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Semiconducting Photothermal Nanoagonist for Remote-controlled Specific Cancer Therapy

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Abstract. Nanomedicines have shown success in cancer therapy, but the pharmacological actions of most nanomedicine are often nonspecific to cancer cells because of utilization of the therapeutic agents that induce cell apoptosis from inner organelles. We herein report the development of semiconducting photothermal nanoagonists that can remotely and specifically initiate the apoptosis of cancer cells from cell membrane. The organic nanoagonists comprise semiconducting polymer nanoparticles (SPNs) and capsaicin (Cap) as the photothermally-responsive nanocarrier and the agonist for activation of transient receptor potential cation channel subfamily V member 1 (TRPV1), respectively. Under multiple NIR laser irradiation at the timescale of seconds, the nanoagonists can repeatedly and locally release Cap to multiply activate TRPV1 channels on the cellular membrane; the cumulative effect is the over-influx of ions in mitochondria followed by the induction of cell apoptosis specifically for TRPV1-positive cancer cells. Multiple transient activation of TRPV1 channels is essential to induce such a cell death both *in vitro* and *in vivo*,

because both free Cap and simple Cap-encapsulated nanoparticles fail to do so. The photothermally-triggered release also ensures a high local concentration of the TRPV1 agonist at tumor site, permitting specific cancer cell therapy at a low systemic administration dosage. Our study thus demonstrates the first example of ion-channel-specific and remote-controlled drug delivery systems for cancer cell therapy.

Keywords: Photothermal nanoagonists, ion channels, remote-controlled drug delivery, specific cancer therapy

Nanomedicine provides new opportunities to enhance bioactivity, prolong bioavailability and reduce side effects of therapeutic agents for cancer therapy.¹⁻⁵ A typical approach of nanomedicines involves the encapsulation of drugs into nanoparticles, followed by the specific delivery and controlled release of drugs into the morbid region.⁶⁻⁸ However, their pharmacological actions still rely on the encapsulated therapeutic agents that are often chemotherapeutic drugs (such as doxorubicin, cisplatin or camptothecin) or exogenous nucleic acids (such as DNA, mRNA, small interfering RNA, and microRNA).⁹⁻¹³ These therapeutic agents induce cell death through disruption of DNA function or modulation of gene expression, and thus the intrinsic therapeutic mechanisms of most nanomedicines are nonspecific to cancer cells.¹⁴⁻¹⁶ Instead, specificity of current nanomedicine often comes from passively and actively targeting capability of nanoparticles and biomarker-stimulated release of therapeutic agents.^{17, 18} However, recent meta-analysis suggests that only 0.7% (median) of the administered dose of nanomedicine is delivered to tumor, while the rest is accumulated in healthy organs.¹⁹ This data not only indicates the low specificity of nanomedicine for cancer therapy but also highlights their potential risk of toxicity to

healthy tissues. Thus, other therapeutic mechanisms are highly desired to develop nanomedicine with high specificity.

Protein ion channels play an important role in the regulation of cell functions.²⁰ In particular, transient receptor potential cation channel subfamily V member 1 (TRPV1) has been revealed to be highly overexpressed in normal cells such as mammalian neuronal cells and cardiomyocytes as well as many types of tumors including bladder cancer cells, breast cancer cells and brain cancer cells (especially in glioblastoma).²¹⁻²⁵ TRPV1 can be activated by external stimuli such as heat, low pH, and vanilloids.²⁶ As a nonselective cation channel that prefers Ca^{2+} over other cations,²⁷ activation of TRPV1 channels allows the influx of Ca^{2+} to across the cell membrane into the cell.²⁸ Due to the feasibility of inducing cell apoptosis through over-influx of ions, protein ion channels provide a pathway to initiate cell death from cell membrane rather than from inner organelles, potentially leading to cell-specific therapy.²⁹ In fact, TRPV1 agonists such as capsaicin (CAP) (8-methyl-*N*-vanillyl-6-nomenamide) have been attempted to treat cancer,³⁰ but most studies are limited to *in vitro* studies with high dosages (~60 $\mu\text{g/mL}$), which cause cell deaths due to their chemical toxicity instead of over-influx of ions.³¹⁻³⁴ Moreover, only few studies are related to cancer treatment *in vivo*, which were conducted through gavage or intratumoral administration of TRPV1 agonists, and showed limited therapeutic efficacy even at extremely high dosage (26-90 mg/kg).³⁵⁻³⁸ Inhibition of tumor growth *in vivo* through intravenous administration of Cap has not been demonstrated, mainly owing to the hydrophobicity-resulted poor biodistribution of small-molecule agonists and their potential systemic toxicity at high dosage.^{30, 39}

