Biomaterials 163 (2018) 142–153

Contents lists available at ScienceDirect

Biomaterials

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

RF-assisted gadofullerene nanoparticles induces rapid tumor vascular disruption by down-expression of tumor vascular endothelial cadherin

Xue Li [a, b], Mingming Zhen [a, b, **], Ruijun Deng [a, b], Tong Yu [a, b], Jie Li [a, b], Ying Zhang [a, b], Toujun Zou [a], Yue Zhou [a, b], Zhigao Lu [a, b], Mirong Guan [a, b], Hui Xu [a], Chunying Shu [a, b], Chunru Wang [a, b, *]

[a] Beijing National Laboratory for Molecular Sciences, Key Laboratory of Molecular Nanostructure and Nanotechnology, CAS Research/Education Center for Excellence in Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100190, China
[b] University of Chinese Academy of Sciences, Beijing, 100049, China

ARTICLE INFO

Article history:
Received 23 November 2017
Received in revised form
10 February 2018
Accepted 11 February 2018
Available online 13 February 2018

Keywords:
Antitumor agents
Gadofullerenes
Radiofrequency
Tumor vascular-disruption
VE-Cadherin

ABSTRACT

The tumor vasculature with unique characteristics offers an attractive target for anti-cancer therapy. Herein, we put forward a novel antitumor therapeutic mechanism based on the gadofullerene nanocrystals (GFNCs), the agent we have previously shown to efficiently disrupt tumor vasculature by size-expansion with assistance of radiofrequency (RF). However, the tumor vascular disrupting mechanism of RF-assisted GFNCs treatment was not further studied. In the present work, a rapid tumor blood flow shutdown has been observed by the vascular perfusion imaging *in vivo* and vascular damages were evident 6 h after the RF-assisted GFNCs treatment. Importantly, a significant down-expression of tumor vascular endothelial cadherin (VE-cadherin) treated by RF-assisted GFNCs was further investigated, which caused vascular collapse, blood flow shut-down and subsequent tumor hemorrhagic necrosis. These findings set forth a systematic mechanism on the superior anti-tumor efficiency by RF-assisted GFNCs treatment.

1. Introduction

The tumor vasculature is an attractive target for anticancer therapies, which plays a crucial role in sufficient blood supply to tumor cells [1–3]. The tumor tissues will undergo programmed death without adequate tumor vasculature [4–7]. Specifically, tumor vasculature is composed of only monolayer endothelial cells (ECs) with incomplete or absent basement membrane [8]. As the ECs grow fast, large nano-sized open gaps in the lining of tumor blood vessels are formed, resulting in unusual permeability [9]. Thus, various types of nanomaterials were designed to selectively deliver to the tumor tissues by the enhanced permeability and retention (EPR) effect [10]. In the past decades, nanomedicines have emerged as potential ways in tumor targeting therapeutics [11]. Among these agents, gadofullerenes with superior paramagnetism and anti-tumor activity have been widely used for tumor imaging and therapy [12–14].

Recently, our group found that RF assisted GFNCs may offer the high possibility of destroying abnormal tumor blood vessels by phase transition induced size-expansion. The biocompatible GFNCs with the chosen sizes were deliberately designed with abilities to penetrate into the leaky tumor blood vessels. When applied with RF, phase transition occurred while GFNCs extravasated the tumor blood vessels. And the explosive structural change of nanoparticles generates a devastating impact on abnormal tumor blood vessels. Tumors rapidly became ischemic, necrotic and shrinking after treatment with RF-assistant GFNCs, which could also significantly reduce toxicity and thus avoid the harmful side effects of other anti-

* Corresponding author. Key Laboratory of Molecular Nanostructure and Nanotechnology, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100190, China; University of Chinese Academy of Sciences, Beijing, 100049, China.
** Corresponding author. Key Laboratory of Molecular Nanostructure and Nanotechnology, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100190, China; University of Chinese Academy of Sciences, Beijing, 100049, China.
E-mail addresses: zhenmm@iccas.ac.cn (M. Zhen), crwang@iccas.ac.cn (C. Wang).

https://doi.org/10.1016/j.biomaterials.2018.02.028

vascular treatments [15]. This remarkable tumor therapy approach inspires us to deeply investigate the systematic therapy mechanism of RF-assisted GFNCs treatment.

Here we deeply investigated the anti-vascular therapeutic mechanism under the RF-assisted GFNCs treatment. The markers of luciferase in human liver tumor cells (HepG-2) made it possible to quantitatively observe tumor growth and necrosis via bioluminescence imaging. The H&E staining of tumors demonstrated a high tumor inhibition rate of approximately 88.7% after RF-assisted GFNCs treatment, and the tumor blood flow flux was greatly decreased by the laser Doppler imaging and magnetic resonance imaging (MRI) blood perfusion. Moreover, the results of the transmission electron microscopy (TEM), immunohistochemistry (IHC) and western blot (WB) of VE-cadherin visually demonstrated that the RF-assisted GFNCs superiorly destroyed the tumor vascular endothelial gap junctions, causing endothelial cell exfoliation, red blood cell exosmosis, blood flow termination and eventually tumor hemorrhagic necrosis. In this study, we explored a novel cancer therapeutic strategy with high-performance to induce tumor vascular endothelial damage by gadofullerene nanoparticles, which would extensively extend the related antitumor mechanisms of anti-vascular therapy.

