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Polymeric vector-mediated gene transfection of MSCs for dual bioluminescent and MRI tracking in vivo

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ABSTRACT

MSC's transplantation is a promising cell-based therapy for injuries in regenerative medicine, and in vivo visualization of transplanted MSCs with noninvasive technique is essential for the tracking of cell infusion and homing. A new cationic polymer, poly(ethylene glycol)-block-poly(L-aspartic acid)-grafted polyethylenimine functionalized with superparamagnetic iron oxide nanoparticles (PAI/SPION), was constructed as a magnetic resonance imaging (MRI)-visible non-viral vector for the delivery of plasmids DNA (pDNA) encoding for luciferase and red fluorescence protein (RFP) as reporter genes into MSCs. As a result, the MSCs were labeled with SPION and reporter genes. The PAI/SPION complexes exhibited high transfection efficiency in transferring pDNA into MSCs, which resulted in efficient luciferase and RFP co-expression. Furthermore, the complexes did not significantly affect the viability and multilineage differentiation capacity of MSCs. After the labeled MSCs were transplanted into the rats with acute liver injury via the superior mesenteric vein (SMV) injection, the migration behavior and organ-specific accumulation of the cells could be effectively monitored using the in vivo imaging system (IVIS) and MRI, respectively. The immunohistochemical analysis further confirmed that the transplanted MSCs were predominantly distributed in the liver parenchyma. Our results indicate that the PAI/SPION is a MRI-visible gene delivery agent which can effectively label MSCs to provide the basis for bimodal bioluminescence and MRI tracking in vivo.

1. Introduction

Various factors, such as viral-infection, toxic-injury and autoimmune, may result in acute and/or chronic liver injury. Although the orthotopic liver transplantation (OLT) is the optimum therapeutic option for end-stage liver disease, the OLT is limited by the shortage of donor livers, immunological rejection and complications caused by the lifelong immunosuppression. Instead, MSCs transplantation provides a strategy for the treatment of impaired organ [1,2]. The MSCs can be obtained and expanded fairly easily in culture. These cells have the ability to self-renew, differentiate into various types of cells [3,4], secret many cytokines [5], migrate to sites of injury [6–8], inhibit inflammation [9], and regulate immune response [10]. In addition, MSCs transplantation has advantages of short operation time and less injury for patients in comparison with liver transplantation. It has been reported that MSCs transplantation can effectively treat liver failure and contribute to liver regeneration [11–13]. Due to the lack of technology available for tracing the transplanted MSCs, the clinical applications of MSCs transplantation for livers are limited yet. Therefore, it is crucial to develop an efficient and noninvasive imaging tool to monitor the transplanted MSCs [14].

Both the bioluminescence imaging (BLI) [15] and MR imaging allow the noninvasive tracking of transplanted cells in living organisms. The bioluminescence imaging is an optical imaging approach, which can provide qualitative and quantitative information on cell viability by detecting luciferase reporter gene expression using IVIS system in small animal models [16].

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Nevertheless, the bioluminescence imaging lacks tomographic resolution and is limited to small animals due to photo attenuation and scattering within deep tissues [17]. On the other hand, superparamagnetic iron oxide nanoparticles (SPION) have been used as MRI T_2^* probes for dynamically tracking cells in vivo because of low-intensity signals in T_2^*-weighted MR imaging [18,19]. SPION are nontoxic and biodegradable, and do not affect proliferation and multilineage differentiation properties of the labeled MSCs [20]. In comparison with BLI, MRI has advantages of unlimited depth penetration, high spatial resolution and multiple imaging parameters, but is poor in distinguishing the survival status of transplanted cells. Apparently, combination of the two imaging tools (BLI and MRI) may overcome the disadvantages while retaining the advantages of each to provide better cell tracking in vivo. Therefore, the development of a delivery system that may effectively co-deliver reporter gene and SPION into MSCs is vital.