In this study, we report the development of semiconducting photothermal nanoagonists that target the TRPV1 protein ion channels for specific cancer therapy. Such semiconducting photothermal nanoagonist utilizes semiconducting polymer nanoparticles (SPNs) as the

temperature-responsive nanocarrier to deliver the TRPV1 agonist (Cap) to tumor site in order to ensure a high local concentration of the TRPV1 agonist at tumor site with a relatively low systemic administration dosage. SPNs are an alternative class of photonic biocompatible nanomaterials made from optically-active semiconducting polymers (SPs).⁴⁰⁻⁴³ Due to their structural versatility and excellent optical properties, SPNs have been exploited for a variety of biological imaging applications.⁴⁴⁻⁵¹ Moreover, SPNs have been revealed to have the photothermal conversion efficiency higher than other inorganic nanomaterials such as carbon nanotubes and gold nanorods.⁵²⁻⁵⁴ These properties make SPNs excellent photothermal converters not only to deliver the TRPV1 agonist to tumor site but also to remotely release Cap under near-infrared (NIR) light irradiation to trigger the ion over-flux through TRPV1 channels for specific cancer therapy.

In the following, the synthesis and characterization of the nanoagonist are first described, followed by *in vitro* validation of its ability to remotely releases Cap under NIR light irradiation and specifically activate TRPV1 ion channels to induce cell apoptosis. At last, the proof-of-concept application of the nanoagonist in the treatment of TRPV1-positive tumor in living mice is demonstrated.

To make the semiconducting photothermal nanoagonist responsive to the deep-tissue penetrating NIR light, a new photothermal SP1, poly(silolodithiophene-*alt*-diketopyrrolopyrrole), was designed and synthesized *via* Stille polymerization between monomers 1 and 2 (Figure 1a). Different from its analogue poly (cyclopentadithiophene-*alt*-diketopyrrolopyrrole) (SP2) (Figure 1b), SP1 had the silicon in the backbone which resulted in the redshifted absorption. The molecular weights of SP1 and SP2 were determined to be ~26565 and ~13660 Da, respectively. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol)2000 (DSPE-PEG₂₀₀₀) were used as the lipid coating

for the nanoagonist, well known to have the phase transition temperate at 41°C (DPPC).⁵⁵ Such a composition allowed the nanoagonist to efficiently release Cap under NIR light irradiation at 808 nm. The cap-encapsulated SPN (SPN1-C and SPN2-C) was self-assembled from DPPC, DSPE-PEG₂₀₀₀, SP, and Cap at a feeding mass ratio of 25:5:0.25:0.03 through nanoprecipitation method (Figure 1c & Table S1, Supporting Information). Dynamic light scattering (DLS) showed the similar sizes within the range of 33-40 nm for SPNs (Figure 1d & Table S1, Supporting Information), and transmission electron microscopy (TEM) revealed the spherical morphology of SPNs (Figure 1d, inset).

The optical and photothermal properties of SPNs were studied and compared with gold nanorods (GNRs). As Cap had no absorption in the visible and NIR region, the absorption of SPN1-C was similar to the cap-free SPN (SPN1-0), showing strong absorption in the NIR region (Figure S2, Supporting Information). Owing to the silicon in the backbone, the absorption maximum of SPN1 was red-shifted by 50 to 810 nm relative to that of SPN2 (758 nm) (Figure 1e), which was better aligned with 808 nm laser. GNR had the maximum at 820 nm, serving as a fair control for SPN1. The peak mass extinction coefficient of SPN1 was 72 cm⁻¹ mg⁻¹ mL, similar to that of SPN2 (61 cm⁻¹ mg⁻¹ mL) but 3.6-fold higher than that of GNR (20 cm⁻¹ mg⁻¹ mL). Under continuous laser irradiation at 808 nm, all the nanoparticles showed gradually increased solution temperatures and reached plateau at t = 360 s (Figure 1f). The temperature of SPN1-C was higher than others at each time point, indicating its faster heating capability. The maximum photothermal temperature of SPN1-C could reach to 73.0 °C under the tested experimental conditions, ~1.2-fold higher than those of SPN2-C (60.0 °C) and GNR (61.9 °C) (Figure 1f & 1g), indicating that SPN1-C was a better photothermal converter on a per mass basis. The photothermal conversion efficiency of SPN1-C at 808 nm was calculated to be 37%, ~1.6-fold higher than that of SPN2-C (23%) and

