Bioluminescent Quantum Dots-Induced Photodynamic Therapy in Vitro

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ABSTRACT

Photodynamic therapy (PDT) is an innovative method for cancer treatment which involves the administration of a photosensitizing agent followed by exposure to the visible light. Appreciate light source to induce photosensitizing agent is the key of PDT. However, the external excitation light source is a limitation for clinical application because of the tissue-penetrating property. Besides, the laser is difficult to match the absorption wavelength of each photosensitizer. In this study, we used a luciferase-immobilized quantum dots-655 (QD-Luc) for bioluminescence resonance energy transfer (BRET)-based PDT to solve the mentioned problems. The bioluminescent Luc-QDs can exhibit self-illumination at 655 nm for photosensitizer activation after coelenterazine adding. Thus, HeLa cancer cells were co-treated with QD-Luc and a clinical photosensitizer, Foscan, which can be excited at 652 nm for PDT. Our results showed that the QD-Luc can stimulate the Foscan and the significant cytotoxicity can be observed after coelenterazine adding. In addition, there is no apparent cytotoxicity of photosensitizer, QD-Luc conjugates and coelenterazine, respectively. We believe this novel strategy may improve the limitation of PDT in clinic.

Keywords: bioluminescence quantum dot, luciferase, photodynamic therapy

1 INTRODUCTION

Photodynamic therapy (PDT) is a light-induced chemical reaction that produces localized tissue damage for the treatment of cancerous, as well as other nonmalignant conditions. The activation of photosensitizers in the target tissue is accomplished with a specific light source in the presence of molecular oxygen [1]. Important advantages of PDT over other therapies include the ability to deliver a precisely targeted treatment through selective illumination, the potential for repeated application at the same site if needed, and it is less invasive than surgery. Despite these significant advantages, the biodistribution of photosensitizers is unfavorable and phototoxicity to the skin, mainly caused by an agents’ hydrophobicity and non-selectivity, is a considerable limitation of their use [2]. An ideal photosensitizer should be non-toxic if it has not been irradiated. However, it should generate a large amount of damage to cells when exposed to a specific light source of a long wavelength, which has advantages in the treatment of deeper lesions [3]. In addition, this agent should accumulate quickly in the target tissue, demonstrate rapid clearance, and tumor selectivity must also be considered [4].

Hydrophobic photosensitizers tend to aggregate in aqueous solution. This results in less photoactivation and generates less 'O2 in solution, which subsequently influences the phototoxicity towards tumor cells [5]. Recently, nanomaterials such as polymer-drug conjugates [6], liposomes [7], nanoparticles [8, 9], and polymeric micelles [3, 10, 11] have been considered as potential carriers for hydrophobic drug delivery and may resolve the aforementioned problems. Polymeric micelles composed of amphiphilic block copolymers possess many attractive properties in drug delivery systems, such as good biocompatibility and high stability in vitro and in vivo, and can be successfully used for the encapsulation of various poorly soluble agents [12]. These nanosized micelles consist of a hydrophilic outer shell and a hydrophobic inner core that can be used to incorporate lipophilic drugs in aqueous solution for intravenous administration [13-15]. Often, these micelles are coassembled with unique macromolecules that can allow the targeted delivery to tissues of interest. The guided delivery of these cytotoxic drugs can be accomplished by a variety of means including magnetic [16] or ligand-directed methods [17]. Furthermore, the controlled release of drugs in response to environmental cues can be achieved by using micelles formed from stimuli-sensitive copolymers [18-21].

Although the aggregation property of hydrophobic photosensitizers in aqueous can be resolved by nanotechnology, the tissue-penetrating property of light source, which is one of the three components of PDT, still needs to be improved. Recently, Quantum dots have been of great interest to many researchers as a popular fluorescent imaging probes due to their unique optical properties and photochemical stability compared to common organic fluorophores [22-27]. In general, quantum dots emit photons by external light source sustained excitation. A new type of quantum dot conjugate that can luminesces through bioluminescence resonance energy transfer (BRET) has been reported by Prof. Rao et al. [28]. In BRET process, quantum dots accept energy form luciferase catalyzed coelenterazine through non-radiation
energy transfer. In the present paper, the possibility of BRET-induced PDT was evaluated in vitro. We believe this novel strategy using self-illuminating QDs may improve the limitation of light penetration for PDT.

