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The use of peptide-delivery to protect human adipose-derived adult stem cells from damage caused by the internalization of quantum dots

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Abstract

Label of human bone mesenchymal stem cells with CdSe/ZnS quantum dots (QDs) had been demonstrated to impair cell functions and activities. In the present study, QDs delivered by two different routes, Pep-1-labeled QDs (LQ) and PolyFect transfected QDs (TQ), were utilized to assess the effects of delivery mechanisms on various cellular responses of the QDs-internalized human adipose-derived adult stem cells (hADAS). Examination of labeled cells by flow cytometry and laser scanning confocal microscopy showed that LQ had higher fluorescence intensity due to the cluster formation and their distribution in cytoplasma while TQ were preferentially accumulated at perinuclear regions. The fluorescence intensity of the LQ group was still higher than that of the TQ group at 28 days after labeling, though cellular LQ were partitioned after initial cell division. Pep-1 but not PolyFect delivery facilitated QDs to escape from lysosome degradation. Pep-1 delivery of QDs rescued the cells from the negative effects caused by the internalized QDs on cell proliferation and on the expressions of CD29 and CD105 as well as osteogenic and chondrogenic-associated lineage markers. The same effect was also observed in the expression of alkaline phosphatase activity, calcium deposition and secretion of chondrogenic matrices (GAG and collagen type II) in micromass culture. These indicated that Pep-1-delivered QDs may serve appropriately to track the hADAS employed in cell therapy/tissue engineering applications. The results also suggested that the endo-/lysosome degradation of QDs may depend on different surface coatings and critically influence the differentiation of hADAS.

Keywords: Quantum dots; Pep-1; Human adult adipose-derived stem cells (hADAS); Lysosome; CD markers; Differentiation

1. Introduction

Labeling cells or tagging biomolecules by fluorescence is crucial for either the real-time tracing of stem cells in vivo or the biochemical assay in vitro [1–4]. The inorganic fluorophores, CdSe/ZnS quantum dots (QDs), are colloidal nanocrystals most commonly used in biological applications due to the unique optical properties such as high fluorescence intensity as well as resistance to photobleaching and chemical degradation [5,6]. Numerous studies have established a variety of techniques to deliver QDs into cells, such as microinjection, endocytic compartment, electroporation, liposome-mediated transfection and special peptide delivery [7–10]. Earlier studies indicated that the incorporation of QDs had no observable deleterious effects in non-mammalian, transformed cells, or in animal models [11–13]. However, recent studies demonstrated that the internalization of QDs inhibited the osteogenesis and chondrogenesis of human bone marrow-derived mesenchymal stem cells (hBMSCs) at the molecule level such as marker-gene expressions [14,15]. In mesenchymal stem cells (MSCs), cell surface receptors such as CD29, CD44, CD73, CD90, CD105, etc. are accredited the prototypical markers [16,17]. Endocytosis of ligand-activated receptors has generally been considered a mechanism to attenuate the intracellular signaling that triggers various cellular responses, e.g. uptake of nutrients, and regulation of protein

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and DNA synthesis [18,19]. Therefore, the delivery of QDs can affect cell functions at the molecular level and must be concerned, especially in the applications of MSCs.

Pep-1, KETWWETWWTEWSQPKKKRKV-cysteamine, an amphipathic peptide, was one of the cell penetrating peptide family (CPP). It consists of three domains: hydrophobic tryptophan-rich motif, hydrophilic lysine-rich domain (KKKRKV), and spacer domain (SQP). Pep-1 efficiently delivers a variety of fully biologically active peptides and proteins into cells by the electrostatic and hydrophobic contact of the cell membrane without the need of prior crosslinking or chemical modification. The mechanism through which Pep-1 cell translocation is independent of the endosome pathway [20,21]. Cytotoxicity of the peptide in mammalian cells was not observed [22,23]. Moreover, QDs-labeled live mammalian cells by Pep-1-mediated delivery have been established in recent years [20–24]. This method did not affect cell physiology, the competitive binding of the receptors, reporter genes, receptor internalization and intracellular calcium release in different cell lines [23].

The distribution of QDs in cytoplasma depended strongly on their surface coating and pH stability. Up to date, the influence of cellular distribution of QDs on the cell morphology, viability and functions of MSCs remains unknown. Therefore, this study contrasted the effect of Pep-1-delivered QDs versus that of the transfected QDs (TQ) on human adipose-derived adult stem cells (hADAS). The distribution and metabolism in cytoplasma as well as re-distribution of cellular QDs during cell division were examined. The proliferation, cell surface marker expressions and differentiation (osteogenesis and chondrogenesis of the QDs internalized cells) were also analyzed. By this approach, we were able to demonstrate for the first time that QDs delivered by Pep-1 were free from the unfavorable effects of TQ on the differentiation of hADAS.