Transport vectors have been categorized into two groups: viral and non-viral vectors [21]. In spite of the superior gene transfection efficiency [22], application of viral vectors was restricted because of their shortcomings of potential carcinogenicity, immunogenicity and complicated synthesis procedure [23]. The non-viral vectors, as an alternative safe and efficient gene carrier, arouse a wide concern [24]. Most of them are synthesized by chemical methods and can be divided into cationic lipids and cationic polymers based on their chemical structures [25]. Cationic polymers have exhibited tremendous potential as effective gene delivery vehicles [26].

In the present study, we first synthesized a polymer-based non-viral vector PAI, in which polyethylenimine was grafted onto the block copolymer of poly(ethylene glycol) and poly(L-aspartic acid) by disulfide bond linkages. The polyethylenimine side chains possess abundant protonated amine groups, which can mediate electrostatic interactions with the polyanionic backbone of pDNA to enable gene complexation and delivery. PEGylation was performed to enhance the vector biocompatibility by shielding the positive charges of PEI. Then, the polymer was complexed with the superparamagnetic iron oxide nanoparticles (SPION) to generate a MRI-visible non-viral vector (PAI/SPION) for gene delivery. Finally, PAI/SPION was complexed with reporter genes for MSCs labeling. The physicochemical properties of PAI/SPION/pDNA complexes were checked. The labeled MSCs were transplanted into rats with acute liver injury via a SMV injection, and then their migration and homing were monitored using bioluminescence and MR imaging.

2. Material and methods

2.1. Materials

The synthetic process of the polymeric vectors (PAI and PAI/SPION) was described in detail in Supplementary information. The IVIS® Spectrum system (Caliper Life Science, Hopkinton, USA) was employed for in vivo imaging measurements. The plasmids pCMV-Luciferase2-mKate2 co-expressing luciferase and RFP were used in this study. The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Kumamoto, Japan). The low-glucose Dulbecco's modified eagle medium (DMEM), Isocove's modified Dulbecco's medium (IMDM), Penicillin–Streptomycin, Fetal bovine serum (FBS) and Dulbecco's phosphate buffered saline (PBS) were purchased from Gibco BRL (Carlsbad, CA, USA). The fluorescent staining agent 4′,6-diamidion-2-phenylindole (DAPI) was purchased from Roche (Roche, Germany). Oregen Green 488 and popo-3 were purchased from Molecular Probes (Eugene, OR, USA). The rabbit polyclonal anti-luciferase antibody was purchased from Abcam (Cambridge, England). HRP-conjugated goat anti-rabbit antibody were purchased from Cell Signaling Technology (Danvers, CST, USA). The 0.25% trypsin-ethylene diamine tetra acetic acid (EDTA) solution, examethasone, L-ascorbic acid phosphate, glycerophosphate, isobutylmethylxanthine and indomethacin were purchased from the Sigma–Aldrich company (St. Louis, MO, USA). The phycoerythrin (PE)-conjugated antibodies against CD29, CD45, CD90 antigens were purchased from BioLegend (San Diego, CA, USA). The PE-conjugated antibody against CD34 antigen was purchased from Santa Cruz Biotechnology (Dallas, Texas, USA).

2.2. Preparation of plasmid-encapsulated PAI/SPION complexes

The plasmids pCMV-Luciferase2-mKate2, co-expressing luciferase and red fluorescent protein (RFP), were used in the present study. The plasmid DNAs were expanded in *Escherichia coli* (*E. coli* strain DH5α) and purified with EndoFree Plasmid Giga Kits (QIAGEN, CA, USA) according to the manufacturer's protocol. The concentration of the plasmid was assessed by UV spectrophotometry at 260 nm. The purified plasmid was kept in ultrapure water at a concentration of 2.5 μg/μL. The plasmid solution and the delivery vector solution (PAI/SPION) were mixed according to various N/P ratios (nitrogen of non-viral vector/phosphate of plasmid), and then the mixture was kept at room temperature for 30 min prior to use (Fig. 1).