GNR (23%). In addition, the maximum temperatures of SPN1-C and SPN2-C remained nearly the same for at least 5 heating and natural cooling circles (Figure S3, Supporting Information), demonstrating their excellent photothermal stability. In contrast, GNR showed gradually decreased maximum temperature due to their vulnerability to laser-induced deformation.⁵⁶ These data demonstrated that SPN1-C had higher photothermal conversion efficiency, faster heating capability, and superior photothermal stability as compared with SPN2-C and GNR. Thereby, SPN1s were used as the photothermal nanoagonists for the following *in vitro* and *in vivo* experiments.

Figure 1. Synthesis and characterization of semiconducting photothermal nanoagonist (SPNs). (a) Synthetic route of SP1 *via* Stille polymerization under the reaction conditions (i) PdCl₂(PPh₃)₂ and 2,6-di-*tert*-butylphenol, 100 °C for 6 h. (b) Chemical structures of SP2. (c) Schematic illustration of the synthesis of SPNs. (d) Representative DLS profiles of SPN1-C. Inset: representative TEM

image of SPN1-C. (e) Absorption of SPN1-C, SPN2-C and GNR. (f) The temperature of SPN1-C, SPN2-C and GNR as a function of laser irradiating time. (g) Photos of SPNs and GNR solution (top). Thermal image of SPNs and GNR at their maximum temperatures (down). The concentrations of SPNs and GNR are $15\ \mu\text{g mL}^{-1}$ in $1\times\text{PBS}$ ($\text{pH} = 7.4$). The concentrations of SPNs were calculated based on SP. The laser irradiation wavelength was at 808 nm with a power of $1\ \text{W cm}^{-2}$.

To test the ability of photothermally-triggered release of SPN1-C, Dio, a lipophilic membrane dye, was used as the model drug to incorporate into SPNs. The Dio-doped SPN (SPN1-D) had two new absorption peaks at 460 and 488 nm (Figure S2, Supporting Information), confirming the existence of Dio within nanoparticles. Upon increasing the solution temperature, the fluorescence of SPN1-D at 501 nm gradually decreased (Figure 2b). This was ascribed to the environment-sensitive fluorescence of Dio, indicating the release of Dio from SPN1-D.⁵⁷ Moreover, a sharp transition was observed between 41-44 °C, showing that the phase transition temperature (T_c) of SPNs was at about 42 °C. Similar fluorescence decrease was observed for SPN1-D under continuous laser irradiation at 808 nm (Figure 2c). In contrast, no fluorescence decrease was observed for the SP1-free nanoparticle (NP1-D) under the same experimental conditions (Figure S4, Supporting Information). Thus, the photothermal heat of SPNs was able to increase the temperature, melt the lipid layer and result in the controlled release of Dio (Figure 1f & Figure 2c). Furthermore, the photothermally triggered release of Dio was examined in cell culture. Upon laser irradiation for 5 min, the strong green fluorescence was detected in the cell membranes, confirming that Dio was rapidly released from SPN1-D and inserted to the cellular membranes (Figure 2d). These data implied that the SPN1s had the ideal photothermal feature to remotely activate the release of hydrophobic molecules into the microenvironment.

Figure 2. *In vitro* validation of photothermally-triggered release of model drug from SPNs. (a) Schematic illustration of photothermally triggered release of Dio from SPN1-D. (b) Fluorescence intensities of SPN1-D at 501 nm after incubation at different temperature for 5 mins. (c) Fluorescence intensities of SPN1-D before and after laser irradiation for 5 mins with a power of 1 W cm⁻². Inset: the corresponding fluorescence images of SPN1-D before and after laser irradiation. (d) Confocal laser scanning microscopy (CLSM) images of SPN1-D in U373 cells before and after laser irradiation of SPN1-D for 5 mins. The temperature of the medium was monitored during laser irradiation and was kept between 41.5 - 42.5 °C. The final concentration of SP for SPN1-D was 15 µg mL⁻¹. The final concentration of Dio for SPN1-D was 1 µg mL⁻¹.

