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Mesenchymal stem cell-based cell engineering with multifunctional mesoporous silica nanoparticles for tumor delivery

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ABSTRACT

Stem cell engineering, the manipulation and control of cells, harnesses tremendous potential for diagnosis and therapy of disease; however, it is still challenging to impart multifunctionalization onto stem cells to achieve both. Here we describe a mesenchymal stem cell (MSC)-based multifunctional platform to target orthotopic glioblastoma by integrating the tumor targeted delivery of mesenchymal stem cells and the multimodal imaging advantage of mesoporous silica nanoparticles (MSNs). Rapid cellular uptake, long retention time and stability of particles exemplify the potential that the combination of MSNs and MSCs has as a stem cell-based multifunctional platform. Using such a platform, we verified tumor-targeted delivery of MSCs by *in vivo* multimodal imaging in an orthotopic U87MG glioblastoma model, displaying higher tumor uptake than particles without MSCs. As a proof-of-concept, this MSC platform opens a new vision for multifunctional applications of cell products by combining the superiority of stem cells and nanoparticles for actively targeted delivery.

1. Introduction

Over the past few decades, various nanomaterials have provided a versatile platform in biomedical applications, especially for the diagnosis and therapy of tumor. However, some nanoparticles (NPs) with outstanding properties for a powerful platform in tumor theranostics show poor delivery and tissue penetration into tumors due to their inappropriate size, shape and/or surface chemistry [1–3]. As such, the search for universal vehicles/carriers can enhance the potential diagnosis and therapy strategies. Previous investigations have demonstrated the unique tumor-tropic properties of mesenchymal stem cells (MSCs) as a vehicle/platform for targeted delivery of anticancer agents to tumor models, such as glioblastoma [4], breast cancer [5], colorectal cancer [6], and melanoma [7]. Genetic engineering is currently the major use of MSCs as carriers for imaging and therapeutic agents. However, transduction of MSCs, especially for viral transfection, can lead to unwanted transformation, significantly increasing the risk of secondary malignancies [8]. Recently, stem cell engineering using NPs allows the development of a simple and generalizable strategy for targeted delivery with low cytotoxicity, establishing a new direction for the modification of cell products [9–12]. In order to improve the quality and accuracy of disease management, the idea of multifunctionalization, the integration of complementary strengths from multiple imaging and therapeutic techniques, has recently gained popularity [13]. However, it is still challenging to impart multifunctionalization to stem cell products by direct chemical modification, which is limited by several major factors, such as the sensitivity of stem cells to the reaction environment and dynamic fluids of cellular components. In addition, the future success of stem cell-based cell therapy requires not only the well-controlled *in vivo* behavior of stem cells, but also the understanding of their *in vivo* dynamic fates [14]. Various imaging techniques have been developed for tracking the *in vivo* fates of stem cells, but each imaging modality has its own strengths and limitations [15–17]. Yet, until now, combining the targeted delivery of MSCs with multimodality imaging of NPs to construct an MSC-based multifunctional stem cell platform (MSC-platform) for *in vivo* systemic tumor-targeted delivery has not been examined.

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In this report, we introduce an MSC-platform that combines the tumor tropism of stem cells and multimodality imaging of hyaluronic acid-based polymer (HA) coated MSNs (HA-MSNs) with FITC, NIR dye ZW800, Gd^{3+} and ^{64}Cu imaging agents for optical, magnetic resonance (MR) and positron emission tomography (PET) imaging. To construct the MSC-platform, the multimodal HA-MSN nanoplatform was firstly established. Subsequently, MSCs were labeled with the nanoplatform, and the interactions of MSCs with particles were investigated, including the cellular uptake mechanism, the retention time, intracellular fates and cytotoxicity of particles. To further demonstrate the potential for *in vivo* applications, the orthotopic U87MG glioblastoma xenograft was used as a model to explore tumor-tropic ability of the MSC-platform.

