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The inhibition of osteogenesis with human bone marrow mesenchymal stem cells by CdSe/ZnS quantum dot labels

Shu-Chen Hsieh[a], Fung-Fang Wang[b], Ching-Shih Lin[a], Yu-Ju Chen[a], Shih-Chieh Hung[c], Yng-Jiin Wang[a,*]

[a]*Institute of Biomedical Engineering, National Yang-Ming University, Taipei 112, Taiwan*
[b]*Institute of Biochemistry, National Yang Ming University, Taipei 112, Taiwan*
[c]*Department of Orthopedics and Traumatology, Veterans General Hospital, Taipei 112, Taiwan*

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Abstract

CdSe/ZnS quantum dots (QDs) have recently been used as cell tracers for long term imaging of live cells. A number of studies indicate that introduction of quantum dots to cells have no apparent deleterious effects on the morphology or growth of cells. In the present study, the human bone marrow mesenchymal stem cells (hBMSCs) were used as a model to examine the effects of QDs on the growth and osteogenic differentiation of the cells. The CdSe/ZnS QDs were delivered into hBMSCs by liposome-mediated transfection with high efficiency; analysis by transmission electron microscopy revealed that the internalized QDs could be located in the endosome-like vesicles. Uptake of QDs into hBMSCs did not affect the proliferation and cell cycle distribution of the cells. When induced to differentiate along the osteogenic lineage, the QD-containing-hBMSCs were shown to have mineral deposition on the extracellular matrix. However, the cells displayed lower alkaline phosphatase activity as compared to those without QDs. Analysis by reverse transcriptase polymerase chain reaction further demonstrated that the expression of two osteogenic markers, osteopontin and osteocalcin, was significantly inhibited. Together our results show that the presence of QDs in hBMSCs prevents the full response of the cells to induced osteogenic differentiation.

Keywords: Quantum dots; Human bone marrow mesenchymal stem cells; Cell growth; Osteogenesis; Gene expression

1. Introduction

Quantum dots (QDs) have recently been explored as labeling agents of cells and tissues for biological imaging. As compared to conventional organic probes, these fluorescent agents can be excited by a wider range of wavelengths and exhibit narrower emission bandwidths [1–3]. Of particular interests are the uniquely strong luminance and high photostability exhibited by CdSe/ZnS [4]. Various techniques, such as microinjection, electroporation, scraping, transferrin-assisted transport and liposome-mediated transfection, have been employed to deliver QDs into cells [5]. Despite the increasing popularity of QDs as cell labeling agents, their cytotoxicity and the possibility of aberrant effects on gene expression remain a major concern.

A number of studies indicate that, as a fluorescent probe for cultured cells and animal models, the internalized QDs have no apparent deleterious effects on the morphology and the growth of cells. For example, it has been shown that when QDs are introduced into *Xenopus* [1] or HeLa cells [6], normal growth and differentiation of the cells are not affected. However, that conclusion, derived from morphological observations and analysis based on gross growth properties of cells, omitted any detailed characterization of cellular activities at the molecular level, such as gene expression. In addition, the cells employed in the above-mentioned studies are either non-mammalian or cancer cells. In the present study, we used human bone marrow mesenchymal stem cells (hBMSCs) as a cell model to explore the effect of QDs on the growth and induced

*Corresponding author. Tel.: +886 2 28267019; fax: +886 2 28210847.
E-mail address: wang@ym.edu.tw (Y.-J. Wang).

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osteogenesis of the cells. A number of criteria including enzyme activity and marker gene expression were examined to evaluate the process of osteogenesis. We found that despite a normal cell growth and cell cycle distribution displayed by the QD-containing cells, the expression of osteocyte specific marker genes, osteopontin and osteocalcin, and the activity of alkaline phosphatase (ALP) are significantly suppressed by the introduction of QDs into hBMSCs. This is the first report showing that the uptake of QDs may affect cellular responses to extracellular stimuli, suggesting that the effects of QDs on various aspects of cell activities should be carefully evaluated when used as a long-term cell tracer.

2. Materials and methods

2.1. Cell culture and the delivery of QDs into hBMSCs

hBMSCs immortalized with HPV16 E6/E7 [7] were cultured in 10-cm dishes containing DMEM-LG (GIBCO) with 100 U/ml penicillin, 10 μg/ml streptomycin and 10% fetal bovine serum (FBS) (GIBCO), the cells were maintained in a humidified cell culture incubator at 37 °C with 5% CO_2/95% air. Cells were subcultured at a 1:3 ratio every 5 days. For transfection, 7×10^5 cells were seeded in 10-cm dishes for 24 h and transfected with 1.625 μg of CdSe/ZnS (Ocean Optics, Carboxyl EviTag with emission of 520 nm) using Lipofectamine PLUS reagent (Invitrogen) according to the procedures suggested in the user's guide. Tat-peptide mediated transfection was performed by incubating 3×10^4 cells with HIV-derived Tat cell-permeable peptide (GRKKRRQRRRPPQ, synthesized by Genesis) and QDs. Cells were washed after 3 h and cultured in DMEM-LG containing 10% FBS for another 24 h before differentiation was induced.

