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The interaction of bacterial magnetosomes and human liver cancer cells *in vitro*

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ABSTRACT

As the biogenic magnetic nanomaterial, bacterial magnetic nanoparticles, namely magnetosomes, provide many advantages for potential biomedical applications. As such, interactions among magnetosomes and target cells should be elucidated to develop their bioapplications and evaluate their biocompatibilities. In this study, the interaction of magnetosomes and human liver cancer HepG2 cells was examined. Prussian blue staining revealed numerous stained particles in or on the cells. Intracellular iron concentrations, measured through inductively coupled plasma optical emission spectroscopy, increased with the increasing concentration of the magnetosomes. Transmission electron microscopy images showed that magnetosomes could be internalized in cells, mainly encapsulated in membrane vesicles, such as endosomes and lysosomes, and partly found as free particles in the cytosol. Some of the magnetosomes on cellular surfaces were encapsulated through cell membrane ruffling, which is the initiating process of endocytosis. Applying low temperature treatment and using specific endocytic inhibitors, we validated that macropinocytosis and clathrin-mediated endocytosis were involved in magnetosome uptake by HepG2 cells. Consequently, we revealed the interaction and intrinsic endocytic mechanisms of magnetosomes and HepG2 cells. This study provides a basis for the further research on bacterial magnetosome applications in liver diseases.

1. Introduction

Bacterial magnetosomes, a type of biologically synthesized magnetic nanoparticles, have numerous advantages, such as homogeneity, biocompatibility and suitable surface properties [1–4] that make them a good candidate for many biomedical and pharmaceutical applications, such as tumor hyperthermia, drug delivery, biomedical imaging and bioseparation [5–8].

The intrinsic mechanisms of interactions between nanoparticles and target bio-organisms at a cellular level should be elucidated to evaluate the biocompatibility of nanoparticles and develop their biomedical applications. Nanomaterials possess unique physical, chemical and biological properties that greatly differ from their bulk materials because nanomaterials are extremely small [9]. The interaction of nanoparticles with cells and biological tissues is closely related to various nanoparticle properties, such as particle size, shape, coating, surface charge and composition [10–12]. Cell type, state and cellular environment can also affect cell-nanoparticle interactions [13–16]. Therefore, the inherent interaction of magnetosomes and target cells should be carefully addressed because of the unique particle properties of bacterial magnetosomes, such as biomembrane coating, to improve their bioapplications and evaluate their biocompatibilities.

The most direct interacting pathway of nanoparticles and cells is the active endocytosis, rather than simple passive permeation [17,18]. Endocytosis is an active and energy-dependent cellular process by which extracellular substances are engulfed. This process depends on dynamic cell membrane deformation processes, such as ruffling and budding. Wrapped vesicles are pinched off from the plasma membrane and then transported to target organelles to internalize particles and macromolecules [17].

Several endocytic mechanisms are involved in the uptake of extracellular materials, including nanoparticles. Endocytosis is classified into two common mechanisms on the basis of cargo size: phagocytosis or cell eating and pinocytosis or cell drinking. Phagocytosis is primarily responsible for the uptake of large particles (size $> 0.5\ \mu m$), such as bacteria, cell debris and apoptotic cells. This process is typically carried out by only few professional phagocytes. Pinocytosis is ubiquitous to almost all eukaryotic cells and mainly considered as a means of taking up fluid surrounding the cell surface and the subsequent internalization of the solutes [19]. Pinocytosis is

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further divided into several classes, including macropinocytosis, clathrin-mediated endocytosis (CME), the caveolae-mediated endocytosis and other clathrin- and caveolae-independent endocytic pathways [20,21]. However, the underlying endocytic mechanisms of bacterial magnetosomes still remain unclear.

Several kinds of nanoparticles can accumulate in liver tissues; as such, these particles are promising drug carrier targeting liver tumours [22,23]. Nevertheless, whether bacterial magnetosomes can interact with liver cancer cells has yet to be fully elucidated. Therefore, this study examined the interaction and intrinsic mechanism of bacterial magnetosomes and human live cancer HepG2 cells.

2. Materials and methods

2.1. AMB-1 culture and magnetosome extraction

Magnetospirillum magneticum AMB-1 (ATCC 700264, Manassas, VA, USA) was cultured in accordance with previously described methods [24] to extract bacterial magnetosomes. AMB-1 cells were grown microaerobically in enriched *Magnetospirillum* growth medium in the dark at 28 ± 1 °C, harvested through centrifugation at 5000×*g* for 10 min and re-suspended in 0.1 M phosphate buffer saline (1×PBS, pH 7.4). The bacterial cells were then fragmented through ultrasonication (180 W, 4 s work, 4 s interval, 99 repetitions) thrice by using an ultrasonic apparatus (Ningbo Xinzhi Biotechnology, Zhejiang, China). Bacterial magnetosomes were extracted from the solutions by utilizing an NdFeB magnet (50 mm×50 mm×25 mm, with a magnetic flux density of approximately 0.2 T at the surface centre; Shenyang General Magnetic Co., Ltd., Shenyang, China). The extracted magnetosomes were initially washed with 1×PBS thrice and then with ddH_2O thrice. Afterward, the extracted magnetosomes were resuspended with ddH_2O at a stocking concentration of 10 mg/mL, sterilized by autoclaving and stored at 4 °C.