2. Materials and methods

2.1. Fabrication and characterization of dye-doped MSNs

60 nm dye-doped MSNs were synthesized by modifying previous methods [18,19]. Briefly, APS-dyes were synthesized by labeling 3-aminopropyl triethoxysilane (APS) with active groups of dyes (Scheme S1). For example, APS (50 mg) was labeled with FITC (1 mg) and ZW800 (3 mg) in 200 μL of *N*, *N*-dimethylformamide (DMF) solution containing 2% diisopropylethyalamine (DIPEA), respectively. The reaction mixture was stirred at room temperature for 6 h. The resulting APS-FITC and APS-ZW800 were mixed for additional 2 h. Separately, cetyltrimethylammonium bromide (CTAB) (0.27 mmol) was dissolved in 70 mL of deionized water and 14.29 mmol of $NH_3 \cdot H_2O$ (28%–30%) was added with magnetic stirring for 10 min at room temperature. Half of tetroethyl orthosilicate (TEOS) (0.72 mmol) was then added with vigorous stirring for 30 min. 100 μL of APS-dyes was added, and the additional TEOS was added with vigorous stirring for 4 h. The resulting particles were collected by centrifugation and then washed three times with deionized water and ethanol, respectively. Unconjugated dyes were completely removed through centrifugation steps. The MSNs were obtained by removing CTAB in acidic ethanol (1 mL of concentrated HCl in 40 mL of ethanol) for 24 h. The particles were washed three times with deionized water and then stored at 4 °C. The resulting particles were observed by transmission electron microscopy (TEM). The UV–Vis and fluorescence spectrum of particles was recorded on a Genesys 10s UV–Vis spectrophotometer and an F-7000 fluorescence spectrophotometer (HiTachi, Japan), respectively.

2.2. Loading of Gd^{3+} and surface coating of dye-doped MSNs with HA-based polymer

For the loading of Gd^{3+} into porous channels, the dyes doped MSNs were incubated with $GdCl_3$ overnight. Then, the particles were collected by centrifugation and then washed three times with deionized water.

The synthesis of HA-CA was reported in previous study [20]. In a typical reaction, a hydrophobic bile acid, 5β-cholanic acid was conjugated onto water soluble HA polymer via amide formation. First, 5β-cholanic acid was converted to aminoethyl 5β-cholanoamide (EtCA) and reacted via EDC and NHS chemistry with the carboxylic acids of hyaluronic acid. For the surface funtionalization of MSNs by HA-CA, a 10:1 weight ratio of MSNs to HA-CA was optimized. Briefly, 5 mg of MSNs were dispersed in 1 mL PBS containing 0.5 mg HA-CA. After 5 min' standing, the particles were aggregated in solution. Then, the solutions were dispersed via probe sonication using a VCX-750 ultrasonic processor (Sonics & Materials, Newtown, CT). The probe was driven at 40% of the instrument's maximum amplitude in an ice-bath. The particles were well dispersed in solution around 2 min sonication. After sonication, the solution was divided into 500 μL aliquots and purified by a disposable PD-10 desalting column (GE Healthcare).

2.3. ^{64}Cu-labeling

For the ^{64}Cu-labeling, DOTA-NH2 was firstly conjugated onto HA-MSNs through amide formation in the presence of EDC and HOBt. Subsequently, HA-MSN-DOTA were labeled with ^{64}Cu. $^{64}CuCl_2$ was converted to $Cu(OAc)_2$ by adding 0.5 mL of 0.4 M ammonium acetate (NH_4Ac, pH 5.5) to 20 μL $^{64}CuCl_2$. $Cu(OAc)_2$ (1 mCi) was added into a solution of HA-MSN-DOTA and incubated for 1 h with constant agitation. The labeled particles were purified with a PD-10 column to remove unreacted ^{64}Cu molecules. The labeled efficiency was calculated based on the radiation dosimeter readings before and after purification. The radio-labeling yield was 50–60%.

2.4. Preparation of MSCs

Isolation and culture of MSCs were performed by following our previous protocol [21]. Briefly, balb/c mice were sacrificed by cervical dislocation. The marrow was harvested by inserting a syringe needle (27-gauge) into one end of the bone and flushing with PBS. The bone marrow cells were filtered through a 70 mm nylon mesh filter. After collection by centrifugation, cells were dispersed into MesenCult® MSC Basal Medium (Mouse) (STEMCELL™ Technology), a standardized basal medium for the *in vitro* culture of mouse mesenchymal stem cells. Then, cells were cultured at 37 °C in a humidified atmosphere containing 95% air and 5% CO_2. The adhered cells were split when they reached 80%–90% confluence. The MSC surface marker expression profile was confirmed by FACS prior to the use of MSCs. For the preparation of MSC-fluc, the cells were isolated from balb/c mice transfected luciferase. The culture of cells was same with MSCs.