2.2. Transmission electron microscopy (TEM)

Cells were cultured on a 400-mesh grid and fixed with 2.5% glutaldehyde for 4 h at room temperature. The cells were rinsed with phosphate buffered saline (PBS), fixed with 1% osmium tetraoxide for 1 h and washed with distilled water. The cells were then pre-stained with 0.5% uranyl acetate overnight, dehydrated and molded at 60 °C for 36 h by G1 epoxy. The samples were thin sectioned with a diamond saw and milled by ion beam to produce a thickness of 50 nm. The samples were post-stained with lead citrate for 10 min and viewed under transmission electron microscopes. For low magnification, a JEOL JSM-1200EXII TEM was used, and for high resolution, the Philips Tecnai F30 Field Emission Gun Transmission Microscope (FEG-TEM) was used.

2.3. Induction of osteogenic differentiation

hBMSCs were induced to osteogenic differentiation as follows: approximately 5,000–6,000 cells/cm^2 were cultured with DMEM-LG supplemented with 50 μg/ml ascorbic acid (Sigma), 10 mM β-glycerolphosphate (Sigma), 0.1 μM dexamethasone (Sigma) for a period of 4 weeks, with the medium changed every 4 days.

2.4. Flow cytometry analysis

Cells were harvested and fixed with ice-cold 70% ethanol for 16 h. The cells were then washed once with PBS, treated with RNase A (1 mg/ml), and stained with 20 μg/ml propidium iodide. Cells were subjected to flow cytometry analysis (BD FACS Calibur), and the cell cycle distribution was analyzed by the Cell Quest software (Becton Dickinson).

2.5. Von Kossa staining

Cells were washed twice with PBS and fixed in 4% paraformaldehyde for 15 min at room temperature. The cells were washed with deionized water, incubated with 5% silver nitrate (Sigma) and exposed under ultraviolet light for 45 min. Afterwards the cells were washed with deionized water twice, counterstained with 10 × diluted Meyer's hemalum solution (Merck) for 10 min and washed with 95% ethanol, and then observed under microscopical examination.

2.6. Alkaline phosphatase activity

Cells in 48-well plates were washed twice with PBS and ruptured with 150 μl of 0.01% SDS at 37 °C for 10 min. Then *p*-nitrophenyl phosphate solution (Calbiochem, 350 μl) was added to each well. The reaction was terminated by adding 500 μl of 0.05 N NaOH after 30 min at 37 °C. The absorbance at 405 nm was measured with a microtiter plate reader, and the values were normalized by viable cell counts obtained by WST-1 assay (see below). Induction is calculated by dividing the enzyme activity in the presence of osteogenic induction by that in its absence. For cell viability assay, the WST-1 reagent (Roche) was added into the culture wells as recommended by the instruction manual, the absorbance at 420–480 nm was measured with a microtiter plate reader.

2.7. RNA extraction and RT-PCR analysis

Total RNA was extracted from cells using REzol (Protech) as recommended by the user's guide. RNA (3 μg) was reverse transcribed with Super Script II (Invitrogen) for 45 min at 42 °C in the presence of oligo-dT primer (25 μg/ml), dNTP (0.5 mM) and DTT (0.01 M). PCR was performed with gene specific primers designed from the sequence of each cDNA. The sequences were as follows: for osteocalcin (405 bp), sense primer: 5′-CGCAGCCACCGAGACACCAT-3′ and antisense primer: 5′-GGGCAAGGGCAAGGGGAAGA-3′; for osteopontin (323 bp), sense primer: 5′-CCCTTCCAAGTAAGTCCAACGAAAGC-3′ and antisense primer: 5′-CTGGATGTCAGGTCTGCGAAACTTC-3′. And for GAP DH (371 bp), sense primer: 5′-AAGTATGACAACAGCCTCAAGA-3′, and antisense primer: 5′-CACCACCTTCTTGATGTCATCA-3′. The reaction mixture was heated initially at 95 °C for 5 min, followed by 25–40 cycles of denaturation at 95 °C for 30–40 s, annealing at 55–58 °C for 30 s and elongation at 72 °C for 30–45 s, with an additional 7-min incubation at 72 °C after completing the last cycle. The amplified DNA was loaded to a 1.5% agarose gel pre-stained with ethidium bromide. After electrophoresis, the DNA bands were photographed under UV light.

3. Experimental results

3.1. Delivery of CdSe/ZnS QDs into hBMSCs

We first explored the techniques for introducing CdSe/ZnS QDs into hBMSCs. It has been shown that QDs can be delivered into cells by various methods including microinjection, electroporation, scraping and liposomal transfection [1,5,8,9]. Uptake of these particles into cells via endocytosis could also be facilitated by surface modification of the QDs. At high concentrations, QDs could significantly increase osmotic pressure, which is detrimental to cells. In the present study, a low dose of 15 nM was used to avoid the side effect caused by osmotic shock. As indicated in Table 1, without the assistance of a delivery vehicle, hBMSCs were unable to take in any significant amounts of the aqueous CdSe/ZnS QDs. Addition of calcium phosphate did not seem to facilitate the uptake of