Biomaterials 146 (2017) 136–145

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

DNA nanotechnology-based composite-type gold nanoparticle-immunostimulatory DNA hydrogel for tumor photothermal immunotherapy

Tomoya Yata [a], Yuki Takahashi [a], Mengmeng Tan [a], Hirotaka Nakatsuji [b], Shozo Ohtsuki [a], Tatsuya Murakami [c], Hiroshi Imahori [b], Yuka Umeki [a], Tomoki Shiomi [a], Yoshinobu Takakura [a], Makiya Nishikawa [a, d, *]

[a] Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, 606-8501, Japan
[b] Department of Molecular Engineering, Graduate School of Engineering, Kyoto University, Nishikyo-ku, Kyoto, 615-8510, Japan
[c] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama, 939-0398, Japan
[d] Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan

ARTICLE INFO

Article history:
Received 1 May 2017
Received in revised form
7 September 2017
Accepted 8 September 2017
Available online 9 September 2017

Keywords:
Injectable hydrogel
Photothermal immunotherapy
CpG motif
Laser responsive immunoadjuvant
DNA nanotechnology

ABSTRACT

Success of tumor photothermal immunotherapy requires a system that induces heat stress in cancer cells and enhances strong anti-tumor immune responses. Here, we designed a composite-type immunostimulatory DNA hydrogel consisting of a hexapod-like structured DNA (hexapodna) with CpG sequences and gold nanoparticles. Mixing of the properly designed hexapodna and oligodeoxynucleotide-modified gold nanoparticles resulted in the formation of composite-type gold nanoparticle-DNA hydrogels. Laser irradiation of the hydrogel resulted in the release of hexapodna, which efficiently stimulated immune cells to release proinflammatory cytokines. Then, EG7-OVA tumor-bearing mice received an intratumoral injection of a gold nanoparticle-DNA hydrogel, followed by laser irradiation at 780 nm. This treatment increased the local temperature and the mRNA expression of heat shock protein 70 in the tumor tissue, increased tumor-associated antigen-specific IgG levels in the serum, and induced tumor-associated antigen-specific interferon-γ production from splenocytes. Moreover, the treatment significantly retarded the tumor growth and extended the survival of the tumor-bearing mice.

1. Introduction

Photothermal immunotherapy is a promising modality for cancer treatment and combines local photothermal therapy and immunostimulation [1,2]. Decades ago, a therapeutic strategy based on localized photothermal stress focused on simply ablating cancer cells, like surgical excision, by heat stress induced by a near infrared (NIR) laser and photosensitizing materials [3]. It was believed that the higher temperature could be used to achieve better therapeutic outcomes. However, increased evidence has revealed that heating tumor tissues to a moderate temperature of 40–45 °C has many therapeutic benefits, including cancer cell death and immunostimulation [4,5]. Heat stress at this temperature range arrests cell proliferation and induces cell death through protein denaturation and changes in cell membranes [6–8]. In addition to these effects, recent studies have demonstrated that cancer cells that have suffered from moderate heat stress upregulate the expression and release of heat shock proteins (HSPs), inducing diverse immune reactions [9]. Extracellular Hsp70, a member of the HSPs that plays the role of a molecular chaperone within cells, is able to bind directly to CD40, Toll-like receptor (TLR)-2, and TLR4 on antigen presenting cells (APCs) to induce proinflammatory cytokine production and antigen uptake by the APCs [10–13]. Moreover, Hsp70 is recognized by natural killer (NK) cells and enhances their proliferation and cell lysis activity [14–17]. Hsp70 also binds with tumor-associated antigens (TAAs) through its polypeptide binding domain located at the C-terminal. The Hsp70-TAA complex is recognized by HSP receptors, such as LOX-1 and CD91, on dendritic cells, macrophages, and other APCs [10,13],

* Corresponding author. Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan.
E-mail address: makiya@rs.tus.ac.jp (M. Nishikawa).

http://dx.doi.org/10.1016/j.biomaterials.2017.09.014
0142-9612/

and promotes the cross presentation of TAA to $CD8^+$ T cells via MHC class I to lead to tumor-specific $CD8^+$ T cell responses [18–20].

Progress in DNA nanotechnology has increased attention towards DNA as a material to be used in the design of novel unique nanostructures for biomedical applications. Recent studies have demonstrated that constructing DNA nanostructures significantly modulates the biological activity of DNA [21–23]. We previously reported that the formation of polypod-like structured DNA (polypodna) using oligodeoxynucleotides (ODNs) containing CpG sequences, a well-known ligand for TLR9, potentiated the activity of the CpG ODN to induce cytokine release from TLR9-positive cells, which was associated with increased cellular uptake of the ODNs [24,25]. Recently, we developed a novel technology to prepare DNA hydrogels through self-assembling of nanostructured DNAs, such as polypodnas, and demonstrated that this DNA hydrogel was useful as a novel vaccine adjuvant with many advantages: safety, injectability, biodegradability, tolerability, ability to stimulate innate immunity, and delivery potential of antigens to APCs [26]. Since the self-assembling technology is based on the hybridization between two DNA ends, the technology can be applied to construct composite materials using two or more different nanomaterials containing DNA for hybridization.