2 MATERIALS AND METHODS

2.1 Preparation of Luciferase-immobilized Quantum Dots

Luc-immobilized QDs (QD-Luc) were prepared according to a procedure published previously [29]. Briefly, carboxylate-containing quantum dots (QD655) are mixed with EDC (N-(3-dimethylaminopropyl)-N’-ethylenediamine hydrochloride) (4000 equivalents to QDs) and Luc protein (40 equivalents to QDs) in phosphate buffer (pH 7.4) and reacted for 1 h at room temperature. Afterwards, excessive EDC and protein (MW: 37 kDa) were removed by filtration with a MWCO 100 K filter (Millipore) at 2,700 g for 1 minute at 4 °C. The washed conjugates were then measured for their bioluminescence using a fluorometer and checked mobility using agarose gel electrophoresis. Expose 1 µg coelenterazine to QD-Luc and collected the luminescence signals with excitation light source disabled.

2.2 Determination the Particle Size and Zeta Potential of QD-Luc

Particle size of QD-Luc was analyzed by Zetasizer (Malvern-zetasizer 3000hs, Malvern, UK). All measurements were performed at 25 °C at a measurement angle of 90°, in triplicate. The measurement yields a hydrodynamic diameter for the nanoparticles. The zeta potential of the nanoparticles was also determined by Zetasizer (Malvern-zetasizer 3000hs, Malvern, UK).

2.3 Culture Conditions

HeLa cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with a 1% penicillin-streptomycin-neomycin solution, 10% fetal bovine serum, and 10% bovine calf serum. The cells were cultured at 37°C in a 5% CO₂ incubator and were sub-cultured 2–3 times every week.

2.4 Cytotoxicity of QD-Luc Conjugates w/o or w/ Coelenterazine

For cytotoxicity studies, cells were first seeded into 96-well plates at a density of 8,000 cells per well and cultured for 24 hours. To determine the cytotoxicity of Luciferase-immobilized QDs with or without coelenterazine, cells were incubated in media containing QD-Luc for 3 hours. Afterwards, Foscan loaded micelle (2 µg/mL) were added to wells then incubated in 5% CO₂ humidified atmosphere at 37 °C. After 3 hours incubation, cells were washed by PBS twice. The BRET-induced PDT was achieved as the experimental procedure shown above plus coelenterazine (2 µg). Twenty-four hours after treatment, cytotoxicity was determined using MTT assay and the results were measured using a SpectraMax M2® multi-detection microplate reader (Molecular Devices, Sunnyvale, CA).

3 RESULTS

3.1 Agarose Gel Electrophoresis of Luc-immobilized Quantum Dots

The Luc-immobilized QDs were prepared via amide couplings between the QDs and Luc. The coupling reagent EDC is utilized to mediate the amide-bond formation between amino groups present on the surface of the protein and the carboxylates of the QDs. In agarose gel electrophoresis result shown in Fig. 1, different mobility was distinct between conjugates and unconjugated QDs. Thus, Luc was successfully immobilized onto QDs surface in according to the changing of surface charge of QDs [30].

![Figure 1: Agarose gel electrophoretogram of samples from conjugation reaction. Lane 1, unconjugated QDs; Lane 2, mixture of QDs and coupling reagent EDC; Lan 3, purified QD-Luc conjugates.](image)

In BRET, the energy transfer is often presented as the BRET ratio, defined by the acceptor emission relative to the donor emission [29]. Fig. 2 shows emission spectrum of the self-illuminating QDs containing two peaks, with one peak, at 480 nm, from Luc, and the other peak, at 655 nm, from the QDs. Area A was the integrated total emission (from 600 nm to 710 nm) from the QDs and area B was the integrated total emission from Luc (390–600 nm). The BRET efficiency is defined by the ratio of A to B [28]. The BRET ratio of our prepared QD-Luc conjugate was about 0.92.
3.3 Particle Size and Zeta Potential of QD-Luc Conjugates

Our results showed that the particle size of QD-Luc conjugates was around 19–25 nm and the zeta potential was about -10 ~ -15 mV (Fig. 3). The size of QD-Luc conjugates were slight larger than that of unconjugated QDs (around 17 nm).

3.4 Cytotoxicity of QD-Luc Conjugates-Induced Photodynamic Therapy

Fig. 4 shows the QD-Luc conjugates-induced PDT. Obviously, the cytotoxicity of QD-Luc/photosensitizer can be enhanced by increasing the concentration of coelenterazine. This enhancement of cytotoxicity may due to the more photons emitted from BRET of QD-Luc plus coelenterazine. In addition, no apparent cytotoxicity was observed by photosensitizer, QD-Luc conjugates and coelenterazine, respectively.

4 CONCLUSIONS

Our results showed that the QD-Luc can stimulate the Foscan and the significant cytotoxicity can be observed after coelenterazine adding. In addition, there is no apparent cytotoxicity of photosensitizer, QD-Luc conjugates and coelenterazine, respectively. We believe this novel strategy may improve the limitation of PDT in clinic.

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