The ability of SPN1-C to activate TRPV1 ion channels was examined using U373 glioma cells and HeLa cells as the TRPV1 positive and the negative cells, respectively^{21, 53}. The Ca²⁺ influx was monitored in real-time using a fluorescent turn-on intracellular indicator (Fluo-8) (Figure 3a). Under laser irradiation, no fluorescence increase of Fluo-8 in U373 cells was observed for the

unloaded SPN (SPN1-0) and the Cap-encapsulated but SP1-free nanoparticles (NP1-C) (Figure 3b); in contrast, significant fluorescence increase was detected for SPN1-C (Figure 3b & Figure S5, Supporting Information). Furthermore, no fluorescence increase was observed when U373 cells were pretreated with a specific TRPV1 antagonist (capsazepine, Cpz) to block the TRPV1 ion channels, or the TRPV1-negative cells (HeLa and NIH-3T3) were tested (Figure 3b & Figure S6, Supporting Information). Thereby, Ca^{2+} influx was only initiated for U373 in the presence of SPN1-C under NIR laser irradiation, validating that TRPV-1 ion influx was specifically activated by the NIR light triggered release of Cap from SPN1-C. Additionally, real-time quantification of Fluo-8 fluorescence revealed that the TRPV-1 ion influx could be rapidly activated within 15 s, and remained active for 20 s under NIR laser irradiation (Figure 3c). Such ion influx could be repeatedly activated and silenced at least for 10 cycles (Figure 3d), and ~5-10 % Cap could release from SPN1-C for each cycle (Figure S7, Supporting Information), implying the feasibility of the multiple activation of the TRPV-1 ion influx for the same cells. These results clearly confirmed that SPN1-C acted as a photothermal nanoagonist to specifically and repeatedly trigger the ion influx of TRPV-1 positive cancer cells.

Figure 3. *In vitro* activation of TRPV1 ion channels by NIR light triggered release of Cap from SPNs. (a) Schematic illustration of photothermal mechanism of activation of TRPV-1 Ca^{2+} channels in cells. The intracellular concentration of Ca^{2+} was monitored in real-time by using Fluo-8 as the indicator, which turned on its fluorescence upon binding with Ca^{2+} . (b) Fluorescence images of U373 or HeLa cells treated with different SPNs including SPN1-C, SPN1-0, and NP1-C before and after laser irradiation at 808 nm ($\sim 0.4 \text{ W cm}^{-2}$) for 35 s. (c) The fluorescence intensities of Fluo-8 as a function of laser irradiation time for SPN1-C, SPN1-0, and NP1-C. (d) The fluorescence intensity of Fluo-8 as a function of the cycle number of laser irradiation for SPN1-C. The final concentrations of SP for SPNs were $15 \mu\text{g mL}^{-1}$. The final concentrations of Cap for SPN1-C and NP1-C were $3 \mu\text{g mL}^{-1}$.

To evaluate the toxicity of SPN1-C, cell viability assays were examined for U373 cells (TRPV1 positive), HeLa cells (TRPV1 negative) and mouse fibroblast NIH-3T3 cells (normal cells) (Figure 4b). Note that all the nanoparticles including SPN1-0, SPN1-C and NP1-C were intrinsically

nontoxic to cells (Figure 4b and Figure S7, Supporting Information). Laser irradiation was carefully controlled in a discontinuous manner (irradiation for 35 s at the interval of 25 s) not only to maintain the temperature below 43 °C s to minimize the direct toxicity caused by photothermal effect, but also to induce the multiple activation of ion influxes as confirmed in Figure 3. With such a multiple stimulation under mild photothermal condition, the cell viabilities of U373 were ~76 and 21% for SPN1-0 and SPN1-C, respectively; while the viabilities of the TRPV1-negative cells were similar for SPN1-0 and SPN1-C (72% and 68% for HeLa, 57% and 58% for NIH-3T3). Thus, only the nanoagonist (SPN-C) could specifically kill TRPV1-positive cancer cells under photothermally triggered release of Cap. This was further verified by the increased cell viability when U373 cells were protected with the TRPV1-specific antagonist (Cpz) before treatment (Figure S8, Supporting Information). By contrast, similar to the previous reports, free Cap (3 $\mu\text{g mL}^{-1}$) showed very limited mortality (~15%) for U373 cells regardless of laser irradiation (Figure S7, Supporting Information).²¹ This should be attributed to the fact that addition of the same amount of free Cap could only activate the TRPV1 ion channels once.²¹ Thus, these data indicate the importance of multiple activation of TRPV1 ion channels and the necessity of photothermally triggered release of Cap in the initiation of TRPV1-specific toxic cascade.

