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HER2 monoclonal antibody conjugated RNase-A-associated CdTe quantum dots for targeted imaging and therapy of gastric cancer

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ABSTRACT

Successful development of safe and effective nanoprobes for targeted imaging and selective therapy of in-situ gastric cancer is a great challenge. Herein, one kind of multifunctional HER2 monoclonal antibody conjugated RNase A-associated CdTe quantum dot cluster (HER2-RQDs) nanoprobes was prepared, its cytotoxicity was evaluated. Subcutaneous gastric cancer nude mouse models and in-situ gastric cancer SCID mouse models were established, and were intravenously injected HER2-RQDs nanoprobes, the biodistribution and therapeutic effects of HER2-RQDs *in vivo* were evaluated. Results showed that HER2-RQDs nanoprobes could selectively kill gastric cancer MGC803 cells, could target imaging subcutaneous gastric cancer cells at 3 h post-injection, and in-situ gastric cancer cells at 6 h post-injection, and could inhibit the growth of gastric cancer tissues and extended survival time of gastric cancer bearing mouse models, which is closely associated with destroying functional RNAs in cytoplasm by RNase A released from HER2-RQDs nanoprobes, preventing protein synthesis and inducing cell apoptosis. High-performance HER2-RQDs nanoprobes exhibit great potential in applications such as in-situ gastric cancer targeted imaging, and selective therapy in the near future.

1. Introduction

Stomach cancer is the fourth most common cancer and the second leading cause of cancer-related death in the world [1–3]. It remains difficult to cure effectively, primarily because most patients present with advanced diseases [4]. Therefore, how to recognize and track or kill early gastric cancer cells is a great challenge for early diagnosis and therapy of patients with gastric cancer.

We have tried to establish an early gastric cancer pre-warning system since 2005 [5,6]. We hoped to find early gastric cancer cells *in vivo* by multi-mode targeting imaging techniques [7–12]. Our previous studies showed that subcutaneous gastric cancer tissues with 5 mm in diameter could be recognized by using BRCAA1-conjugated fluorescent magnetic nanoprobes, and also could be treated by using hyperthermia caused by magnetic nanoparticles under external magnetic field [13,14]. However, so far those reports associated with targeted imaging and therapy of gastric cancer mainly are based on the subcutaneous stomach cancer models, few report is based on the in-situ gastric cancer models. In fact, in-situ gastric cancer animal models maybe more fitful for actual stages of human gastric cancer than subcutaneous gastric cancer models, and represent the real course of development of tumor *in vivo*. Therefore, the mouse models with in-situ gastric cancer play important roles on the development of new contrast reagents and therapeutic drugs.

Nanotechnology makes an important contribution towards cancer prevention, diagnosis, imaging, and treatment [15]. It not only offers unprecedented capability of carrying multiple diagnostic and therapeutic payloads in the same package, but also facilitates targeting delivery into specific sites and across complex biological barriers [16,17]. The multifunctional integrated system combines different properties such as tumor targeting, imaging, and selective therapy in an all-in-one system, which will provide more useful multimodal approaches in the battle against cancer [18,19]. Quantum dots is one kind of nanomaterial which have abroad application prospects in cellular imaging [20–22], immunoassays [23], DNA hybridization [24], and optical barcoding [25], due to its significant advantages including good photostability, strong fluorescent intensity, and various emission wavelength. Furthermore, quantum dots also have been used to study the interactions between protein molecules or detect the dynamic course of signal transduction in live cells by Fluorescence

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Resonance Energy Transfer (FRET) [26–28]. Therefore, quantum dots is regarded as one kind of prospecting candidate to trace and treat cancer cells *in vivo*. In our previous works, we successfully used RNase-A as the template to assist synthesize CdTe quantum dot clusters, and the prepared quantum dot clusters exhibited the property of inhibiting growth of tumor cells *in vitro* [29]. This result brings one kind of novel method for imaging and treating gastric cancer. We further hope to verify the ability of RNase-conjugated QDs in imaging and treating gastric cancer *in vivo*.

