-treated C6 glioma cells. *Histochem Cell Biol.* 2006; 125:183–191. [PubMed: 16283354]
38. Mashima T, Naito M, Noguchi K, Miller DK, Nicholson DW, Tsuruo T. Actin cleavage by CPP-32/apopain during the development of apoptosis. *Oncogene.* 1997; 14:1007–1012. [PubMed: 9070648]
39. Oshima RG. Apoptosis and keratin intermediate filaments. *Cell Death Differ.* 2002; 9:486–492. [PubMed: 11973607]
40. Marceau N, Schutte B, Gilbert S, Loranger A, Henfling ME, Broers JL, Mathew J, Ramaekers FC. Dual roles of intermediate filaments in apoptosis. *Exp Cell Res.* 2007; 313:2265–2281. [PubMed: 17498695]
41. Lang SH, Hyde C, Reid IN, Hitchcock IS, Hart CA, Bryden AA, Villette JM, Stower MJ, Maitland NJ. Enhanced expression of vimentin in motile prostate cell lines and in poorly differentiated and metastatic prostate carcinoma. *Prostate.* 2002; 52:253–263. [PubMed: 12210485]
42. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. *Histochem Cell Biol.* 2008; 129:705–733. [PubMed: 18461349]
43. Liu T, Wu LY, Choi JK, Berkman CE. Targeted photodynamic therapy for prostate cancer: Inducing apoptosis via activation of the caspase-8/-3 cascade pathway. *Int J Oncol.* 2010; 36:777–784. [PubMed: 20198319]
44. Zhang M, Zhang Z, Blessington D, Li H, Busch TM, Madrak V, Miles J, Chance B, Glickson JD, Zheng G. Pyropheophorbide 2-deoxyglucosamide: a new photosensitizer targeting glucose transporters. *Bioconjug Chem.* 2003; 14:709–714. [PubMed: 12862422]

Fig. 1.
Structures of Ppa and Ppa-CTT-54.

Fig. 2. Changes of α -tubulin and β -tubulin in LNCaP cells after targeted PDT with Ppa-CTT-54.0. **(A)** Immunofluorescence analysis: targeted PDT induced the rearrangement of microtubules (red) to ring-like perinuclear structures following PDT (0, 15, 30 min). **(B)** The nucleolar translocation of α - and β -tubulin (red) at 1 h or 2 h post-PDT. The nuclei were counterstained with H342 (blue). The cellular imaging was visualized by confocal microscopy; distance scale is 20 μ m.

Fig. 3.

Changes of actin in LNCaP cells after targeted PDT with Ppa-CTT-54.0. **(A)** F/G actin staining: cytoplasmic G-actin (green) was immediately destroyed following PDT. In contrast, the F-actin (red) appeared mainly beneath the cellular membrane and persisted longer than G-actin. **(B)** Immunofluorescence detection of actin: normal cytoplasmic and membrane distribution of actin (green) in the control cells with a rapid loss of cytoplasmic actin and slower loss of membrane-localized actin in PDT-treated cells with increasing time (0, 15 and 30 min) following PDT. The nuclei were stained with H342 (blue). The cellular imaging was visualized with a confocal microscopy; distance scale is 20 μ m. **(C)** Western blot analysis: the amount of actin decreased in PDT-treated cells following PDT (0, 15, 30 min), compared to control cells (C). An increase of cleaved actin was observed in PDT-treated cells.

Fig. 4. Changes of cytokeratin 8 and 18 in LNCaP cells after targeted PDT with Ppa-CTT-54.0. **(A)** Immunofluorescence analysis: targeted PDT induced the immediate loss of cytoplasmic intermediate filament networks (cytokeratin 8 and 18, red). A small increase of membrane-localized Cytokeratin 8 and 18 was observed in PDT-treated cells (0, 15 and 30 min). Cell nuclei were stained with H342 (blue). The cellular imaging was visualized with a confocal microscopy; distance scale is 20 μ m. **(B)** Western blot analysis: compared to the control sample, the amount of aggregated (irreversible) and cleaved cytokeratin 8 increased in PDT-treated cell samples following PDT (0, 15, 30 min). In contrast, cytokeratin 18 remained stable except for a small increase of aggregated forms (irreversible) in PDT-treated cell samples.

Figure 5.