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Retronectin enhances lentivirus-mediated gene delivery into hematopoietic progenitor cells

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Abstract

Genetic modification of hematopoietic stem cells holds great promise in the treatment of hematopoietic disorders. However, clinical application of gene delivery has been limited, in part, by low gene transfer efficiency. To overcome this problem, we investigated the effect of retronectin (RN) on lentiviral-mediated gene delivery into hematopoietic progenitor cells (HPCs) derived from bone marrow both in vitro and in vivo. RN has been shown to enhance transduction by promoting colocalization of lentivirus and target cells. We found that RN enhanced lentiviral transfer of the VENUS transgene into cultured c-Kit$^+$ Lin$^-$ HPCs. As a complementary approach, in vivo gene delivery was performed by subjecting mice to intra-bone marrow injection of lentivirus or a mixture of RN and lentivirus. We found that co-injection with RN increased the number of VENUS-expressing c-Kit$^+$ Lin$^-$ HPCs in bone marrow by 2-fold. Further analysis of VENUS expression in colony-forming cells from the bone marrow of these animals revealed that RN increased gene delivery among these cells by 4-fold. In conclusion, RN is effective in enhancing lentivirus-mediated gene delivery into HPCs.

Keywords: Retronectin; Hematopoietic progenitor cells; Lentivirus; Gene delivery; Intra-bone marrow injection; Colony-forming activity

1. Introduction

Efficient gene transfer techniques offer the possibility to manage both malignant and non-malignant human diseases. Since hematopoietic stem cells (HSCs) are capable of differentiating into specialized blood cells as well as undergoing self-renewal, these cells are important targets for gene delivery. Several gene therapy trials have been conducted to investigate the use of HSCs in the treatment of cancers, immunodeficiencies, and AIDS [1–3]. Virus-mediated gene transfer is essential to stably integrate the gene of interest into the genome of target cells and to sustain long-term expression [4]. Retrovirus-mediated gene transfer has been widely used since this method is safe and allows for stable vector integration and expression with simple manipulation [5–7]. However, this gene transfer method is problematic due to low gene transfer efficiency into hematopoietic cells [8]. The human immunodeficiency-based lentivirus vector (LV) was developed to improve gene transfer efficiency into HSCs, including both dividing and non-dividing cells [9–12]. Multiple trials have been conducted with this vector to investigate newly discovered cytokines, highly expressed alternative viral receptors, and a modified vector.

Previous gene delivery studies have shown that fibronectin promotes colocalization between stem cells and vector particles and increases gene delivery efficiency [13,14]. In addition,

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fibronectin fragments containing the RGDS in segment-1 (CS-1) and heparin-binding domains induce colocalization of virus particles and HSCs, promoting efficient gene transduction into HSCs [15]. The biological activity of fibronectin significantly enhances the phenotypic and functional improvement of murine bone marrow hematopoietic cells. In fact, in vitro culture and in vivo transplantation tests have shown that fibronectin can increase expansion of primitive HSCs/HPCs by 800-fold [16]. Fibronectin is abundantly expressed in the bone marrow microenvironment and supports cell-to-cell adhesion as well as cell-to-matrix adhesion to influence homing, cell survival, and proliferation [17]. The CS-1 domain binds to HSCs/hematopoietic progenitor cells (HPCs) via VLA-4 integrin, and this binding is lost upon differentiation. The RGDS domain binds to HSCs/HPCs as well as many differentiated cells via VLA-5 integrin [18,19]. On the other hand, the heparin domain binds to virus particles. RN is a fibronectin fragment containing all three of these domains (i.e., RGDS, CS-1, and heparin domains). This fragment not only supports colocalization of target cells expressing integrins and virions, but also enhances retroviral gene delivery into murine and human HSCs [5,20].

This study was conducted to investigate whether HSCs can be genetically modified through intra-bone marrow (IBM) injection of mice with a combination of RN and LV encoding a VEGF transgene. Our data show that co-injection of lentivirus and RN increases the efficiency of gene delivery into HSCs. Our data also reveal that RN enhances gene delivery in cultured bone marrow-derived c-Kit$^+$ Lin$^-$ HPCs and augments the colony-forming ability of these cells. These findings suggest that RN supports in vivo lentiviral gene transfer into HPCs in bone marrow.