Herein, we selected human gastric cancer cells (MGC803) to prepare subcutaneous and in-situ gastric cancer mouse models, prepared the quantum dot nanoprobes (HER2-RQDs) by coupling the RNase A-associated CdTe quantum dot clusters with the HER2 monoclonal antibody, and investigated as-prepared HER2-RDQs nanoprobes' ability of targeted diagnosis and selective therapy of in-situ gastric cancer bearing mouse models. Finally we explored the possible molecular mechanism of imaging and treating gastric cancer based on HER2-RDQs nanoprobes.

2. Materials and methods

2.1. Preparation of the HER2-RQDs nanoprobes

Ribonuclease-A-Conjugated CdTe Quantum Dot Clusters (RQDs) were synthesized according to our previous reports [29]. HER-2 monoclonal antibody was modified with sulfhydryl group according to reference [30]. The HER-2 monoclonal antibody with thiol group were conjugated with RQDs by using two-step reaction method reported by Kok [31]: the N-succinimidyl iodoacetate (SIA) molecules were coupled to the primary amino groups of RNase A, and then the thiolated HER-2 monoclonal antibody were bonded to the iodoacetyl groups, resultant HER2-RQDs nanoprobes were purified by centrifugation at 1000 g for 5 min. Fluorescence spectra of RQDs and prepared HER2-RQDs nanoprobes were analyzed by using a Hitachi F-4600 fluorescence spectrophotometer at excitation wavelength of 400 nm. The fluorescent image of HER2-RQDs nanoprobes were captured with the digital camera under the irradiation of hand-held UV lamp. The gel electrophoresis analysis for HER2-RQDs was done in a 15% acrylamide gel at 130 V for 80 min.

2.2. In vitro gastric cancer cells targeted by HER2-RQDs nanoprobes

The MGC803 cells were cultured in a DMEM medium supplemented with 10% FBS and 1% penicillin–streptomycin at 37 °C for 2 days. The cells were collected and plated onto 18 mm glass cover slips in a 12-well tissue culture plate and were allowed to grow for 24 h, and the addition of 500 μl of medium containing 200 μg/mL of HER2-RQDs nanoprobes were added into each well and followed by 30 min of incubation, after rinsing the cells 3 times, these cell monolayers were fixed with 4% paraformaldehyde, stained with Hoechst 33258, coated with glycerol, and sealed with another cover slip. Parallel experiments were carried out by using RQDs and normal gastric mucous cell line GES-1 cells as controls. Finally, these cells were imaged with a laser confocal scanning microscope (Leica TCS SP5). All images were taken at the same magnification under constant lighting conditions.

2.3. Biocompatibility of HER2-RQDs nanoprobes

MGC803 cells and GES-1 cells were cultured in the 96-well plate at the concentration of 5000 cells per well and incubated in a humidified 5% CO_2 balanced air incubator at 37 °C for 24 h, 100 μL of DMEM medium containing 200 μg/mL of RQDs or HER2-RQDs, 10% FBS and 1% penicillin–streptomycin were added in each well. After 3 h incubation, the medium were replaced with the fresh cell medium and were allowed to grow for 12, 24, 48, 72 h. Cell viability was evaluated by cell counting kit-8, the optical absorbance of the solution was measured at 450 nm with 96-well microplate reader (Perkin–Elmer). After MGC803 cells were treated with HER2-RQDs and RQDs for 48 h, part of the cells were collected and embedded into paraffin, made into the ultra-thin slices, and then observed with TEM.

2.4. Western blot analysis of HER2-RQDs nanoprobes treated MGC803 cells

MGC803 cells were incubated with DMEM medium containing 200 μg/mL RQDs and HER2-RQDs for 48 h, meanwhile GES-1 cells were incubated with DMEM medium containing 200 μg/mL HER2-RQDs. 48 h later, MGC803 cells and GES-1 cells were respectively collected and lysed with protein lysis buffer. Equal amounts of sample lysates were separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore). The membrane was blocked with 0.1% BSA in Tris-Buffered Saline Tween-20 (TBST) buffer, and incubated overnight at 4 °C with specific primary antibodies such as anti-survivin antibody, anti-caspase-3 antibody and anti-β-actin antibody. Subsequently, the membrane was washed with TBST buffer and incubated with horseradish peroxidase-conjugated secondary antibodies. Bands were detected with an Enhanced chemiluminescence kits (Amersham, ECL kits).

