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Mutual sensitization mechanism and self-degradation property of drug delivery system for in vitro photodynamic therapy

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ABSTRACT

In this manuscript, a photosensitive anti-cancer drug, hypocrellin A (HA), and TiO_2 nanoparticles were co-loaded on the surface of graphene oxide (GO) as a photosensitive drug delivery system. *In vitro* studies have demonstrated the active uptake of the system into the mitochondrial of tumor cells. Such system has mutual sensitization mechanism to greatly improve the reactive oxygen species (ROSs) generation ability of the complex by visible light irradiation and, thereby, the strong photodynamic therapy (PDT) efficacy. Furthermore, during such PDT process, GO can be destroyed by the ROSs, which would helpful for the metabolism of this drug delivery system.

1. Introduction

PDT is an emerging modality for the cancer treatment, which is based on the generation of reactive oxygen species (ROSs), such as singlet oxygen ($^1O_2$) and superoxide radicals ($O_2^{\bullet-}$), by excitation of administered photosensitizers (PSs) with light. ROSs are responsible for oxidizing various cellular compartments including plasma, mitochondria, lysosomal, and nuclear membranes, etc., resulting in irreversible damage of tumor cells (Chen et al., 2014; Krzykawska-Serda et al., 2014; Luque et al., 2014; Ocakoglu et al., 2015; Park et al., 2015; Schmitt et al., 2015). Hypocrellin A (HA) (Fig. S1), a perylenequinonoid pigment isolated from *Hypocrella bambusae sacc.*, has been proposed as potential PSs for PDT because of its high ROSs generation ability (Gao et al., 2012; Zhou et al., 2010b). However, natural HA is hydrophobic, and therefore, preparation of pharmaceutical formulations for parenteral administration is highly hampered (Baba et al., 2007). Therefore, for its delivery, special formulations are required to make their aqueous dispersion, using surfactants or other nanoparticle-based delivery vehicles, such as oil dispersions (micelles), liposomes, polymeric micelles, hydrophilic drug-polymer complexes, inorganic nanoparticles, etc. (Kitagishi et al., 2015; Li et al., 2015).

Recently, graphene oxide (GO), a two-dimensional carbon nanomaterial, has attracted increasing interest for its potential applications in biomedical fields such as drug delivery, cellular imaging, photothermal therapy and biosensors (Ciriza et al., 2015; Mianehrow et al., 2015; Sharker et al., 2014). Reports indicated that GO possesses well water solubility and good biocompatibility. GO is obtained by the oxidation of graphite, and therefore contains many hydroxyl, carboxylic acid and other reactive groups amenable to ligand conjugation, cross-linking and other modifications, rendering GO tailored for drug delivery applications (Chung et al., 2013; Kim et al., 2012; Singh et al., 2012).

Our previous research indicated that hypocrellins, including HA, can be loaded on GO through noncovalent absorption because the large π-conjugated structure of GO can result in π–π stacking interaction with the benzene ring portion of HA. And the hydroxyl, epoxide and carbonyl groups of hypocrellins can form hydrogen bond with the carboxyl, hydroxyl, or epoxide groups of GO (Zhou et al., 2015a,b; Zhou et al., 2011). Besides, the loading efficacy of HA on GO was very high and the result complex can well disperse in aqueous systems for a long time. However, experiments indicated that after loading on the surface of GO, the photodynamic activity of HA, including ROSs generation ability and anticancer efficiency, were reduced. One possible way to further raise their photosensitization ability is to modulate the photophysical property of HA by introducing a certain intermolecular interaction to increase the ROSs generation efficacy.

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Typically, in cancer treatment, integration of multimodal treatment or sensitizing strategies leading to synergistic or combined effects is a promising approach to enhance anticancer efficacy; and so, co-assembly of multifunctional or synergetic sensitization agents for systematic therapy has attracted increasing interest (Gao et al., 2012). TiO_2 nanoparticles show weak toxicity in dark. However, by UV-light irradiation, these particles produce electrons and holes, which leading to the formation of ROSs and inducing toxicity to cancer cells (Hu et al., 2012). Reports indicated that modifying TiO_2 with a carbonaceous substance, including GO, on the surface can induce visible light responsive activity (Hu et al., 2012). Furthermore, according to the literature, the complex of TiO_2 and graphene-based nanosheets can be prepared by many methods (Fan et al., 2011; Guo et al., 2011; Jiang et al., 2011; Morales-Torres et al., 2012; Neppolian et al., 2012), and some of them are very gentle, which would not affect the drugs that have already loaded on the surface of GO. More importantly, TiO_2 can interact with many pigments, including hypocrellins, to form stable complex. And after forming such complex, the ROSs generation ability of hypocrellins by exposing to visible light was also improved.