Here, we designed a novel photothermally active and immunostimulatory agent for tumor photothermal immunotherapy using the self-assembling technology. Gold nanospheres (AuNS) and gold nanorods (AuNR), two major gold nanoparticles that show surface plasmon resonance with high extinction coefficients and have been widely used in photothermal therapy, were selected as photosensitizers, because they are safe and have high affinity to DNA, that is, gold nanoparticles can directly load DNA on their surface [27,28]. Photothermal and immunostimulatory properties of the AuNS-DNA hydrogel were first examined in detail *in vitro*, and then the antitumor activity was examined in tumor-bearing mice using the AuNR-DNA hydrogel.

2. Methods

2.1. Preparation of AuNP-DNA hydrogel

AuNS (average diameter of 50 nm) and AuNR (dimensions of 38 by 10 nm) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All ODNs were purchased from Integrated DNA Technologies, Inc. (Coralville, IA, USA). The sequences of the ODNs used are summarized in Table 1. To prepare ODN-modified AuNS, i.e., AuNS-ODN (cg) and AuNS-ODN (gc), ODN (cg)-oligoA or ODN (gc)-oligoA, a CpG or GpC ODN with polyadenine sequence, was adsorbed onto the surface of AuNS, respectively, according to the method previously reported by Juewen Liu et al. [29]. Briefly, 500 mM citrate·HCl buffer (pH 3) was added to the mixture of AuNS and ODN to a final concentration of 10 mM, and the mixture was incubated at room temperature for 3 min. Then, the pH of the mixture was adjusted back to neutral by adding 500 mM HEPES buffer (pH 7.6), and the mixture was incubated for another 10 min at room temperature. The AuNS-ODNs were collected by centrifugation at 20,000 × *g*, and washed with purified water. Separately, four types of hexapodnas, i.e., hPODNA (cg)-A, hPODNA (gc)-A, hPODNA (cg)-B, and hPODNA (gc)-B, were prepared by mixing six equimolar ODNs for each preparation as previously reported. Here, hPODNA (cg)-B and hPODNA (gc)-B contain the 8-nucleotide-long single-stranded 5′-ends complementary to the 5′-end of ODN (cg)-oligoA and ODN (gc)-oligoA, and the 8-nucleotide-long single-stranded 5′-ends of hPODNA (cg)-A and hPODNA (gc)-A are non-complementary to (and the same sequence as) ODN (cg)-oligoA and ODN(gc)-oligoA. Again, (cg) and (gc) indicate that the hexapodnas contain the CpG or GpC sequence, respectively. The formation of these hexapodnas was assessed by polyacrylamide gel electrophoresis (PAGE) as previously reported [25]. Then, the following six types of samples were prepared by mixing equivolumes of two components at room temperature: (1) AuNS-hydrogel (cg), (2) AuNS-hydrogel (gc), (3) AuNS-ODN/hPODNA (cg), (4) AuNS-ODN/hPODNA (gc), (5) DNA hydrogel (cg), and (6) DNA hydrogel (gc). The combinations of the components used for each sample preparation are summarized in Table 2. AuNS-hydrogel (cg), AuNS-hydrogel (gc), DNA hydrogel (cg), and DNA hydrogel (gc) are the formulations that are intended to form hydrogels, whereas AuNS-ODN/hPODNA (cg) and AuNS-ODN/hPODNA (gc) are the mixtures of AuNS-ODN and hexapodna, which will not form a hydrogel. AuNR-containing formulations were prepared by mixing AuNR-ODN and hPODNAs.

2.2. Scanning electron microscope imaging

The structure of the AuNS-hydrogel (cg) was observed by scanning electron microscopy as reported previously [30]. Briefly, the AuNS-hydrogel (cg) was fixed with 2% glutaraldehyde at room temperature overnight, dehydrated with increasing concentrations of ethanol, which was replaced with butyl alcohol, and freeze-dried. The dried material was observed using a field-emission scanning electron microscope (SEM) (TM3000, Hitachi, Tokyo, Japan).

2.3. UV absorption spectra

UV–visible absorption spectra were measured on a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA).

2.4. Evaluation of photothermal behavior

AuNS-hydrogel (cg) was exposed to continuous wave laser irradiation at 532 nm at two different levels of strength: 1 or 2 W/cm^2 (Verdi-V10, COHERENT, Santa Clara, CA, USA). The temperature of the AuNS-hydrogel (cg) was monitored using a contact thermometer (TT-508, TANITA, Tokyo, Japan).

2.5. Evaluation of laser-responsive disruption of AuNS-hydrogel

AuNS-hydrogel (cg) in phosphate buffered saline (PBS) was exposed to laser irradiation at 532 nm at 1–2 W/cm^2 (Compass 315M – 100, Coherent, Luebeck, Germany). Supernatants were periodically sampled for the evaluation of the disruption of the hydrogel. The size of released products was assessed by PAGE analysis after staining with SYBR Gold (Molecular Probes, Eugene, OR, USA). The amounts of released components, i.e., DNA and AuNP, were determined using a NanoDrop 2000 spectrophotometer by measuring the absorbance at 260 and 532 nm, respectively.

2.6. Cell culture

Murine macrophage-like RAW264.7 cells were cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, 0.2% sodium bicarbonate, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. Murine dendritic DC2.4 cells (kindly provided by Dr. K. L. Rock, University of Massachusetts Medical School, Worcester, MA, USA) were cultured in RPMI medium supplemented with 10% heat-inactivated fetal bovine serum, 0.2% sodium bicarbonate, 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, 0.5 mM monothioglycerol, and 0.1 mM non-essential amino acids. These cells were plated on 96-well culture plates at a density of 5×10^4 cells/well, and cultured for 24 h prior to use.