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A FRET-guided, NIR-responsive bubble-generating liposomal system for *in vivo* targeted therapy with spatially and temporally precise controlled release

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ABSTRACT

The nonspecific distribution of therapeutic agents and nontargeted heating commonly produce undesirable side effects during cancer treatment since the optimal timing of triggering the carrier systems is unknown. This work proposes a multifunctional liposomal system that can intracellularly and simultaneously deliver the therapeutic drug doxorubicin (DOX), heat, and a bubble-generating agent (ammonium bicarbonate, ABC) into targeted tumor cells to have a cytotoxic effect. Gold nanocages that are encapsulated in liposomes effectively convert near-infrared light irradiation into localized heat, which causes the decomposition of ABC and generates CO_2 bubbles, rapidly triggering the release of DOX. Additionally, a hybridized Mucin-1 aptamer is conjugated on the surface of the test liposomes, which then function as a recognition probe to enhance the uptake of those liposomes by cells, and as a molecular beacon to signal when the internalized particles have been maximized, which is the optimal time for photothermally triggering the release of the drug following the systemic administration of the liposomes. Empirical results reveal that this combined treatment effectively controls targeted drug release in a spatially and temporally precise fashion and so significantly increases the potency of the drug while minimizing unwanted side effects, making it a promising treatment for cancer.

1. Introduction

Conventional chemotherapy commonly causes the nonspecific distribution of therapeutic agents, resulting in significant toxicity toward healthy tissues and limited therapeutic efficacy because the dose at the tumorigenic site is low. Molecularly targeted nanodelivery systems such as liposomes (Lips) reduce potential toxicity and enhance effective therapy [1]. To deliver the drug successfully to diseased tissues, Lips must stably encapsulate the drug in circulation but be triggered to release the contained drug after they have accumulated in the targeted cells [2]. The release of drugs from carriers can be triggered by the application of heat using a hot water bath [3], microwaves [4], or ultrasound [5]; however, such nontargeted heating frequently has adverse side effects [6]. Additionally, the optimal time for the *in vivo* heat-triggering of drug release following the systemic delivery of Lips is unknown.

This work proposes a novel Lip system that simultaneously delivers both a therapeutic agent, doxorubicin (DOX), and heat into targeted tumor cells; the DOX is rapidly released from the Lips by intracellular heat-triggering, exerting its cytotoxicity. Molecularly targeted delivery is achieved using a surface-conjugated Mucin-1 (MUC1) aptamer, which has a high binding affinity for the MUC1 protein on various tumor cells, including MCF-7 human breast cancer cells [7]. The conjugated MUC1 aptamer acts as a molecular recognition probe, and as an activatable molecular beacon following modification by hybridization with a short DNA sequence with an FITC fluorophore and a Cy3 fluorophore that are covalently attached at either terminus (FITC–MUC1 aptamer and Cy3–DNA)

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(Fig. 1).

In the absence of a target, the distance between the FITC fluorophore (donor) and Cy3 fluorophore (acceptor) at the end of the hybridized MUC1 (h-MUC1) aptamer is short enough to allow Förster resonance energy transfer (FRET on). However, when the aptamer binds to a target cancer cell, it can cause spontaneous conformational change, inducing dissociation of the short DNA sequence with the Cy3 fluorophore from the MUC1 aptamer with the FITC fluorophore (FRET off). Therefore, this h-MUC1 aptamer can serve as an activatable molecular beacon in an FRET-mediated alternation of a fluorescence signal, as a result of cell-membrane protein binding events (Fig. 1). This FRET imaging technique can be used to monitor the *in vivo* tumor accumulation dynamics of the h-MUC1 aptamer-conjugated Lips in real time, enabling the optimal time for the heat-triggered release of the drug following systemic administration of the test Lips to be determined.

Gold nanocages (Au NGs), ammonium bicarbonate (ABC, NH_4HCO_3, which is a CO_2 bubble-generating agent when local temperature >40 °C) [8], and DOX are encapsulated together in the aqueous core of the h-MUC1 aptamer-conjugated Lips (h-MUC1 AuNG-Lips). Upon exposure to near-infrared (NIR) light, the Au NGs generate localized heat (42 °C), which induces the decomposition of ABC, produces CO_2 bubbles, and forms permeable defects in their lipid bilayer, triggering the rapid release of DOX, increasing the intracellular drug concentration above the dose required to kill cancerous cells (Fig. 1).

2. Materials and methods

2.1. Materials

The dipalmitoylphosphatidylcholine (DPPC), cholesterol, polyethylene glycol 2000-distearoylphosphatidylethanolamine (PEG 2000-DSPE), and maleimide-functionalized PEG 2000-DSPE (Mal-PEG 2000-DSPE) were acquired from Avanti Polar Lipids (Alabaster, AL, USA), while DOX was obtained from Fisher Scientific (Waltham, MA, USA). The FITC–MUC1 aptamer and Cy3–DNA were ordered from MDBio (Taipei, Taiwan), and the anti-MUC1, anti-EEA1, and anti-LAMP2 antibodies were purchased from Abcam (Cambridge, MA, USA). All other chemicals and reagents used were of analytical grade.