To understand the toxic mechanism of the semiconducting photohermal nanoagonist (SPN1-C) towards TRPV1-positive cancer cells, the fluorescent probes JC-1, Alex-488 conjugated cleaved caspase-3 antibody and FITC Annexin V were used to evaluate the damage to mitochondrial membrane potential (MMP), the activity of caspase-3, and the evagination of phosphatidylserine, respectively (Figure 4c, 4d, 4e & Figure S9, Supporting Information). Only for SPN1-C mediated laser treatment, the fluorescence signals induced by the depolarized MMP, the activated caspase-3 and the presence of phosphatidylserine in extracellular surface were detected, while no such

signals were observed for the cap-free SPN (SPN1-0) and the SP-free Cap nanoparticles (NP1-C). Moreover, pretreatment of Cpz effectively blocked all these apoptotic related signals. These imaging results proved that the cellular apoptosis was closely associated with SPN1-C activated TRPV-1 ion influx. Accordingly, the toxic mechanism of SPN1-C towards TRPV1-positive cancer cells is proposed as follows: NIR light efficiently triggers the release of Cap from SPN1-C, leading to the rapid increase in the local concentration of Cap in the microenvironment of cells. The released Cap activates the ion influx through TRPV1 channels. As a cumulative result of multiple activation of TRPV1 channels, the Ca^{2+} overload eventually occurs, leading to the depolarization of MMP. Thus, cytochrome *c* is released to activate caspase-3 and eventually cell apoptosis.^{29, 32}

Figure 4. *In vitro* anti-cancer studies on SPNs. (a) Schematic illustration of proposed apoptosis mechanism induced by the semiconducting photothermal nanoagonist (SPN1-C). (b) Cell viability

of U373 cells, HeLa cells, and Mouse fibroblast NIH-3T3 cells after laser irradiation of SPNs for 5 mins (25 s break after 35 s illumination). The concentration of SP for SPN1-C and SPN1-0 was $15 \mu\text{g mL}^{-1}$. The concentration of CAP for SPN1-C and NP1-C was $3 \mu\text{g mL}^{-1}$. $**P < 0.01$; n.s.: no statistically significant differences. (c) MMP of U373 cells analysis using JC-1 mitochondrial membrane dye. U373 cells were treated with SPNs for 5 mins (25 s break after 35 s illumination) under laser irradiation. Red fluorescence implies active mitochondria with the polarized membrane, while green fluorescence implies unhealthy mitochondria with depolarized membrane. (d) The activity of caspase-3 analysis using cleaved caspase-3 antibody. U373 cells were treated with SPNs for 5 mins (25 s break after 35 s illumination) under laser irradiation. Caspase-3 were labeled with Alex-488 conjugated cleaved caspase-3 antibody (Green). The nuclei were counterstain with DAPI (Blue). (e) The evagination of phosphatidylserine analysis using FITC Annexin V apoptosis kit. U373 cells were treated with SPNs for 5 mins (25 s break after 35 s illumination) under laser irradiation. Phosphatidylserine were bound to FITC Annexin V (Green). The laser irradiation wavelength was at 808 nm with a power of $\sim 0.4 \text{ W cm}^{-2}$ and the temperature of the medium was monitored during laser irradiation and was kept between $41.5\text{--}42.5^\circ\text{C}$.