2.2. Measurement of the magnetic hysteresis loop of the purified magnetosomes

For the purified magnetosome sample, approximately 20 μL of magnetosome suspension was deposited and dried on the surface of a small nonmagnetic cover slide (0.22×0.22 cm) in the presence of a strong magnetic field (approximately 2 T produced by a laboratory-made electromagnetic equipment). The magnetic hysteresis loop of the magnetosomes was detected by using a Model 3900 vibrating sample magnetometer (Princeton Measurements Corporation VSM 3900, Princeton, NJ, USA).

2.3. Cell culture and treatments

Liver hepatocellular carcinoma cell line HepG2 (No. 3111C0001CCC000035; China Infrastructure of Cell Line Resources, Beijing, China) was maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco/Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Australian origin, Hyclone Laboratories, Logan, UT, USA), 100 IU/mL penicillin (Sigma-Aldrich, St Louis, MO, USA) and 100 μg/mL streptomycin (Sigma-Aldrich) at 37 °C in 5% CO_2. HepG2 cells were seeded with an appropriate density in cell culture medium and incubated for 24 h. The medium was then replaced with the magnetosome working solution for the succeeding experiments. The magnetosome stocking solution (10 mg/mL) was dispersed sufficiently through sonication at 40 kH, 300 W for at least 5 min in a water bath (Ningbo Xinzhi Biotechnology) just before incubation with the cultured cells.

For low-temperature treatment, the cells were pre-incubated at 4 °C for 10 min, treated with 0, 100, 200 and 400 μg/mL magnetosomes and incubated respectively at 4 °C and 37 °C for another 3 h. For endocytosis inhibitor treatment, HepG2 cells were pretreated with different inhibitors in the cell culture media, and the media were replaced with magnetosome working solutions. The pretreated concentration and period of each inhibitor were as follows: 10 μg/mL cytochalasin B preteated for 2 h; 2 μM rottlerin pretreated for 30 min; 200 μM monodansyl cadaverine (MDC) pretreated 15 min; and 50 μg/mL nystatin preteated 15 min. All the inhibitors were obtained from Sigma-Aldrich.

2.4. Transmission electron microscopy (TEM)

The cells incubated with or without 100 μg/mL magnetosomes for 24 h were fixed in a suspension with 2.5% glutaraldehyde in 1×PBS for at least 1 h, dehydrated with graded ethanol and propylene oxide and embedded in Epon (SPI-Pon 812 Epoxy Embedding kit, SPI Supplies, West Chester, PA, USA). Thin sections (70 nm) were stained with uranyl acetate and lead citrate and then observed using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan). To determine the morphological characteristics of AMB-1 or isolated magnetosomes, we collected and re-suspended AMB-1 cells with 1×PBS. We diluted the extracted magnetosome solution with ddH_2O and prepared the sample for TEM by drying a drop of AMB-1 or magnetosome suspension on a copper grid-supported transparent carbon foil.

2.5. Prussian blue staining

Prussian blue staining kit (Beijing Solarbio Science & Technolgy, Beijing, China) was applied to detect iron, and experiments were conducted according to the manufacturer's instruction. In Brief, HepG2 cells treated with magnetosomes were washed with 1×PBS thrice to remove the free particles and then fixed with 4% paraformaldehyde. The magnetosomes interacting with the cells were stained with Prussian blue staining solution, and the cytoplasm was stained with eosin solution. The cells were subsequently observed under an inverted optical microscope system (Olympus, Tokyo, Japan), and cellular images were captured.

2.6. Inductively coupled plasma optical emission spectrometry (ICP-OES)

Intracellular iron concentration was quantified through inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6300, Thermo Scientific, MA, USA). HepG2 cells were treated with 0, 50, 100, 200 and 400 μg/mL magnetosomes for 24 h and washed with 1×PBS thrice to remove the free magnetosomes. The cells were digested with trypsin, counted, harvested through centrifugation and washed with 1×PBS twice. The supernatants were then removed. The cell samples were dissolved completely with concentrated nitric acid (65–68%) and heated for 4 h at 85 °C. After the concentrated nitric acid evaporated, the samples were diluted with 1% nitric acid to obtain the final volume of 5 mL. The iron concentration was determined from standard solutions with known concentrations. The statistical data of iron concentrations were expressed as mean ± standard deviation. The experiments were repeated thrice.

3. Results and discussion

3.1. Characterization of magnetotactic bacteria and magnetosomes

TEM were conducted to observe the morphological characteristics of bacterial magnetosomes directly. In Fig. 1A and B, a kind of magnetotactic bacteria, *Magnetospirillum magneticum* AMB-1 contained the endogenous magnetosomes arranged in chains along the long axis of the cell body. The TEM images of the extracted magnetosomes showed that the particles exhibited good dispersion and did not aggregate together severely (Fig. 1C and D). The magnetosomes were also surrounded by a 2–3 nm thick membrane, which was not