2.5. Preparation of subcutaneous nude mouse models with bioluminescence gastric cancer cells

Bioluminescence MGC-803 cells were presented by Dr. Wang Chunli, cultured with DMEM medium containing 20% FBS, 1% penicillin–streptomycin at 37 °C in a 5% CO_2 incubator for 48 h. The cells were trypsinized with 0.25% trypsin–ethylene diaminetetra acetic acid (EDTA), 1×10^6 Bioluminescence MGC-803 cells were injected subcutaneously into the right anterior flank area of 6- to 8-week-old nude mice. Tumors were allowed to grow to approximately 5 mm in diameter. Bioluminescence images of tumors were achieved by IVIS imaging system at exposure time of 30 s, meanwhile MMPsense750 FAST (Perkin Elmer) fluorescent dye were intravenously injected into mouse models, 6 h later, the mice were performed fluorescence imaging with IVIS system (Xenogen) under the excitation wavelength of 749 nm and emission wavelength of 775 nm.

2.6. Construction of in-situ SCID mouse models with gastric cancer

All animal experiments were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University (NO.SYXK2007-0025). SCID mice (male, 18–22 g, 4–5 weeks old) were obtained from the Shanghai LAC Laboratory Animal Co. Ltd., Chinese Academy of Sciences (NO. SCXK2007-0005) and housed in SPF grade animal center.

Subcutaneous bioluminescence gastric cancer tissues at the exponential growth phase loaded by nude mice were resected aseptically. Necrotic tissues were cut away, and the remaining tumor tissues were scissor-minced into pieces about 1–2 mm in diameter in Hanks' balanced salt solution, and each piece was adjusted to be 50 mg with scissors.

SCID mouse models with in-situ gastric cancer were prepared by orthotopic implantation of histological intact human gastric cancer tissues according to a previous protocol [32]. Mice were anesthetized with 4.3% trichloraldehyde hydrate, and an incision was made through the left upper abdominal pararectal line and peritoneum. The stomach wall was carefully exposed, and a part of the serosal membrane, about 3 mm in diameter, in the middle of the greater curvature of the glandular stomach was mechanically injured using scissors. A tumor piece of 50 mg was then fixed on the injured site of the serosal surface with a 7-0 Dexon transmural suture. The stomach was then returned to the peritoneal cavity, and the abdominal wall and skin were closed with 5-0 Dexon sutures. The animals were kept in an SPF environment.

2.7. Evaluation of the growth of orthotopic implanted gastric cancer

The survival state of the mice were observed and recorded every day. And the body weights of the mice were weighted and recorded every week. Four weeks later, the post-implanted mice were injected with MMPsense750 FAST (Perkin Elmer) intravenously and 6 h later the mice were performed fluorescent imaging with IVIS system (Xenogen) under the excitation wavelength of 749 nm and emission wavelength of 775 nm at lateral position and prone position. Meanwhile, the mice were monitored bioluminescence imaging by IVIS imaging system at exposure time of 30 s.

2.8. Distribution of HER2-RQDs in subcutaneous nude mouse models with gastric cancer

The HER2-RQDs were imaged *in vitro* under the condition of excitation wavelength of 465 nm and emission wavelength of DSRed before starting *in vivo* imaging. Afterwards, 200 μL 200 μg/mL HER2-RQDs nanoprobes were injected into the mice via the tail vein, PBS were injected into the mice via the tail vein as controls. Mice were respectively monitored in a non-invasive manner with Xenogen IVIS spectrum at 0.5, 1, 3, 6, 12 and 24 h post-injection to get fluorescence images, regions of interest (ROI) were drawn over the signals, and average radiant efficiency was quantified in $p/s/cm^2/sr$. The tissue to background ratio (TBR) value was calculated as shown in equation: TBR = (Tumor signal-background signal)/(background signal). Finally, fluorescence images of the major organs in trial group such as lung, heart, liver, spleen, kidneys and tumor were performed.

The mice were sacrificed 24 h later, and bloods were immediately collected from the heart, mixed with 1 mg EDTA in microfuge tubes, and frozen in liquid nitrogen. Then, organs such as lung, heart, liver, spleen, kidneys and tumor were collected, and frozen in liquid nitrogen. Blood and tissue samples were digested by nitric acid (67%, ultrapure reagent grade) and hydrogen peroxide (30%, ultrapure reagent grade), and further analyzed by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Elemental X7) system for quantifying the concentration of RQDs.