2.5. Cellular uptake of particles

MSCs were plated 24 h before the start of the experiment in chamber slides at a density of 5×10^3 cell/cm^2. After incubation with 100 μg/mL particles for 2 h, the MSCs were incubated by changing fresh medium for 2 h. The cells were fixed with Z-fix solution (Anatech, Battle Creek, MI) for 15 min. Then, the cells were incubated with 0.1% Triton X-100 in PBS at room temperature for 5 min and subsequently incubated with Alexa Fluor®568 phalloidin (Invitrogen) for staining F-actin for 20 min, followed by 1.5 mg/mL DAPI staining at room temperature. The slides were observed and 3D imaging was acquired with an Olympus confocal microscope (Olympus FV10i). As a control experiment, the cell labeling using CellTracker (Invitrogen) was performed by following the standard protocol.

Quantitative analysis of cellular uptake was performed by flow cytometry (FACS). 50 μg/mL particles were incubated with the MSCs for the indicated time points, and then removed by washing three times. Subsequently, the cells were collected by centrifugation and redispersed in PBS buffer. The fluorescence intensity of 10,000 cells was quantified by FACS.

For the cell retention study, the procedures were similar with cellular uptake of particles. Briefly, the particles were incubated with cells for 2 h and subsequently removed by PBS washing three times. The cells were incubated by changing fresh medium for indicated time points, and then were stained similar with the cellular uptake procedures. The images were acquired by the confocal microscopy.

Quantitative analysis of retention ability of particles was performed by flow cytometry (FACS). 50 μg/mL particles were incubated with the MSCs for 2 h, and then removed by washing three times. The cells were continued to culture by adding fresh medium for indicated time points. Subsequently, the cells were collected by centrifugation and redispersed in PBS buffer. The fluorescence intensity of 10,000 cells was quantified by FACS.

2.6. Intracellular fate

MSCs were incubated with 100 μg/mL particles for the indicated time points. Cells were collected and the supernatant was removed. The cell pellets were fixed in a 0.1 M PBS solution containing 2% gluteraldehyde and 2.5% paraformaldehyde for 2 h. They were then rinsed with 0.1 M PBS, embedded in 2% agarose gel, post-fixed in 4% osmium tetroxide solution for 1 h, rinsed with distilled water, stained with 0.5% uranyl acetate for 1 h, dehydrated in a graded series of ethanol (30, 60, 70, 90, and 100%), and embedded in epoxy resin. The resin was polymerized at 60 °C for 48 h. Ultrathin sections (50–70 nm) obtained with an ultramicrotome were stained with 5% aqueous uranyl acetate and 2% aqueous lead citrate and imaged under TEM.

For the co-localization of particles and lysosome, cells were incubated with 100 μg/mL particles for the indicated time points. The cells were washed twice with PBS. Then, the lysosome of cells was stained with LysoTracker™ following the manufacturer's protocol (Invitrogen/Molecular Probes). Briefly, the cells were incubated with 50 nM LysoTracker™ for 1 h. The cells were washed twice with PBS, and the fresh medium was added. The live cell imaging was observed with an Olympus confocal microscope (Olympus FV10i).

2.7. Cytotoxicity

The cytotoxicity of HA-MSNs was evaluated using the standard MTT assay protocol. Briefly, MSCs were incubated with a various concentrations of HA-MSNs (0.0625–1 mg/mL) for 24 h. The medium was replaced with 200 μL fresh media including 20 μL of MTT solution (5 mg/mL), and the incubation proceeded for 4 h. The media was then removed, and 150 μL dimethyl sulfoxide (DMSO) was added into each well to dissolve the internalized purple formazan crystals. An aliquot of 100 μL was taken from each well and transferred into a fresh 96-well plate. The absorption at 570 nm was measured using a microplate reader. The absorption from the control cells was set as 100% cell viability.

The luciferase was used as a model to study the effect of particles on protein expression. The MSCs transfected luciferase was were plated 24 h before the start of the experiment in 96 well plates at a density of 1×10^4 cell/well. MSCs were incubated with a various concentrations of HA-MSNs (0.0625–1 mg/mL) for 24 h. The medium was replaced with 100 μL fresh media. The cells in the plate were imaged using a Xenogen IVIS-100 system (Caliper Life Sciences, Hopkinton, MA, USA) after addition of the substrate D-luciferin (5 μL per well of 3 mg/mL stock).