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Magnetic graphene-based nanotheranostic agent for dual-modality mapping guided photothermal therapy in regional lymph nodal metastasis of pancreatic cancer

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ABSTRACT

Although regional lymph nodes (RLN) dissection remains the only way to cure pancreatic cancer metastasis, it is unavoidably associated with sizable trauma, multiple complications, and low surgical resection rates. Thus, exploring a treatment approach for the ablation of drug-resistant pancreatic cancer is always of great concern. Moreover, reoperative and intraoperative mapping of RLN is also important during treatment, because only a few lymph nodes can be detected by the naked eye. In our study, graphene oxides modified with iron oxide nanoparticles (GO-IONP) as a nanotheranostic agent is firstly developed to diagnose and treat RLN metastasis of pancreatic cancer. The approach was designed based on clinical practice, the GO-IONP agent directly injected into the tumor was transported to RLN via lymphatic vessels. Compared to commercial carbon nanoparticles currently used in the clinic operation, the GO-IONP showed powerful ability of dual-modality mapping of regional lymphatic system by magnetic resonance imaging (MRI), as well as dark color of the agent providing valuable information that was instrumental for surgeon in making the preoperative plan before operation and intraoperatively distinguish RLN from surrounding tissue. Under the guidance of dual-modality mapping, we further demonstrated that metastatic lymph nodes including abdominal nodes could be effectively ablated by near-infrared (NIR) irradiation with an incision operation. The lower systematic toxicity of GO-IONP and satisfying safety of photothermal therapy (PTT) to neighbor tissues have also been clearly illustrated in our animal experiments. Using GO-IONP as a nanotheranostic agent presents an approach for mapping and photothermal ablation of RLN, the later may serve as an alternative to lymph node dissection by invasive surgery.

1. Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the United States in 2012 and has one of the worst prognosis among various types of cancers, with only 5% of patients surviving for 5 years after diagnosis and treatment [1,2]. Lymph node metastasis is the main metastatic pattern of pancreatic cancer and considered as the major cause of death [3]. Typically, pancreatic cancer first metastasizes to sentinel lymph nodes, and then quickly and latently spreads to other nearby lymph nodes (called regional lymph

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nodes). RLN dissection remains the only treatment approach providing a chance for a cure [2,3]. However, less than 20% of patients are diagnosed with pancreatic cancer at an early resectable stage [2,4]. Even after radical resection for early stage pancreatic cancer, local recurrences and systemic metastases often occur within one year [2]. To improve the prognosis, many modalities such as surgery, radiotherapy, chemotherapy and a combination of all these modalities, have been used for the treatment of pancreatic cancer, unfortunately their outcome has been still very pessimistic [5,6]. Furthermore, these modalities can yield many side-effects, such as lymphatic leakage, body weight loss resulting from oral intake disturbance, systemic toxicity and a destructive "bystander" effect to neighboring cells [7–9].

Compare to radiotherapy, chemotherapy and surgical management, photothermal therapy (PTT) is less invasive and has attracted widespread attention [10]. PTT applies photo-absorbing agents to convert optical energy into heat, leading to the 'burning' of cancer cells [11–17]. Our research groups and the other have developed a large number of nanomaterials as PTT agents, such as various gold-based nanomaterials [10,18–21], carbon nanotubes [22] and graphene [23], all of which show strong optical absorbance in the NIR tissue optical transparency window. According to the previous study, sentinel lymph nodes model was always selected for the PTT of pancreatic cancer metastasis [10]. Actually, this doesn't meet clinical requirements. In the clinical practice, the only mapping and treatment of sentinel lymph nodes is not sufficient. More importantly, the potential metastasis of RLN should be traced and ablated. However, the treatment of metastatic lymph nodes by PTT has been rarely reported, mainly because the location of metastases in RLN are challenging due to their small size and fuzzy boundary with surrounding adipose and connective tissue [24]. In clinical practices, carbon nanoparticles, isosulfan blue and radioactive colloids (such as Tc-99 m sulfur colloid) are the most popular lymph tracing agents [24–27]. However, these lymph nodes mapping methods have some apparent disadvantages and limitations. For example, the visible dyes (carbon nanoparticles and isosulfan blue) have limit tissue penetration and are difficult to detect deeper lymph nodes [26]. Radioactive colloids have the harm of ionizing radiation and low spatial resolution [26,28]. To date, MRI has also been widely used for the preoperative location of lymph nodes, which provides perfectly three-dimensional soft tissue details [26,27,29–32]. Considering the cost effectiveness, it is difficult to use the complicated setup of MRI for real-time visualization during the surgical procedure, while dye mapping has the convenient and visual advantages. Unfortunately the versatile RLN tracing agents, with a combination advantage of MRI and dye mapping greatly needed for PTT are still scarce.

Theranostics is a recently proposed concept that combines therapeutics and diagnostics, aiming to improve therapeutic efficiency with better planning and prognosis. Many versatile nanomaterials incorporating PTT and diagnostic imaging functions have been explored as a new generation platform in the field of nanomedicine [17,32,33]. Previously, Marites et al. and Zheng et al. have developed Gd-based or IONP-based dual-modality MRI and fluorescence imaging for sentinel lymph nodes mapping [26,28]. To our knowledge, the papers focused on dual-modality imaging for RLN were rarely reported, let alone both diagnosis and PTT.