So, we proposed that we can prepare a HA delivery system, which is composed of GO, HA and TiO_2 nanoparticle. Such system can greatly improve the disperse ability of HA in aqueous system and increase its photodynamic activity, simultaneously. To demonstrated this concept, firstly, we prepared HA loaded GO (HA–GO), driving for π–π stacking and intermolecular hydrogen bond, according to our previous reports. Then, TiO_2 were loaded on HA–GO (HA–TiO_2–GO) driving for electrostatic interaction.

We suppose that the photodynamic activity of HA–TiO_2–GO would stronger than HA–GO because of the following reasons. Firstly, the photodynamic activity of HA–GO will be improved because of the sensitization effect of TiO_2 to HA. Besides, modifying TiO_2 with GO can induce visible light responsive activity to generate ROSs of TiO_2, which would further increase the PDT activity of HA–GO. As expected, our results indicated that TiO_2, HA and GO can form a stable complex. Furthermore, the $^1O_2$ generation efficiency and the in vitro PDT efficacy of HA–TiO_2–GO were greatly enhanced comparing with HA-GO. Interestingly, during such PDT process, GO can be destroyed by $^1O_2$, which would helpful for the metabolism of this drug delivery system (Fig. 1).

2. Experimental

2.1. Chemicals

Graphite powder (99.9995%, 325 mesh) was from Alfa Aesar. 9,10-anthracenedipropionicacid was from Sigma; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) were from Amosco; Dulbecco's minimum essential medium (DMEM) and fetal bovine serum (FBS) were from Gibico. Hoechst 33342, trypan blue and 2′7′-dichlorofluorescin diacetate (DCFH-DA) was from Beyotime. Tetra-*n*-butyl titanate and ADPA was from Sigma. All the reagents were analytical grade and used without further purification.

2.2. Preparation of GO

Aqueous dispersion of GO was prepared using the modified Hummer's method. Typically, graphite flakes were oxidized using strong oxidants such as H_2SO_4, $KMnO_4$, $NaNO_3$, and H_2O_2 under vigorous stirring. The sample was washed using 3% hydrochloric acid in Millipore™ water with further washing by ultracentrifugation and vortexing. The details of the synthesis process have been discussed in details elsewhere.

2.3. Preparation of TiO_2

TiO_2 was prepared by sol–gel method. Typically, methanol (15 mL) anhydrous acetic acid (100 μL) and tetra-*n*-butyl titanate (200 μL) were mixed by string. 800 μL Millipore™ water was added to initiate the hydrolysis process and the system stirred for about 20 h. The entire reaction was carried out at room temperature. At the end of the process, a white translucency indicating the formation of nanoparticles was observed. After the formation of nanoparticles, redundant acetic acid and by product were removed by dialysis the solution against methanol in a 12–14 k Da cutoff cellulose membrane for 24 h. The resulted TiO_2 was preserved in methanol with the concentration of 3 g/L. The solid of TiO_2 can be got by vacuum drying to remove methanol.

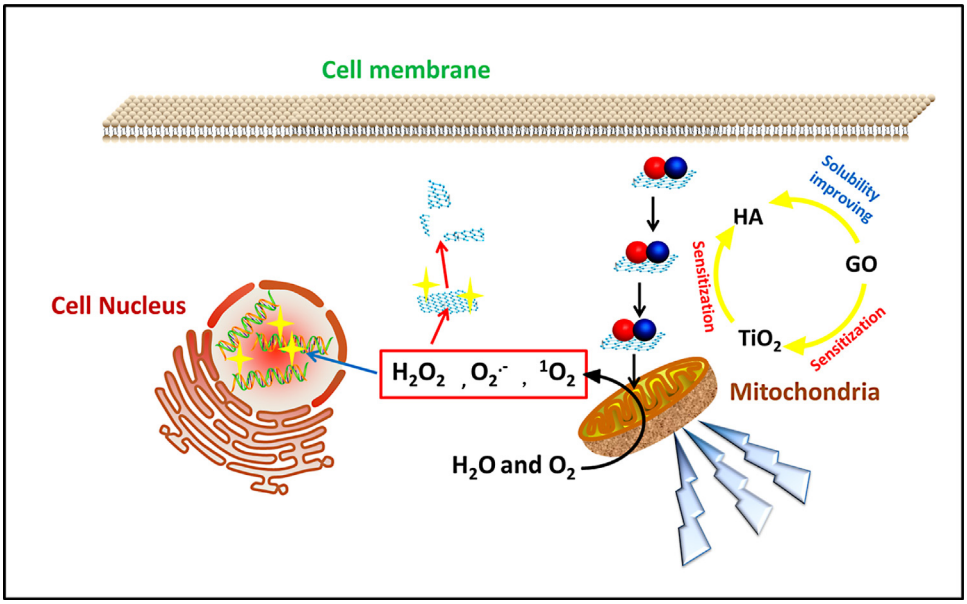

Fig. 1. Schematic representation of the mutual sensitization process, self-degradation and anticancer mechanism of HA-TiO_2-GO.