The therapeutic capability of the semiconducting photothermal nanoagonist (SPN1-C) was tested on U373 (TRPV1 positive) and HeLa (TRPV1 negative) xenograft tumor mouse models and compared with other controls including the Cap-free SPN (SPN1-0), the Cap nanoparticles (NP1-C) and free Cap at the same dosages based on SP or Cap (Figure 5a&5b). The total dosage of Cap was 0.72 mg kg^{-1} , $\sim 36\text{--}125$ times lower than the previous studies,³⁵⁻³⁸ which could avoid the nonspecific chemical toxicity of Cap itself to normal organs. U373 or HeLa cells were inoculated subcutaneously to BALB/c nude mice at the left flank. When the tumor volume reached about $60\text{--}80 \text{ mm}^3$, the mice were randomly allocated to different groups and treated with the

different formulations through tail vein for the first time, and this date was designated as Day 1. The tumor-bearing mice were treated through tail vein for 2 additional times on Days 3 and 5 (Figure 5a). Because the accumulation of SPN1-C and SPN1 reached maximum at 8 h post-injection as confirmed by real-time photoacoustic imaging (Figures S10&S11, Supporting Information), photothermally triggered release of Cap was conducted at this time point with laser irradiation at 808 nm. The laser irradiation time was controlled to be 5 min in a discontinuous manner (25 s break after 35 s illumination) so that the temperature of tumor areas was controlled below 43 °C and the release of cap was multiply triggered (Figures 5c&5d). This temperature was high enough to trigger the efficient release of Cap from SPN1-C as witnessed by *in vitro* studies, but insufficient to induce nonspecific photothermal ablation of cancer cells.^{55, 58} The tumor sizes for different treatment groups were continuously monitored every other day for 19 days (Figures 5e&5f). Without laser irradiation, all the treatment showed negligible inhibition on both U373 and HeLa tumors (Figures 5e &5f), indicating that these nanoparticles themselves did not have anticancer therapeutic effect, consistent with the *in vitro* results. With laser irradiation, SPN1-0 only showed a subtle inhibition (no statistically significant difference relative to the saline-treated mice) on the growth of both U373 and HeLa tumors, and failed to completely inhibit the tumor growth (Figures 5e&5f). This again proved that the mild photothermal effect of SPN1-0 (between 41.5-42.5 °C) had negligible effect on tumor inhibition under the tested experimental conditions. In contrast, SPN1-C effectively suppressed the growth of U373 tumor (TRPV1 positive) but not HeLa tumor (TRPV1 negative) under the same laser irradiation condition (Figures 5e&5f). Such a selective tumor inhibition of the nanoagonist (SPN1-C) implied that its therapeutic mechanism was specific to TRPV1 ion channels, and related to the photothermally-triggered releases of Cap. Moreover, the fact that no inhibition effect was observed for the cap nanoparticles (NP1-C)

indicated the importance of in-situ remote-controlled release of Cap in the microenvironment of tumor for such a specific cancer therapy.

The immunohistochemical staining revealed a large fraction of apoptotic cells for U373 tumor of SPN1-C treated mice after laser irradiation, but only a limited number for SPN1-0 treated mice (Figure 5g). No obvious apoptosis was observed for other control groups (without laser irradiation or saline treatment mice with laser irradiation, Figure S12, Supporting Information) as well as for TRPV1 negative HeLa tumors regardless of treatments (Figure 5g). In addition, no tissue damage was detected for normal organs such as liver, spleen, and kidney (Figure S13, Supporting Information), and no significant weight loss was observed for all groups (Figure S14, Supporting Information), demonstrating that the biosafety of SPN1-C in cancer therapy. These histological data verified at the cellular level that SPN1-C effectively induced the apoptosis of TRPV1 positive cancer cells, which was in accordance with the *in vivo* observation of inhibited tumor growth (Figure 5e&5f).

Figure 5. *In vivo* tumor therapy using semiconducting photothermal nanoagonist. (a) Schematic illustration of semiconducting photothermal nanoagonist (SPN1-C) therapy in tumor models. (b) Schematic illustration of the mechanism of anti-tumor responses induced by SPN1-C in TRPV1 positive (U373 cells) or TRPV1 negative (HeLa cells) tumor models. (c) Mean tumor temperature during laser irradiation after systemic administration of saline and different SPNs at post injection time of 8 h of U373 tumor-bearing mice. Error bars were based on standard error of mean (SEM) ($n = 4$). The laser irradiation wavelength was at 808 nm with a power of 0.3 W/cm^2 . (d) IR thermal images of U373 tumor-bearing mice under 808 nm laser irradiation (0.3 W/cm^2) after systemic administration of saline and different SPNs at post injection time of 8 h. (e, f) U373 tumor growth curves (e) and HeLa tumor growth curves (f) of different groups of mice after systemic treatment with saline, different SPNs and free Cap with and without laser irradiation. The saline and different SPNs were injected to mice every 2 days for 3 times. Error bars were based on standard error of mean (SEM) ($n = 4$). $**P < 0.01$; n.s.: no statistically significant differences. (g) Immunofluorescence staining of U373 tumor slices and HeLa tumor slices with antibody to cleaved caspase-3. The tumors were acquired at day 19 after treatment with saline, NP1-C, SPN1-0, or SPN1-C with laser irradiation. Green fluorescence indicates the signal from cleaved caspase-3 staining, while the blue fluorescence is from the nucleus staining.

