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AuNP flares-capped mesoporous silica nanoplatform for MTH1 detection and inhibition

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ABSTRACT

The human mutT homologue MTH1, a nucleotide pool sanitizing enzyme, represents a vulnerability factor and an attractive target for anticancer therapy. However, there is currently a lack of selective and effective platforms for the detection and inhibition of MTH1 in cells. Here, we demonstrate for the first time a gold nanoparticle (AuNP) flares-capped mesoporous silica nanoparticle (MSN) nanoplatform that is capable of detecting MTH1 mRNA and simultaneously suppressing MTH1 activity. The AuNP flares are made from AuNPs that are functionalized with a dense shell of MTH1 recognition sequences hybridized to short cyanine (Cy5)-labeled reporter sequences and employed to seal the pores of MSN to prevent the premature MTH1 inhibitors (S-crizotinib) release. Just like the pyrotechnic flares that produce brilliant light when activated, the resulting AuNP flares@MSN (S-crizotinib) undergo a significant burst of red fluorescence enhancement upon MTH1 mRNA binding. This hybridization event subsequently induces the opening of the pores and the release of S-crizotinib in an mRNA-dependent manner, leading to significant cytotoxicity in cancer cells and improved therapeutic response in mouse xenograft models. We anticipate that this nanoplatform may be an important step toward the development of MTH1-targeting theranostics and also be a useful tool for cancer phenotypic lethal anticancer therapy.

1. Introduction

Redox dysregulation in cancer cells results in reactive oxygen species (ROS) overproduction, damaging DNA and free bases in deoxyribonucleotide triphosphates (dNTPs) pools [1–3]. The MutT Homolog1 protein, MTH1, can effectively degrade oxidized nucleotides to prevent their incorporation into DNA, and thereby is important for minimizing cancer-associated damage in the dNTP pool as required for cancer cell survival. Experimental evidence has shown that inhibition of MTH1 activity selectively and effectively kills cancer cell lines, but is considerably less toxic to normal cells, which validates MTH1 as an “Achilles heel” for cancer cells and a new target for cancer treatment [4,5]. However, the localization and relative abundance of MTH1 mRNA in different cell types, as well as the regulation of intracellular release of MTH1 inhibitors, are poorly characterized. Northern blots [6] and reverse transcription polymerase chain reaction (RT-PCR) [4,5] are commonly used to detect MTH1 mRNA transcripts. However, these methods require millions of cells and are not applicable to real-time studies of live single cells. Although in situ staining [6,7], green fluorescent protein (GFP) [8], and fluorescence resonance energy transfer (FRET) sensors [8] have been used to detect MTH1 mRNA in living cells, these probes are often difficult to transfect, require additional agents for cellular internalization, and are unstable in cellular environments. This leads to a high background signal and an inability to detect targets. Small molecules such as TH287, TH588, SCH51344 and S-crizotinib inhibit the MTH1 catalytic activity [4,5], but the therapeutic effects may be limited by their insolubility and instability in cellular environments as well as their poor penetration into tumor sites. Moreover, these inhibitors lack selectivity and distribute indiscriminately into all cells, requiring a high dose to significantly affect tumor volume in mouse xenografts. The uncontrolled release mechanism makes it difficult for MTH1 inhibitors to reach their full therapeutic efficacy. Therefore, the detection and visualization of MTH1 mRNA in living cells and controlled release of the inhibitors according to MTH1 mRNA levels remains a challenge.

Nanomaterials have showed their powerful ability to construct

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multifunctional platforms for intracellular research [9,10]. Gold nanoparticles (AuNPs) conjugated to short, fluorophore-labeled oligonucleotide duplexes can be intracellular "nanoflares" and are of significant interest because of their strong fluorescence signals, low background and probe stability [11,12], which enables the visualization and quantification of MTH1 mRNA in living cells, without adding external transfection agents. Another motivation for employing AuNP flares as an MTH1 probe is that they have the potential of becoming a switch to control the release of MTH1 inhibitors in response to intracellular MTH1 mRNA levels. Surface-functionalized, end-capped mesoporous silica nanoparticles (MSNs) are promising scaffolds to construct mRNA-responsive controlled release systems, because of their unique mesoporous structures, large surface areas, tunable pore sizes, and good biocompatibility, both *in vitro* and *in vivo* [13–15]. These end-capped MSNs with "controlled release" properties have been synthesized with different pore-blocking caps such as organic molecules [16,17], supramolecular assemblies [18,19], nucleotides [14,20] and nanoparticles (NPs) [21,22]. AuNPs in particular have successfully controlled the opening and closing of the pore entrance of MSN through either a reversible pH-dependent boronate ester bond [23], an acetal linker [24] or photo-controlled electrostatic interactions [25]. We hypothesized that the AuNP flares could be employed as caps to encapsulate MTH1 inhibitors within the porous channels of the MSNs, which will be selectively opened in the presence of MTH1 mRNA targets.