Herein, we developed magnetic GO as a nanotheranostic agent for mapping RLN and PTT of pancreatic cancer metastasis. Owing to its unique physical–chemical properties, GO has been extensively developed for applications in a large number of fields including biomedicine [34,35]. GO and its derivatives have shown great promise in the nanocarriers for drug [36–38] and gene delivery [39,40], PTT [14,23,41,42] as well as biomedical imagining [11,14,43]. By growing IONP on the surface of GO, the GO-IONP composites have been applied as a nanotheranostic agent for photothermal destruction of cancers [11]. However, no attention has been focused to the feasibility of GO-IONP as a dual-modality lymphatic mapping and PTT agent for the treatment of metastatic lymph nodes in previous reports. In this work, GO-IONP was functionalized with polyethylene glycol (PEG). The obtained GO-IONP-PEG with intrinsic black color serving as a visual dye, and superparamagnetism serving as a T_2-MRI contrast agent, was firstly employed to solve a clinical problem of how to locate abdominal lymph nodes. Meanwhile, treatment efficacy of GO-IONP-PEG was also investigated through dual-modality mapping guided PTT. Meanwhile, we systematically assessed the safety of PTT to neighboring tissue and toxicity of GO-IONP-PEG for the major organs in nude mice.

2. Materials and methods

2.1. Materials

N-(3-dimethylaminopropyl-N′-ethylcarbodiimide) hydrochloride (EDC) was purchased from Sigma–Aldrich. NH_2-polyethylene glycol 5000 (NH_2-PEG5000) was purchased from Seebio Biotech, Inc. (Shanghai, China). Iron (III) chloride hexahydrate, sodium acetate trihydrate, sodium polyacrylate, ethylene glycol (EG) and diethylene glycol (DEG) were purchased from Sinopharm Chemical Reagent Co., Ltd. CCK-8 and Calcein AM and propidium iodide (PI) were purchased from KeyGen BioTech (Nanjing, China). RPMI-1640 medium, fetal bovine serum (FBS), Penicillin-Streptomycin solution and Trypsin-EDTA solution were purchased from Gibco (Tulsa, OK, USA). Carbon nanoparticles were purchased from Laimei Pharmaceutical Co., Ltd. (Chongqing, China). All the other chemicals were analytical grade, and purified water was produced by a Millipore water purification system.

2.2. GO-IONP coated with PEG

GO and GO-IONP were synthesized according to the previous reports [44,45]. In order to increase hydrophilicity, the nanomaterials of GO-IONP were modified with NH_2-PEG5000 by covalently linked method [40]. Briefly, 5 mg of GO-IONP and 25 mg of NH_2-PEG5000 were dispersed with 5 mL of deionized water, and then sonicated in an ultrasonic bath for 5 min at room temperature. The mixture was added with EDC (0.5 mg/mL) following another sonication for 5 min, and then was stirred gently for 30 min at room temperature. Following the second time addition of EDC (1 mg/mL), the mixture was sonicated for 5 min then stirred at room temperature for 6 h. Finally, the acquired GO-IONP-PEG was washed and purified using Millpore ultrafiltration tube (MWCO = 100 kDa) for 5 times, and redispersed in deionized water.

2.3. Materials characterization

The transmission electronic microscopy (TEM) images were taken by a Philips CM300 transmission electron microscope operating at an acceleration voltage of 200 kV. Atomic force microscopy (AFM) (XE-100, Park system) was used to observe the morphology of films. ICP-AES was performed to determine the concentration of iron contents in our GO-IONP nanocomposites by a P-4010 spectrometer (Hitachi, Japan). The magnetization characterization of the GO-IONP-PEG was measured using a vibrating sample magnetometer on a Model 6000 physical property measurement system (Quantum, USA) at 300 K. Raman spectra were recorded using a Renishaw spectrometer (model Invia Reflex) with 632.8 nm laser excitation. The Fourier-transform infrared (FTIR) spectra were recorder on a Magna-550 spectrometer (Nicolet, USA). The samples were dried and mixed with KBr to be compressed to a plate for measurement. UV/Vis absorption spectra were measured on a UV-3150 ultraviolet–visible spectrophotometer (Shimadzu, Japan). T_2-weighted images of GO-IONP-PEG nanocomposites with different iron concentration were obtained under a 3-T clinical MRI scanner. The stability of GO-IONP-PEG was also examined both in PBS and serum-containing cell medium at 37 °C for 72 h.

2.4. In vitro temperature evaluation caused by NIR laser irradiation

150 μL aliquots with various concentration of GO-IONP-PEG were deposited into wells of a 48-well cell culture plate. Wells were illuminated using an 808 nm continuous-wave NIR laser (Changchun New Industries Optoelectronics Technology, Changchun, China) with 2 and 3 W/cm^2 for 5 min. Pre- and postillumination temperatures were taken by thermocouple.

2.5. Cell experiment

The BxPC-3 cells, human pancreatic cancer cells, originally obtained from the American Type Culture Collection, were routinely cultured in RPMI-1640 cell medium supplemented with 10% FBS, 100U/mL penicillin and 100 μg/mL streptomycin, at 37 °C in 5% CO_2 and 95% air atmosphere with >95% humidity. The cells (1×10^4 cells per well) were seeded in 96-well plates and incubated with various concentrations of GO-IONP-PEG for 24 h. Then, cell viabilities were determined by

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