Two optically-active semiconducting polymers (SP1-2) were synthesized and transformed into water-soluble nanoparticles (SPN1-2) with strong NIR absorption. Simple replacement of one carbon atom in the polymer backbone with silicon red-shifted the maximum absorption from 760 nm for SPN2 to 810 nm SPN1 due to the stronger electron donating ability of silicon, resulting in a better alignment with the common 808-nm laser. The photothermal conversion efficiency of SPN1 (37%) was 1.6-times higher than the GNRs (23%) and generally higher than two-

dimensional nanomaterials such as MoS₂ nanosheets⁵⁹ and WS₂ nanosheets⁶⁰. With its excellent photostability, SPN1 was indeed an outstanding photothermal agent. Utilization of temperature-responsive lipids to coat SPN1 led to a controlled drug-delivery system wherein the release of cargo could be remotely triggered *via* photothermal signal.^{61,62} Despite the extensive applications of SPNs in sensing, imaging and therapy,⁴⁴⁻⁵¹ this is the first design of photothermally-triggered delivery system based on SPNs.

Although potential of ion-channel agonists for cancer therapy has been realized, no *in vivo* studies have been conducted through intravenous administration of Cap, while those based on oral and intratumoral treatment showed limited anticancer efficacies and required extremely high dosage of Cap between 26 to 90 mg Kg⁻¹.³⁵⁻³⁸ This is reasonable because ion-channel agonists such as Cap often have poor water-solubility and are not suitable for intravenous administration alone. Moreover, *in vitro* studies have revealed that other TRPV1-independent apoptotic pathways are activated when the dosage of Cap is higher than 15 µg mL⁻¹. Such nonspecific toxicity of Cap has been reported to induce substantial toxicity when intravenous administration of free Cap is conducted at a relatively high dosage.^{39, 63}

The poor bioavailability and nonspecific toxicity issues of Cap were solved when it was encapsulated in SPN1 and formulated into a photothermal nanoagonist (SPN1-C). At the total dosage of Cap (0.72 mg kg⁻¹) that was ~36-125 time lower than those used for oral and intratumoral treatment, SPN1-C could be administered *via* intravenous injection and home to tumor through EPR effect in living mice due to their favorable size. Under NIR laser irradiation at the timescale of seconds, SPN1-C could repeatedly release Cap to multiply activate TRPV1 ion channels on the cellular membrane, cumulatively causing the over-influx of ions in mitochondria and subsequently inducing specific cell apoptosis for TRPV1-positive cancer cells. As a result, SPN1-C effectively

and specifically inhibited the growth of TRPV1-positive U373 tumor but not TRPV1-negative HeLa tumor (Figure 5e&5f), while free Cap failed to inhibit neither tumor at the same dosage (Figure 5e&5f). Moreover, the ineffectiveness of the Cap-encapsulated but SP-free nanoparticle (NP-C) in tumor inhibition proved the necessity of light-triggered multiply activation of TRPV1 ion channels to maintain a high concentration of Cap in the microenvironment to initiate apoptosis. The high specificity and low dosage of SPN1-C eventually resulted in specific cancer therapy with minimized systematic toxicity.

In summary, we have introduced semiconducting photothermal nanoagonists as a kind of ion-channel targeted nanomedicine to specifically initiate cell apoptosis through cell member rather than inner organelles. Such SPN-based nanoagonists represent the first remote-controlled drug delivery system that permits ion-channel-specific cancer therapy in living mice. In view of completely organic ingredients, composition versatility and easy surface modification of SPNs, the design concept of semiconducting photothermal nanoagonists could be extended to treat other diseases such as atherosclerosis or fatty liver disease when relevant agonists are used.^{64,65}

ASSOCIATED CONTENT

Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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