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Photothermal ablation of tumor cells using a single-walled carbon nanotube–peptide composite

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ABSTRACT

Single-walled carbon nanotubes (SWCNTs) are known to have great potential for biomedical applications such as photothermal ablation of tumor cells in combination with near-infrared (NIR) irradiation. In this study, the photothermal activity of a novel SWCNTs composite with a designed peptide having a repeated structure of H-(-Lys-Phe-Lys-Ala-)$_7$-OH [$(KFKA)_7$] against tumor cells was evaluated in vitro and in vivo. The SWCNT-$(KFKA)_7$ composite demonstrated high aqueous dispersibility that enabled SWCNTs to be used in tumor ablation. The NIR irradiation of SWCNT-$(KFKA)_7$ solution resulted in a rapid temperature increase dependent on the SWCNTs concentration up to 50 μg/ml. Three minutes of NIR irradiation of a colon 26 or HepG2 cell culture incubated with SWCNT-$(KFKA)_7$ resulted in remarkable cell damage, while that by single treatment with SWCNT-$(KFKA)_7$ or NIR irradiation alone was moderate. The intratumoral injection of SWCNT-$(KFKA)_7$ solution followed by NIR irradiation resulted in a rapid increase of the temperature to 43 °C in the subcutaneously inoculated colon 26 tumor based on thermographic observation and remarkable suppression of tumor growth compared with treatment with only SWCNT-$(KFKA)_7$ injection alone or NIR irradiation alone. These results suggest the a great potential of an SWCNT–peptide composite for use in photothermal cancer therapy.

1. Introduction

Carbon nanotubes (CNTs) have been widely studied from the viewpoint of potential medical applications because of their unique and useful physical, chemical, electrical, and mechanical properties [1,2]. Attempts have been made, for example, to utilize the intrinsic hyperthermic property of CNTs induced by near-infrared (NIR) irradiation for the photothermal ablation of cancer cells [3]. In general, however, studies on the use of CNTs in biological, medical, and pharmaceutical applications have not advanced because of the high hydrophobicity of CNTs, which makes them incompatible with living organisms or biological settings. To improve the poor dispersibility of CNTs into aqueous media, we have developed a novel composite material of single-walled carbon nanotubes (SWCNTs) with artificially designed peptides and evaluated its chemical and physicochemical characteristics with an aim toward biomedical application [4]. The formation of the composite of SWCNTs with peptide (SWCNT–peptide) was confirmed by atomic force microscopy, transmission electron microscopy, and molecular modeling [4].

In an ongoing series of investigations, we have evaluated the utility of SWCNT–peptide in various aspects of biomedical application including tumor ablation. Near-infrared light (NIR) at a region of 700–900 nm in wavelength is known to be relatively harmless to the body even though it penetrates deep into the tissue [5]. The electromagnetic wave in this region shows minimal absorption by media such as hemoglobin (absorption <650 nm) and water (absorption >900 nm) [6], whereas SWCNTs can effectively absorb NIR and convert its energy into heat [3]. Because of this feature, SWCNTs would seem to be promising for use in noninvasive photothermal cancer therapy under NIR irradiation [7–9].

Among tested peptides in previous report, composite with H-(-Lys-Phe-Lys-Ala-)$_7$-OH [$(KFKA)_7$] showed satisfactory dispersibility and stability in water for injection [4]. The expected binding to tumor tissue based on electrostatic interaction [10] and the possibility of introducing various functions such as controlled release of anticancer agents [4,10] further encourage the application of $(KFKA)_7$ in cancer ablation. Thus, the $(KFKA)_7$ peptide was employed to solubilize and thereby improve

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the therapeutic effects of SWCNT, and the prepared SWCNT-$(KFKA)_7$ composite was evaluated for its photothermal characteristics and antitumor activity in combination with NIR laser irradiation in this study.

2. Materials and methods

2.1. Ethics statement

All animal experiments were carried out in accordance with Guide for the Care and Use of Laboratory Animals by the U.S. National Institutes of Health (Bethesda, MD) and the Guidelines for Animal Experiments of Kyoto University (Kyoto Japan). The protocol was approved by the Kyoto University Animal Experimentation Committee (iCeMS Kyo-7-4). All surgery was performed under sodium pentobarbital anesthesia.

2.2. Materials

Purified SWCNTs (HiPco; Lot No. P0343) were purchased from Carbon Nanotechnologies (Houston, TX). The $(KFKA)_7$ peptide shown in Fig. 1 was designed by expecting self-assembled wrapping of SWCNTs [4] and synthesized by GL Biochem (Shanghai, China) with more than 90% purity. Triton X-100 was purchased from Sigma-Aldrich (St. Louis, MO). RPMI 1640 medium, Dulbecco's modified Eagle's medium (DMEM), and Hanks' balanced salt solution (HBSS) were obtained from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum was purchased from MP Biomedicals (Irvine, CA). Other chemicals were purchased from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemicals (Osaka, Japan).

2.3. Preparation of SWCNTs solution

Dispersion of SWCNTs was prepared by sonicating SWCNTs with $(KFKA)_7$ peptide in aqueous media. One milligram of SWCNTs and 10 mg of $(KFKA)_7$ peptide were weighed and put into a test tube. Then 5 ml of saline or dextrose solution was added to the test tube, and sonication was performed for 1 h with an ultrasonic disruptor UD-201 (TOMY Digital Biology, Tokyo, Japan) on ice.