2. Materials and methods

2.1. LV preparation

The LV contained the packaging construct pMDLg/pRRE, which deleted all accessory genes (*vif*, *vpr*, *vpu*, and *nef*) and regulatory genes (*tat* and *rev*) [21,22]. Human immunodeficiency virus type 1 expressing the VENUS gene under the control of the human polypeptide chain elongation factor-1α (EF-1α) promoter was produced by transiently transfecting 293T cells with envelop plasmid [pRSV-REV harboring the vesicular stomatitis virus G glycoprotein (VSV-G) gene], packaging plasmid (pCAG-HIVgp), and transfer plasmid. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) was included in the LV backbone to enhance transgene expression. 293T cells were co-transfected with LVs pseudotyped with VSV-G and the plasmid DNAs using calcium phosphate transfection buffer. Media were replaced 12–16 h after transfection with Dulbecco's modified Eagle's medium (Sigma, St Louis, MO, USA) supplemented with 10% FBS (Gibco, Carlsbad, CA). Virus particles were collected 48 h after transfection and concentrated ~400-fold by centrifugation at 50 000 × *g* for 4 h at 4 °C. To measure virus titer (TU/ml), we transfected HeLa cells with serial dilutions of the viral supernatant and analyzed the percentage of VENUS-expressing cells using FACS vantage SE (Becton–Dickinson, CA).

2.2. Mice

All C57BL/6 mice (5–6 weeks old) were obtained from the Orient Animal Corporation. The mice were maintained under pathogen-free conditions at the Animal Facility of Cell and Gene Therapy Research Center of CHA University. Cages, bedding, and water were sterilized and maintained under laminar flow conditions. Mice aged 6–10 weeks old were used to isolate bone marrow cells for lentiviral gene transfer.

2.3. Hematopoietic progenitor cells' purification and viral transduction

Isolation of c-Kit$^+$ cells from murine bone marrow cells was performed using magnetic cell sorting (MACS). Sorted c-Kit$^+$ cells were prestimulated for 48 h in α-MEM containing 20% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml rmSCF (Peprotech, Rocky Hill, NJ), and 50 μg/ml rmIL-6. Culture plates (24-well plate) were prepared for transduction by coating with 18 μg/ml RN (Takara Shuzo Co., Otsu, Japan) or PBS (control). Well was coated by incubation in RN or PBS at room temperature for 1 h. Nonspecific binding sites on the wells were blocked with 2% BSA for 30 min at room temperature. Wells were then washed three times with PBS. Prestimulated c-Kit$^+$ cells (1×10^5 cells/well) were seeded onto RN-coated wells in 0.6 ml of VENUS lentiviral supernatants containing 50 μg/ml rmSCF and 50 μg/ml rmIL-6. After a 5-h incubation, lentivirus supernatants were replaced with 1.5 ml α-MEM containing 50 μg/ml rmSCF and 50 μg/ml rmIL-6, and the plate was incubated at 37 °C. Transduction efficiencies were assessed by FACS analysis 48 h after transduction.

2.4. Colony-forming capacity assay

Transduced cells were subjected to colony-forming capacity (CFC) assays to assess the efficiency of gene transfer into primitive progenitor cells with proliferative and differentiative capability. Transduced c-Kit$^+$ cells were plated in triplicate onto 35 mm tissue culture plates at a density of 2.5×10^4 cells/ml. Cells were incubated at 37 °C in 5% CO_2 and grown in methylcellulose medium 3434 (Stem Cell Technology, Vancouver, Canada) supplemented with 50 ng/ml rmSCF, 10 ng/ml rmIL-3, 10 ng/ml rhIL-6, and 3 U/ml rhEPO. After 12 days of culture, colonies were scored and photographed in situ under fluorescent inverted microscopes.

2.5. Direct IBM injection with LV

Lentiviral supernatants were concentrated to $1–2 \times 10^9$ particles per milliliter. C57BL/6 mice were sublethally irradiated with 6 Gy from a ^{137}Cs source prior to IBM injection. Mice were anesthetized via intraperitoneal injection of 20 μl rumpun and 20 μl ketamine, and the skin around the right knee