Herein, we present a novel and effective strategy for combined MTH1 detection and inhibition based on AuNP flares-capped MSN nanoplatform. As shown in Fig. 1, the AuNP flares consisted of AuNP functionalized with a dense shell of 20-base MTH1 recognition sequences hybridized to short cyanine (Cy5) dye-labeled reporter sequences via a gold–thiol bond. After entrapping MTH1 inhibitors (S-crizotinib) in the mesopores of MSN, the AuNP flares were immobilized on the exterior surfaces of the MSN through the linkage of reporter sequences. In the bound state, the Cy5 fluorescence was quenched, and the AuNP blocked the pores. In the presence of MTH1 mRNA, the recognition sequences hybridized with this complementary target sequences by forming the longer and more stable duplexes, causing the liberation of AuNP from the reporter sequences and the opening of the pores, which can then produce fluorescent signals correlated with the relative amount of the MTH1 mRNA and release of S-crizotinib in an mRNA-dependent manner. These AuNP flares-capped MSNs were successfully used to detect and quantify MTH1 mRNA and inhibit MTH1 activity both *in vitro* and *in vivo*. To the best of our knowledge, this is the first time that AuNP flares have been employed as pore-blocking caps to construct mRNA-responsive controlled release system based on MSNs, which allows a single nanoplatform capable of both detecting and regulating MTH1. By targeting MTH1, a novel cancer phenotypic lethal, AuNP flares-capped MSNs have the potential to be a useful tool for cancer diagnosis and therapy.

2. Materials and methods

2.1. Materials

DNA oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of these oligonucleotides are shown in Table S1. Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), (3-aminopropyl) triethoxysilane (APTES), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), S-crizotinib and Rhodamine B (RhB) were purchased from Sigma–Aldrich. Hydrogen tetrachloroaurate (III) ($HAuCl_4 \cdot 4H_2O$, 99.99%), Trisodium citrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), Sodium dodecylsulfate (SDS), mercaptoethanol (ME), $MgCl_2$, $CaCl_2$, KCl were obtained from Sinopharm Chemical Reagent. Co. Ltd. (Shanghai, China). Deoxyribonuclease I (DNase I) was purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China). Cell culture products, unless mentioned otherwise, were purchased from GIBCO. DAPI were bought from Beyotime Inst. Biotech, (Haimen, China). All chemicals and solvents used were of analytical grade. Water was purified with a Sartorius Arium 611 VF system (Sartorius AG, Germany) to a resistivity of 18.2 MΩ cm.

2.2. Characterization

High resolution transmission electron microscopy (HRTEM) was carried out on a JEM-2100 electron microscope. N_2 adsorption–desorption isotherms were recorded on a Micromeritics ASAP2020 surface area and porosity analyzer. The samples were degassed at 150 °C for 5 h. The specific surface areas were calculated from the adsorption data in the low pressure range using the BET model and pore size was determined using the

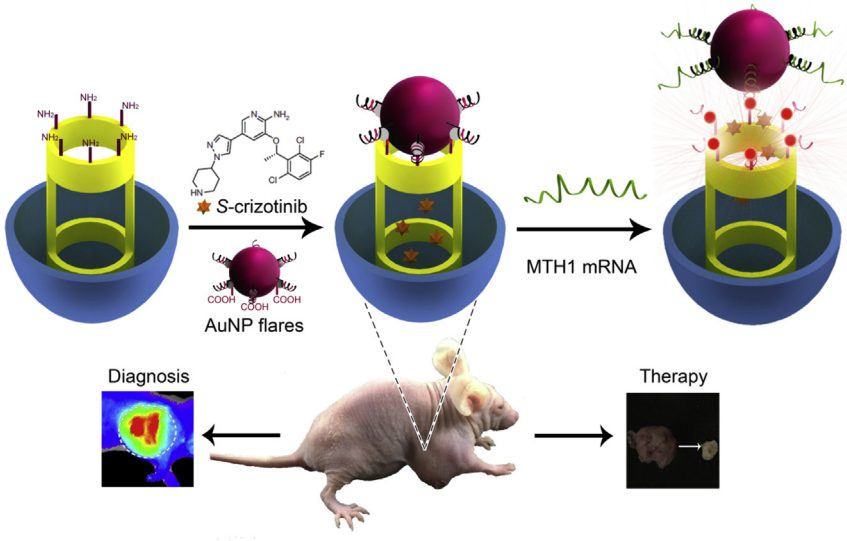

Fig. 1. Schematic illustration of AuNP flares-capped MSN nanoplatform for detection and inhibition of MTH1.