2.4. Quantificaion and size determination of SWCNTs in the solution

The concentration of SWCNTs in the solution was determined from the optical absorbance at 808 nm according to the previous report [3]. An absorptive coefficient of $A_{1mg/ml} = 40.3$ was obtained from the calibration line of the SWCNTs suspension (0–25 μg SWCNTs/ml) prepared with Triton X-100 [4]. The length of SWCNTs was estimated from atomic force microscopic (AFM) image. AFM observation was performed for SWCNTs in the solution using an MFP-3D-SA atomic force microscope (Asylum Technology, Santa Barbara, CA) in AC mode. AC200-TS microcantilevers (Olympus, Tokyo, Japan) with a force constant of $k = 9\ N \cdot m^{-1}$ and a nominal tip radius of less than 10 nm were used.

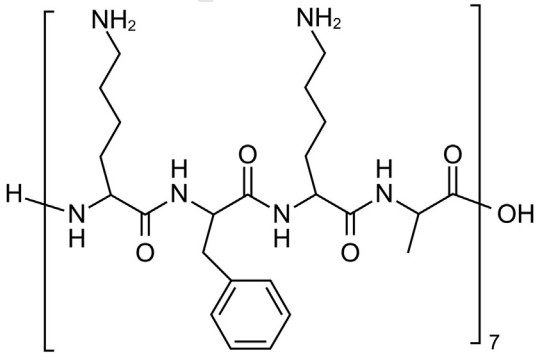

Fig. 1. Chemical structure of $(KFKA)_7$ peptide.

Measurements were performed in air. The size of SWCNT in AFM image was measured using an image analysis software, Image J (Ver. 1.47, http://rsbweb.nih.gov/ij/).

2.5. Photothermal characteristics of SWCNTs solution with NIR irradiation

The photothermal characteristics of the SWCNT-$(KFKA)_7$ composite with NIR laser irradiation was evaluated by continuous temperature monitoring of its aqueous solution. One milliliter of SWCNTs solution supplemented with $(KFKA)_7$ peptide (0–100 μg SWCNTs/ml) in a vial with a diameter of 1.6 cm and cross-section of 2.0 cm^2 was irradiated with an NIR laser of 1.2 W (808 nm) (Femtosecond Titanium Sapphire laser Chameleon-RF; Coherent, Santa Clara, CA) over an exposure area of 0.2 cm^2 (6 W/cm^2). During irradiation, the SWCNTs solution was stirred with a magnetic stirrer and the temperature of the solution was measured each second using a fiber optic temperature sensor Reflex (Neoptix, QC, Canada).

2.6. Cell culture

The murine rectum carcinoma cell line (colon 26) and human hepatocellular carcinoma cell line (HepG2) were cultured in RPMI 1640 medium and DMEM, respectively, under 5% CO_2 at 37 °C. The culture medium was supplemented with 10% fetal bovine serum, 100 IU/ml of penicillin, and 100 μg/ml of streptomycin.

2.7. Cytotoxicity assay for $(KFKA)_7$ peptide

The cytotoxicity of the $(KFKA)_7$ peptide was evaluated by measuring the activities of lactate dehydrogenase (LDH) released from damaged cells to the medium [11,12]. Colon 26 cells and HepG2 cells were seeded in 24-well plates (1×10^5 cells/well) and incubated overnight. Then, the culture medium was removed and 400 μl of medium containing 0–100 μM of $(KFKA)_7$ peptide and 1% FBS were added. After 6 h of incubation, the plates were centrifuged at 250×*g* for 10 min at 4 °C, and the activity of LDH in the supernatant was measured with an LDH Cytotoxicity Detection Kit (Takara Bio, Shiga, Japan). As a positive control, cells were treated with the medium containing 1% Triton X-100 for 6 h, and the amount of released LDH was measured in the same way. The 50% inhibitory concentration (IC_{50}) values of $(KFKA)_7$ peptide against both cell lines were calculated by fitting to a logistic model function.

2.8. In vitro evaluation of cell death induced by SWCNT-$(KFKA)_7$ with NIR irradiation

The damage to tumor cells induced by thermal ablation with SWCNT-$(KFKA)_7$ and NIR irradiation was evaluated in vitro. Colon 26 cells and HepG2 cells (2×10^5 cells/500 μl) were seeded in an 8-well chambered cover glass (Asahi Glass, Tokyo, Japan) and incubated overnight. After changing the culture medium, 1.5 and 5 μl of SWCNTs solution (200 μg SWCNTs/ml) were added to the 400 μl of culture medium of colon 26 cells and HepG2 with final concentrations of 0.75 μg/ml and 2.5 μg/ml, respectively. After 2 h of incubation, the wells were exposed to irradiation with an 808-nm NIR laser for 3 min at 1.2 W, collected in a 1.5-ml tube, and stained with a Live-Dead cell staining kit (Biovision, Mountain View, CA).

The fluorescence microscopic observation was performed using a Biozero Bz-8000 (Keyence, Osaka, Japan) with Ex/Em = 470/535 nm (Live-Dye fluorescing green) and Ex/Em = 540/605 nm (propidium iodide fluorescing red), respectively. Confocal microscopy was carried out with A1RMP (Nikon, Tokyo, Japan) with Ex/Em = 488/525 nm (green) and Ex/Em = 562/595 nm (red), respectively. For flow cytometric analysis, cells were stained with propidium iodide of a Live-Dead cell staining kit and the number of labeled cells was analyzed by a FACSCant II (BD biosciences, San Jose, CA) with Ex/Em = 488/585 nm.

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