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A novel nanocomposite based on fluorescent turn-on gold nanostars for near-infrared photothermal therapy and self-theranostic caspase-3 imaging of glioblastoma tumor cell

Juang Wang[a], Zilong Zhou[a], Fang Zhang[a], Hui Xu[b], Wenli Chen[a], Tingting Jiang[a,*]

[a] School of Life Sciences, Ludong University, Yantai 264025, China
[b] College of Chemistry and Materials Science, Ludong University, Yantai 264025, China

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ABSTRACT

We demonstrated a novel nanocomposite based on fluorescent turn-on gold nanostars for simultaneous tumor targeting photothermal therapy (PTT) and feedback apoptosis imaging of the self-therapeutic effect. In this theranostic agent (AuNS@probe), gold nanostars (AuNSs) and fluorescent dye Atto 655, as a fluorophore/quencher pair, were conjugated to form intermolecular fluorescence quenching in virtue of the linkage of a caspase-3 responsive peptide. Folic acid targeting moiety facilitated the selective accumulation in cancer cells *via* receptor-mediated endocytosis. Upon photo irradiation, AuNS@probe demonstrated excellent photothermal effect and induced cell death with apoptosis related mechanism. The typical apoptosis-effector proteases, caspase-3, was subsequently activated and terminated intramolecular fluorescent quenching process. Obvious fluorescence recovery could be applied to precisely assess the activated caspase-3 expression and the real time therapeutic efficacy. This novel versatile nanocomposite could serve as a theranostic agent for tumor targeting PTT and also provide self-therapeutic monitoring for precise cancer therapeutic applications.

1. Introduction

Photothermal therapy (PTT) has been considered as one promising tumor therapeutic modality due to its minimal invasiveness to normal tissues, high selectivity to diseased sites and great efficacy against cancer resistance [1–3]. Photothermal cancer therapy is based on localized heating generated by a beam of light in near-infrared (NIR) region, which can penetrate tissues deeply due to the relatively low absorption and scattering in the biological transparent window (650–900 nm) [4]. Therefore, a variety of NIR photothermal transducers have been extensively developed as functional platforms for photothermal ablation of tumors *in vitro* and *in vivo*, including gold nanostructures [2,5], copper sulfate nanocrystals [6], carbon-based nanocomposite [7,8] and graphene derivatives [4,9]. Of these photothermal transducers, gold nanostars (AuNSs) have recently emerged and gained significant interest in material science due to their high absorbance at the NIR window and excellent photothermal conversion efficiency, allowing them to serve as robust PTT agents toward more efficient disease diagnosis and therapy [2,4]. Detailed investigations of molecular mechanism revealed that the photothermal treatments trigger cancer cell death by a programmed apoptosis with the activation of the caspase-3 pathway [10]. Caspase-3 is a typical apoptosis-effector proteases and has been identified as a key cellular mediator in the process of apoptosis pathway [11]. Hence, development of sensitive and specific strategies to trace the caspase-3 activity has become an important subject in photothermal mediated apoptosis diagnosis. Up to now, considerable strategies based on fluorescent turn on nanoprobes are able to successfully monitor the caspase cascade in cancer cells during apoptosis [12–14]. In these research works, fluorescent nanoprobes provide the caspase imaging after the activation of some typical apoptosis chem-inducers (such as staurosporine (STS)). This “after-triggered imaging” could also be found when photothermal agents substitute the common used apoptosis inducers. However, relevant researches have reveal that the separate implementation of imaging and therapeutic procedures may cause report delay due to the differences in distribution of the probe and drug [15–18]. To solve this problem, it is highly desirable to develop a novel theranostic approach for self-therapeutic feedback image-guided therapy, which could extensively simplify the therapeutic process and facilitate the development of personalized medicine for simultaneous cancer therapy and therapeutic efficacy evaluation [19–23].

Herein, we presented a novel nanocomposite (designed as AuNS@

* Corresponding author.
E-mail address: jiangtingting@ldu.edu.cn (T. Jiang).

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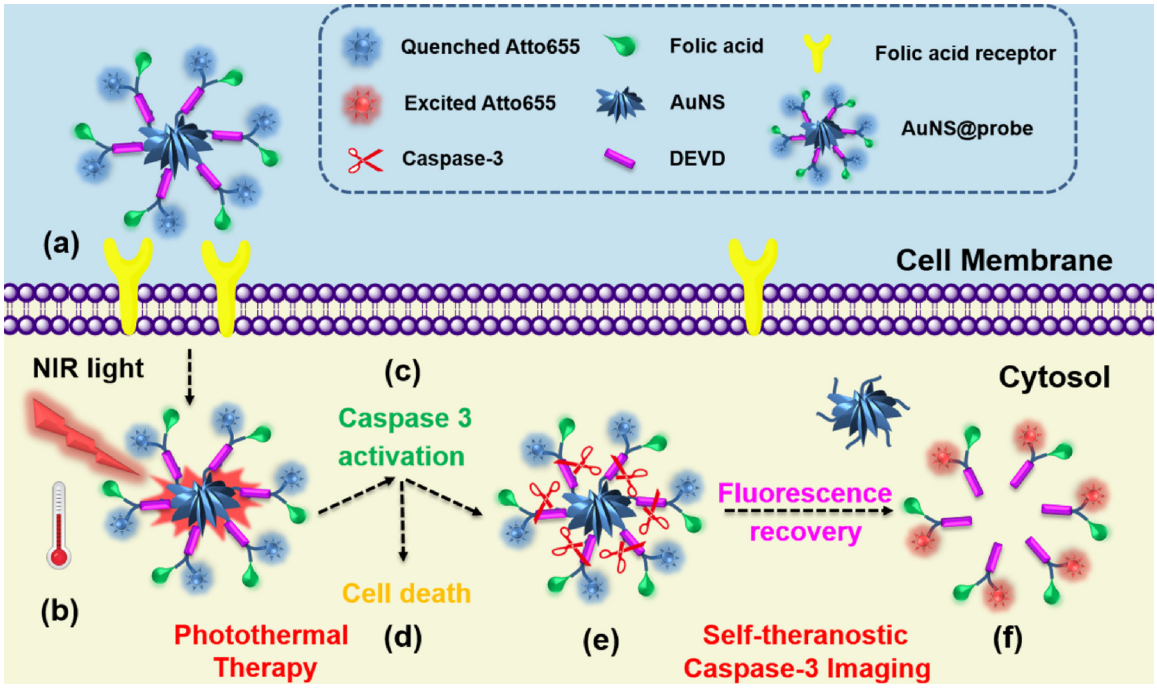