2.3. Agarose gel retardation assay

The complexes (PAI/pDNA) were prepared at various N/P ratios that ranged from 1 to 3. The amount of plasmid for each sample was 1 μg. They were loaded onto the 1% agarose gels with ethidium bromide (0.1 mg/ml) and electrophoresed at 100 V for 45 min. The retardation of plasmid mobility was evidenced using an ultraviolet imaging system (DNR Bio-Imaging Systems, Israel).

2.4. Zeta potential and particle size measurements

The PAI/SPION/pDNA complexes were prepared at various N/P ratios that ranged from 3 to 22. The amount of pDNA for each sample was 1 μg. The zeta potential and particle size of each sample were measured with a Zeta-Plus instrument (Brookhaven Instruments Corporation, USA) at room temperature. The data represent the averages ± standard deviations. All tests were performed in triplicate. The serum stability and the MRI sensitivity of the nanoparticle was evaluated (Supplementary information).

2.5. Animals

The female Sprague–Dawley rats (six-week-old) were purchased from the animal center of Sun Yat-sen University (Guangzhou China). All conditions and handing of animals in this study were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Bioethics Committee of Sun Yat-sen University.

2.6. Isolation and culture of MSCs

MSCs were isolated and cultured as reported previously [27]. In brief, the rats were sacrificed by cervical dislocation. The femurs of the rats were excised aseptically and placed in low-glucose DMEM. The bone marrow tissue was collected by flushing the femurs with DMEM using a 26 gauge needle. After centrifugation at 2000 rpm for 20 min, the precipitate was resuspended in DMEM (supplemented with 10% fetal calf serum, 100 IU/ml penicillin, 100 g/ml streptomycin) and seeded into a culture dish (37 °C, 5% humidified CO_2) (Corning, Schiphol-Rijk, the Netherlands). After 24 h, the nonadherent cells were removed. The culture medium was changed every 3 days. At subconfluence (70%), the cells were detached with trypsin and passaged at the ratio of 1:3 dilution. The final MSCs used were taken at passage 4.

2.7. MSCs phenotype identification

Surface markers expression of cultured bone marrow derived MSCs at primary or later passages were assessed with a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). MSCs were detached using 0.25% trypsin-EDTA solution, and then incubated with phycoerythrin (PE)-conjugated antibodies against CD29, CD34, CD45 and CD90 antigens for 30 min. After being washed with PBS, the cells were analyzed with fluorescence-assisted cell sorter (BD Biosciences, USA). Rat IgG isotype control (R&D Systems, USA) was used as control.

2.8. CCK-8 assay

MSCs were seeded at a density of 5×10^3 cells per well in 96-well plates. Each well contained 100 μl of DMEM medium supplemented with 10% FBS, and the MSCs were cultured for 24 h at 37 °C. The cells were then incubated in 100 μl of fresh medium for 48 h with the PAI/SPION/pDNA complexes prepared at various N/P ratios and Lipofectamine/pDNA complexes prepared as the manufacturer' protocol, respectively. The amount of pDNA in each well was set to 1 μg. After incubation with the complexes, 10 μl of Cell Counting Kit-8 (CCK-8; Kumamoto, Japan) solution was added to each well according to the manufacturer's protocol. The cells were further incubated for 3 h, and the absorbance at 450 nm and 620 nm were recorded with an Infinite F200 Multimode plate reader (Tecan, Crailsheim, Germany).

2.9. MSCs uptake analysis

The uptake of complexes by the MSCs was evaluated by confocal laser scanning microscopy (CLSM) experiments using a Zeiss LSM 510 META microscope (Carl Zeiss, Göttingen, Germany). The PAI/SPION was labeled with Oregon Green 488, and pDNA was labeled with popo3, as reported previously [28]. The MSCs were seeded at a density of 1×10^3 cells in a confocal dish. The popo3-labeled pDNA was combined with the corresponding amount of Oregon Green 488-labeled PAI/SPION