2. Materials and methods

2.1. Cell culture and delivery of quantum dot into hADAS

Fresh human infrapatellar fat pads of the knee were obtained from the surgical specimens discarded after knee arthroplasty in the Veterans General Hospital (Taichung) by following the ethical guidelines. The age of the donors was 40–65 years old. Cells were enzymatically isolated from adipose tissues as described in literature [25,26]. The adipose tissues were cut into several pieces and treated with 200 U/ml collagenase (type I, Sigma-Aldrich, St. Louis, MO, USA) in PBS for 30 min at 37 °C with gentle agitation. The cellular pellet was resuspended in DMEM-LG/Ham's F12 (1:1) (Gibco/BRL, Grand Island, NY, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Biological Industries, Israel), 100 U/ml penicillin, 100 mg/ml streptomycin (Biological Industries, Israel), and filtered through a 70-μm mesh filter (Falcon®, BD Biosciences, Franklin Lakes, NJ, USA) to remove debris. Cells were isolated by centrifugation at 1500 rpm for 10 min and then plated in tissue culture flasks (Falcon®, BD Biosciences, USA) at approximately 3500 cells/ml culture medium. When confluent, the primary cells were trypsinized by 0.25% trypsin/EDTA (Gibco/BRL, Grand Island, NY, USA) and expanded in monolayer for two to four passages to select for adherent cells. Cells were subcultured at a 1:2 ratio every 7 days. The delivery of QDs followed the previous literature with some modification [23]. The water-dispersible QDs were purchased (Quantum Dot Corp., Hayward, CA; probes.invitrogen.com, with an emission of 655 nm), which were made of CdSe/ZnS encapsulated in a neutralized amphiphilic polymer (40% octylamine-modied polyacrylic acid) and coated further with lysine. 1×10^6 cells were seeded in 75 T flask (Falcon®, BD Biosciences, USA). For delivery by Pep-1, 3 μg QDs (0.2 nM) and Pep-1 (Active Motif, Carlsbad, CA, USA) at an optimal concentration ratio of 1:6000 (QDs/Pep-1) were mixed for 5 min at room temperature to form a complex [23]. Then the cells were incubated with the QDs/Pep-1 complex at 37 °C/5% CO_2 for 60 min. For transfection, cells were transfected with the same amount of QDs using PolyFect transfection reagent (Qiagen, Valencia, CA, USA) for 36 h, according to the procedures suggested in the user's guide. After being labeled, cells were washed twice with full growth medium. The amount of Cd element inside the labeled cells was immediately measured by inductively coupled plasma mass spectrometry (ICP, Hewlett Packard 4500, Japan). Cells were incubated further at 37 °C/5% CO_2 in growth medium overnight before all the following experiments were performed.

2.2. Cell imaging by a laser scanning confocal microscope

An inverted laser scanning microscope LSM510 (Axiovert 100, Carl Zeiss, Germany) was optimized for the lens. Laser lines, pinhole settings, and emission filters were used as appropriate for the maximum signal of different labeled cells. In order to observe the aggregation of cellular QDs, the 3D sections were analyzed. At same pinhole settings, for each synaptic site, 17–39 sections were collected every 1.2 μm, with each being a Kalman average of four to five scans. Images of QDs were pseudocolored, using National Institutes of Health image software to merge. Adjustments to brightness and contrast as well as merging of the images were performed with Adobe Photoshop 6.0. To compare the distribution of cellular QDs and aggregation at a given synaptic site in the *z*-plane, the volume data were resampled from a *z*-series of confocal images collected in the dual wavelength mode along a specified line. The integration of line scans through the *z*-axis yielded a longitudinal view of the synaptic site. These reconstructed line scans were pseudocolored and then merged as described above. The number of labeled cells was counted by a regular fluorescence microscope (NIKON H600L, Japan). This was used to calculate the efficiency of QD by two different delivery vehicles.

2.3. Lysosome stain

Labeled cells were subcultured into 8-well Lab-Tek chambered coverglass slides (Nunc, Roskilde, Denmark) for 12 h. Cells were rinsed once with 37 °C cell culture medium. 0.1 μM LysoTracker dye Green (Molecular probe, Invitrogen, Bulington, CA, USA) working solution was used at room temperature and protected from light. Cells were incubated for 15–30 min at 37 °C in the dark or in subdued light. Dead cells were excluded by co-staining with 5 μM propidium iodide (Chemicon, Temecula, CA, USA). The coverslip was sealed by melted wax or nail polish. Cells stained with LysoTracker dyes were observed under the confocal microscope or a fluorescence microscope at an emission of 510 nm. Lysome expression in the labeled cells was quantified by flow cytometry (BD FACScan, San Jose, CA, USA). About 1×10^6 cells were suspended in 0.1 μM dye working solution by briefly vortexing at maximum speed. The other process of staining was as earlier described.

2.4. MTT assay

To assess the proliferation of QDs-labeled cells, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA) assay was performed. The cells were plated at a density of 2×10^4 cells/well in 24-well microtiter plates (Corning, NY, USA) and incubated for 1, 3, 5, and 7 days at 37 °C/5% CO_2. Cells were further washed by PBS twice. MTT solution (5 mg/ml, 1 × PBS) was then added and incubated for 4 h at 37 °C. The supernatant was removed and the aliquoted DMSO