2.2. Preparation of test Lips

To prepare the test Lips, DPPC, cholesterol, PEG 2000-DSPE, and Mal-PEG 2000-DSPE were dissolved in chloroform. The organic solvent was removed using a rotavapor to produce a thin lipid film (10.0 mg) on a glass vial. This film was then hydrated with Au NGs in aqueous ABC with sonication, and then underwent sequential extrusions to obtain Lips with controlled sizes. The free ABC and Au NGs were removed by dialyzing against 10 wt% sucrose solution that contained 5 mM NaCl and were then centrifuged for a short time.

To prepare test Lips that were functionalized with h-MUC1 aptamer (h-MUC1 AuNG-Lips), complementary thiol-containing FITC–MUC1 aptamer and Cy3–DNA sequence were conjugated to Mal-PEG 2000-DSPE on Lips (with a 1:1 M ratio of FITC-MUC1 aptamer/Cy3–DNA sequence:Mal-PEG 2000-DSPE) by forming a thioether linkage [9]. In the preparation of the test Lips that were functionalized with MUC1 aptamer or Cy3–DNA, only thiol-containing FITC-MUC1 aptamer or thiol-containing Cy3–DNA was conjugated to Mal-PEG 2000-DSPE on Lips (with a 1:1 M ratio of FITC-MUC1 aptamer:Mal-PEG 2000-DSPE or Cy3–DNA:Mal-PEG 2000-DSPE). The unreacted aptamer was removed by five rounds of centrifugation using a membrane ultrafiltration filter tube (Vivaspin 2, MWCO: 3 kDa; GE Healthcare, Buckinghamshire, UK).

Subsequently, DOX was mixed with the h-MUC1 AuNG-Lip suspensions with a 0.05 drug/lipid weight ratio and maintained

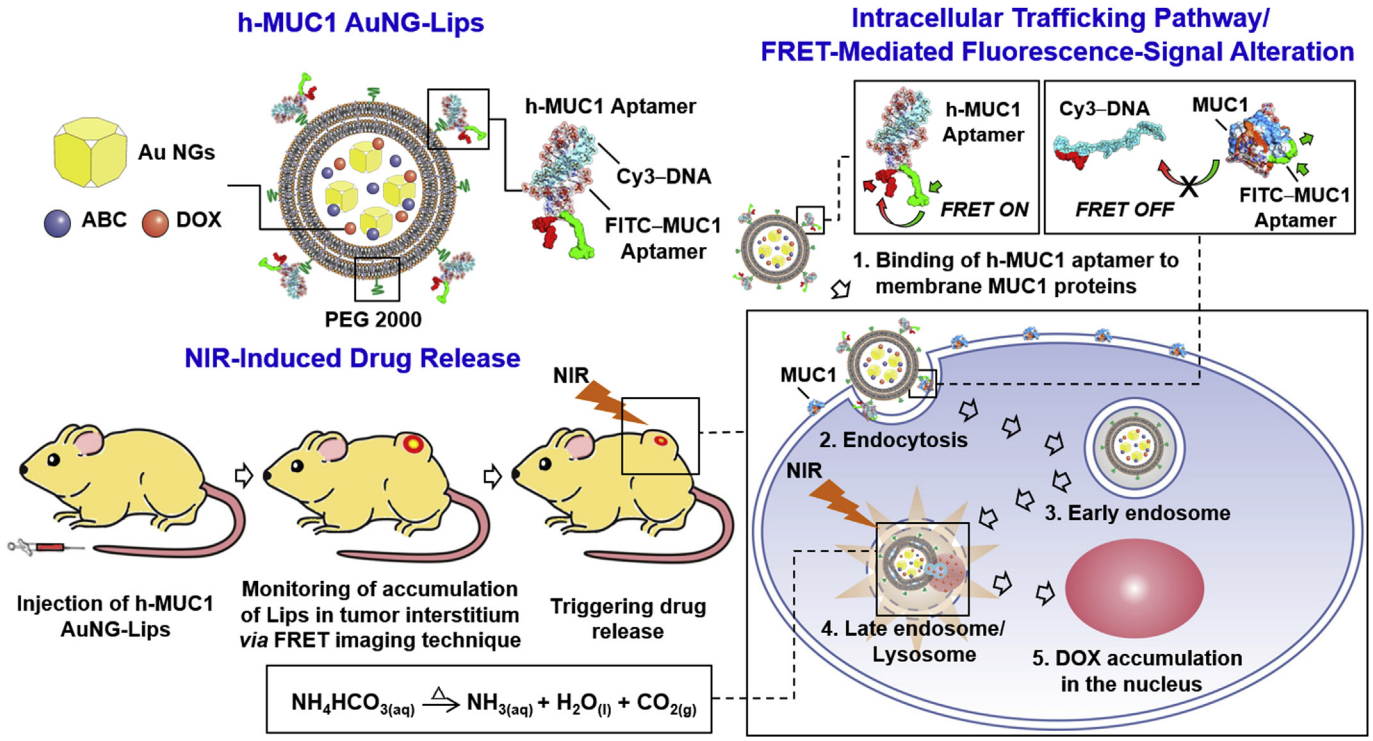

Fig. 1. Composition and structure of an h-MUC1 AuNG-Lip, and its mechanism of simultaneous delivery of heat and DOX into a tumor cell for spatially and temporally precise targeted drug delivery.