2. Materials and methods

2.1. Materials

Solid Gd@C_{82} (99% purity) was obtained from Xiamen Funano Co. Ltd., China. D-luciferin was purchased from Fanbo Biochemical Co. Ltd. (Beijing, China). Dulbecco's minimal essential medium (DMEM) was purchased from Beijing BioDee Biotechnology Co. Ltd., China. Isoflurane was purchased from RWD Life Science Co. Ltd. (Shenzhen, China). Hoechst 33342 and pelltobarbitalum natricum were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gadopentetic acid dimeglumine salt solution (Gd-DTPA) was obtained from Bayer Healthcare Co. Ltd. (Leverkusen, Germany). Anti-CD31 antibody was obtained from Neobioscience Technology Co. Ltd. (Beijing, China). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), and uric acid (UA) assay kits were purchased from Toshiba Medical Co. Ltd., Japan. All reagents and solvents were commercially obtained and used without further purification.

2.2. Preparation and characterization of GFNCs

Water-soluble GFNCs were prepared as previously reported [16,17]. Briefly, the suspension comprised 100 mg of Gd@C_{82}, 7 mL of aqueous H_2O_2 (30%) and 3 mL of NaOH (10%) was vigorously stirred at 50 °C until the black particles gradually dissolved into a yellow solution. The solution was then precipitated with ethanol, centrifuged and dialyzed before being used for treatment. The size and zeta-potential of the GFNCs in different media were characterized by dynamic light scattering (DLS, Malvern, UK) at 25 °C. GFNCs stability was evaluated by taking photos of different solutions before and after centrifugation at 10,000 rpm for 10 min.

2.3. Cell culture

The human liver hepatocellular carcinoma cell line infected with a luciferase reporter (HepG2-luc) was purchased from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). The HepG2-luc cells were cultured with DMEM supplemented with 10% fetal bovine serum (HyClone Company, South Logan, UT), penicillin (100 μg/mL^{-1}), and streptomycin (100 μg/mL, Gibco, Grand Island, NY, USA) in 5% CO_2 at 37 °C in a humidified incubator. The human umbilical endothelial vein cells (HUVECs) were obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China), and cultured under the same conditions as the HepG2-luc cells in Ham's F12k culture medium supplemented with 10% fetal bovine serum, 50 μg/mL endothelial cell growth supplement, and 0.1 mg mL^{-1} heparin sodium.

2.4. Animal models

The animal experiments were performed with BALB/c nude mice. Five-week-old female BALB/c nude mice with body weights ranging from 17 g to 19 g were purchased from the Perking University Laboratory Animal Center, China, and were housed in a temperature-controlled, ventilated and standardized animal room and fed by a professional technician. All animal experiments were conducted according to protocols approved by the Institutional Animal Care and Use Committee. The HepG2-luc cells were cultured to construct tumor models. After being washed with phosphate-buffered saline (PBS), 5×10^6 cells were subcutaneously injected into the right back flank of each mouse. The mice were used in the experiments once their tumors reached a diameter of approximately 6 mm.

2.5. Radiofrequency generator

The key therapeutic instruments that were used in this study were a RFgenerator set including a commercial MXG Analog Signal Generator (from 100 kHz to 1 GHz, N5181A, Agilent, USA), a BLAXH 20 signal amplifier (Bruker, German) and a home-made antenna (2.5 cm in diameter, 0.5 cm in width). The typical waveform of the RF pulses that were applied during treatment is shown in Fig. 1. A spectrum analyzer (DSA 815, RIGOL, China) was used to monitor this waveform. When performing anticancer treatment, we placed the HepG2-luc tumor-bearing mice ($n = 6$) in the center of the home-made antenna after GFNCs were intravenously injected into their tails. RF pulses (200 MHz, 20 Watts of transmit power and 10% pulses) were subsequently applied.

2.6. Cell viability assay

As reported in our previous study, cell viability was evaluated using WST-8 assay with Cell Counting Kit-8 (CCK-8, DOJINDO, Japan). Briefly, the cells were cultured in 96-well plates for 24 h, and medium with different concentrations of GFNCs was added to the plates for another 24 h. DMEM with CCK-8 was then used to evaluate cytotoxicity. The absorbance value at 450 nm was read with a 96-well plate reader (iMark plate reader, Bio-Rad, USA) to determine cell viability. To assess cell viability after RF-assisted GFNCS treatment, the cells were cultured with GFNCs for 3 h and then subjected to RF irradiation for 1 h.

2.7. Laser Doppler perfusion imaging of tumors

Tumor blood flow changes after RF-assisted GFNCS treatment were assessed using a full-field laser perfusion imager (FLPI-2, Moor, UK). The mice were anesthetized with isoflurane at a certain flow to maintain them at a stable respiratory frequency before the measurements. The mice were then placed in a fixed posture and examined under same focus for approximately 30 s. The data were analyzed by Moor FLPI-2 review software, and the relative differences were calculated as the following equation,