Fig. 1. Schematic diagram of the theranostic probe (AuNS@probe) for tumor targeted photothermal therapy and caspase-3-mediated apoptosis imaging. Proposed processes: (a) Folic acid mediated endocytosis of AuNS@probe. (b) Temperature increasing under NIR light irradiation. (c) Apoptosis related caspases-3 activated by photothemal therapy. (d) Cell death mediated by local high temperature and activated caspases. (e) Peptide unit of DEVD cleaved by activated caspases. (f) Apoptosis imaging by turning on Atto 655 fluorescence of AuNS@probe.

probe) based on fluorescent turn-on gold nanostars for photothermal therapy and self-theranostic feedback based on turning-on fluorescence for caspase-3 imaging. As illustrated in the Fig. 1, AuNS@probe was comprised of a targeting moiety (folic acid, FA), a caspase-3 responsive peptide linker (DEVD, Asp-Glu-Val-Asp), a NIR fluorescent dye (Atto 655) and a photothermal transducer (gold nanostars, AuNSs). AuNSs also served as the fluorescent quencher to effectively quench the fluorescence intensity of Atto 655. This hybrid nanocomposite could recognize the tumor cells and go through the cell membrane through the FA receptor mediated endocytosis pathway. The PTT effect of AuNS@probe could induce cell apoptosis through the activation of caspase-3 pathway. And the intramolecular fluorescent quenching process could be subsequently terminated due to the DEVD cleavage activity of the activated caspase-3, giving the recovery of the report fluorescence (Atto 655). The NIR fluorescent output could indicate the apoptosis process and provide an imaging-guided feedback for accurate personalized oncotherapy. Therefore, this novel nanocomposite could potentially serve as a theranostic agent for photothermal cancer treatment as well as an apoptosis imaging probe for monitoring therapeutic efficacy.

2. Experimental sections

2.1. Materials

All chemicals and solvents used were of analytical grade. Atto 655 NHS ester, folic acid, 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and Gold(III) chloride trihydrate were purchased from Sigma–Aldrich (St. Louis, USA). 2-chlorotrityl chloride resin (100–200 mesh, loading: 0.7–1.5 mmol/g), o-benzotriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU), 1-hydroxybenzotriazole (HOBt), N-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids (Fmoc-Asp(OtBu)-OH, Fmoc-Cys(tBu)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Val-OH, Fmoc-Glu(OtBu)-OH and Fmoc-Lys(Dde)-OH) were purchased from GL Biochem. Ltd. (Shanghai, China). Diisopropylethylamine (DIEA), dichloromethane (DCM), trifluoroacetic acid (TFA), *N*, *N*'-dimethylformamide (DMF), Triisopropylsilane and Staurosporine (STS) were obtained from Aladdin Reagent Co. Ltd. (Shanghai, China). Caspase-3 (human recombinant) was purchased from Biovision corp. Caspase-3 inhibitor Ac-DEVD-CHO was purchased from Beyotime Biochem. Ltd. (Shanghai, China). Annexin V-FITC, Propidium Iodide (PI), Hoechst 33258, Dulbecco's phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium-bromide (MTT) were purchased from Invitrogen Corp (New York, USA).

2.2. Cell culture

Human glioblastoma (U87-MG) cells were incubated in DMEM medium containing 10% FBS and 1% antibiotics (penicillin-streptomycin, 10 000 U/mL). All of the cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

2.3. Synthesis of AuNS@probe

The modified peptide (Atto 655-K(FA)SDEVDSC) was synthesized by standard solid phase peptide synthesis (SPPS) (Fig. S2) [17]. The product was purified with high performance liquid chromatography (HPLC) (Fig. S3). Analytical reverse-phase high performance liquid chromatography (HPLC) was performed on a C-18 column (250 × 3.0 mm) at a flow rate of 1.0 mL/min and semi-preparative HPLC was performed on the similar C-18 column (250 × 10 mm) at a flow rate of 3 mL/min. An eluting system consisting of solvent A (water with 0.1% TFA) and solvent B (acetonitrile with 0.1% TFA) was used under a linear gradient to elute the products, which was monitored by UV–vis absorbance at 663 nm. The linear gradient started from 80% solvent A and 20% solvent B, changed to 20% solvent A and 80% solvent B in 15 min and to 0% solvent A and 100% solvent B in the following 3 min, and then back to 80% solution A and 20% solution B in the next 2 min. The molecular weight was confirmed by Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry