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Application of amphiphilic fluorophore-derived nanoparticles to provide contrast to human embryonic stem cells without affecting their pluripotency and to monitor their differentiation into neuron-like cells

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ABSTRACT

Fluorogenic labeling is a potential technique in biology that allows for direct detection and tracking of cells undergoing various biological processes. Compared to traditional genetic modification approaches, labeling cells with nanoparticles has advantages, especially for the additional safety they provide by avoiding genomic integration. However, it remains a challenge to determine whether nanoparticles interfere with cell traits and provide long-lasting signals in living cells. We employed an amphiphilic fluorophore-derived nanoparticle (denoted by TPE-11) bearing a tetraphenylethene (TPE) moiety and two ionic heads; this nanoparticle has an aggregation-induced emission (AIE) effect and the ability to self-assemble. TPE-11 exhibited the property of higher or longer fluorescence intensities in cell imaging than the other two nanomaterials under the same conditions. We used this nanomaterial to label human embryonic stem (hES) cells and monitor their differentiation. Treatment with low concentrations of TPE-11 (8.0 μg/mL) resulted in high-intensity labeling of hES cells, and immunostaining analysis and teratoma formation assays showed that at this concentration, their pluripotency remained unaltered. TPE-11 nanoparticles allowed for long-term monitoring of hES cell differentiation into neuron-like cells; remarkably, strong nanoparticle signals were detected throughout the nearly 40-day differentiation process. Thus, these results demonstrate that the TPE-11 nanoparticle has excellent biocompatibility for hES cells and is a potential fluorogen for labeling and tracking the differentiation of human pluripotent stem cells.

Statement of Significance

This study uses a nanoparticle-based approach to label human embryonic stem (hES) cells and monitor their differentiation. hES cells are distinguished by two distinctive properties: the state of their pluripotency and the potential to differentiate into various cell types. Thus, these cells will be useful as a source of cells for transplantation or tissue engineering applications. We noticed the effect of aggregation-induced emission, and the ability to self-assemble could enhance the persistence of signals. Treatment with low concentrations of TPE-11 nanoparticles showed high-intensity labeling of hES cells, and immunostaining analysis and teratoma formation assays showed that at this concentration, their pluripotency remained unaltered. Additionally, these nanoparticles allowed for long-term monitoring of hES cell differentiation into neuron-like cells lasting for 40 days.

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1. Introduction

Since the first report that human embryonic stem (hES) cells could be cultured in vitro [1], hES cells are known to be able to differentiate into the three germ layers, and cells from these germ layers can develop into a variety of cell types [2,3]. The advantages of hES cells are that they can proliferate indefinitely in culture and be maintained as diploid cells with a normal karyotype [4]. Thus, hES cells are regarded as a source of differentiated cells and have many potential applications for regenerative medicine. This potential therapeutic application largely relies on the efficient and controlled differentiation of hES cells toward a specific cell type and the generation of functional cell populations [5,6]. The differentiation of hES cells in vitro is a long-term process involving many stages and can be influenced by many factors. Many differentiation protocols involve the formation of progenitors through a multiple-step approach. Thus, characterizing and understanding the mixed populations of these progenitor stages will be of increasing importance for stem cell research.

Long-term and noninvasive cell tracking can offer valuable information concerning the differentiation of hES cells [7]. The genetic modification of hES cells is time consuming and laborious, such as approaches involving green fluorescent protein (GFP) labeling or knock-in of reporter genes. hES cells are compact and offer reliable clonal expansion but are resistant to direct vector transfection. Moreover, the use of retrovirus and lentivirus for infection of hES cells faces safety questions owing to their random genome integration and abnormal gene activation [8]. Some nanoparticles with special configuration could be of benefit for the maintenance of hES cells [9,10]. Given the rapid progress taking place in nanotechnology, we hypothesized that a nanoparticle-based approach could be used for the tracking of stem cells and dynamic cellular processes [2,11–13]. We noticed that organic dots with aggregation-induced emission (AIE) [14–17] could enhance fluorescence intensities in living cells and maintain signaling in long-term cell tracking [18]. Herein, we employed a newly developed nanoparticle, TPE-11 [19,20], with characteristics of AIE and the ability to self-assemble, which enhance the signal intensity of the nanoparticle [21–24]. By comparing with the other two nanomaterials, that is retrovirus and lentivirus, TPE-11 exhibited the property of high fluorescence intensities and persistence in cell imaging.

The differentiation of hES cells into neural or neuron-like cells has been well established in vitro [25–27]. Several protocols have been developed to achieve neural induction and differentiation toward specialized neuronal subtypes. We used this model to test whether the TPE-11 nanoparticle could label hES cells without interfering with their pluripotency and track these cells during their differentiation into neural cells. To the best of our knowledge, this is the first time nanoparticles have been used to monitor the dynamic process (occurring for 40 days) of hES cell differentiation into neuron-like cells.

2. Materials and methods

2.1. Comparisons of the three nanomaterials for fluorescence intensities and signal persistence in HeLa cells

Rhodamine B derivative-functionalized graphene quantum dots (RBD-GQDs) and methyl ester-containing tetraphenylethene (TPE-Me) were selected for comparison with TPE-11 in terms of fluorescence intensities and signal persistence in HeLa cells. During the experiment, RBD-GQDs were dissolved in 20 mM Tris-HCl buffer (ethanol:H_2O ratio, 1:1 by volume). TPE-Me was dissolved in DMSO and TPE-11 in water. HeLa cells were cultivated in glass bottom dishes with Dulbecco's Modified Eagle Medium (DMEM, Thermo Fisher) supplemented with 10% fetal bovine serum (FBS, HyClone). These cells were treated with TPE-11, TPE-Me, and RBD-GQDs with the same concentration (8.0 μg/mL) and for the same time (6 h). The cells treated with the three nanomaterials were then divided into two parts. One part was washed twice with PBS and fixed with 4% polyformaldehyde as passage 1. The other part was continuously cultivated for 12 h after the removal of fluorescent probes and passaged in another glass bottom dish. The passaged cells (passage 2) were continuously cultivated for 24 h, washed twice with PBS, and fixed with 4% polyformaldehyde. The cells of both passage 1 and passage 2 were observed under a confocal microscope (LEICA TCS-SP8, Germany) with excitation wavelength of 405 nm for TPE-11 and TPE-Me and that of 561 nm for RBD-GQDs. The mean fluorescence intensities were analyzed using "Leica Application Suite X" software.

2.2. Cell culture of hES cells, nanoparticle treatment, and pluripotency detection

hES H9 cells were obtained from WiCell Research Institute (Madison, WI, USA). These cells were cultivated in a formulated medium (PSCeasy; Cellapy Technology, Beijing, China) with 20 ng/mL of bFGF on Matrigel (BD Biosciences, USA)-coated plates. After incubating H9 cells with 8.0 μg/mL (approximately 6.4 μM) of TPE-11 for 12 h, the medium was replaced with fresh PSCeasy medium. These cells were divided into three parts. One part was washed twice with PBS and digested with Accutase (Sigma-Aldrich, USA) into single cells; these cells were subjected to flow cytometry analysis.

The second part of the TPE-11-treated H9 cell sample was fixed with 4% polyformaldehyde and blocked with 2% horse serum. The hES H9 cells were incubated with primary antibodies (Stage-specific embryonic antigen 4, SSEA-4, and Octamer-binding protein 4 or OCT4) overnight at 4 °C at a dilution of 1:100. The second antibody, goat antimouse Alexa Fluor 647 (Beyotime, China) antibody, was incubated for 40 min under ambient conditions. The hES H9 cells were washed with PBS three times, sealed with glycerol, and observed under confocal laser scanning microscopy (LEICA TCS-SP8, Germany). The fluorescence signals were observed and recorded using lasers with excitation wavelengths of 405 and 561 nm for TPE-11 and pluripotent markers, respectively.

The third part was cultivated with PSCeasy medium for 1 day, which was then changed to defined differentiation medium on the next day and continuously cultured for nearly 40 days.

2.3. Teratoma formation assay

hES H9 cells were cultivated in Matrigel-coated dishes. The hES cells were treated with TPE-11 (8.0 μg/mL) for 12 h or given no treatment (control). Approximately 5×10^6 hES cells treated with TPE-11 or the control cells were injected subcutaneously into immunocompromised SCID mice. Six weeks after injection, teratomas formed and the tissues were harvested. The formed teratomas from both the control and TPE-11 treatment groups were subjected to hematoxylin and eosin (H.E.) staining. The teratomas were also subjected to histological staining to visualize the interior components. All animal handling procedures were approved by the Institutional Animal Care and Use Committee at Peking University.

2.4. Directed differentiation of TPE-11-treated hES cells into neural cells

Our protocol for the differentiation of hES cells into neural cell lineages was based on several previous reports [4,26,28–31] with some modifications. The TPE-11-treated H9 cells were subcultured on Matrigel-coated six-well plates (20–30 cell colonies/well) and

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