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Original article

HIV-1-derived self-inactivating lentivirus vector induces megakaryocyte lineage-specific gene expression

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Abstract

Pluripotent, self-renewing, hematopoietic stem cells are considered good targets for gene modification to treat a wide variety of disorders. However, as many genes are expressed in a stage-specific manner during the course of hematopoietic development, it is necessary to establish a lineage-specific gene expression system to ensure the proper expression of transduced genes in hematopoietic stem cells. In this study, we constructed a VSV-G-pseudotyped, human immunodeficiency virus type 1-based, self-inactivating lentivirus vector that expressed green fluorescent protein (GFP) under the control of the human CD41 (glycoprotein 2b; GP2b) promoter; this activity is restricted to megakaryocytic lineage cells. The recombinant virus was used to infect human peripheral blood $CD34^+$ (hematopoietic stem/progenitor) cells, and lineage-specific gene expression was monitored with GFP measurements. The analysis by FACS determined that GFP expression driven by the GP2b promoter was restricted to megakaryocytic progenitors and was not present in erythrocytes. Furthermore, in the hematopoietic colony-forming assay, GFP expression was restricted to colony-forming units-megakaryocyte (CFU-Meg) colonies under the control of the GP2b promoter, whereas all myeloid colonies (burst-forming units-erythroid, colony-forming units-granulocyte-macrophage, and CFU-Meg) expressed GFP when the transgene was regulated by the cytomegalovirus promoter. These results demonstrated lineage-specific expression after gene transduction of hematopoietic stem cells. The application of this vector system should provide a useful tool for gene therapy to treat disorders associated with megakaryocyte (platelet) dysfunction.

Keywords: Gene therapy; Lentiviral vector; GP2b promoter; Megakaryocyte-specific expression; Peripheral blood $CD34^+$ cell

1. Introduction

Platelets are produced by the shedding of membrane fragments from mature polyploid megakaryocytes that have differentiated from pluripotent hematopoietic stem cells. This differentiation process occurs in multiple steps and includes a commitment to megakaryocytic progenitor cells [1,2]. Platelets play an essential role in the formation of a blood clot. Therefore, various defects in gene expression during megakaryogenesis can result in bleeding disorders, such as thrombocytopenia [3] and thrombasthenia [4]. Currently, a blood transfusion is the only way to restore the shortage of platelets in these diseases. However, multiple treatments are usually required owing to the short half-life of platelets. These factors increase the risk of side effects such as transfusion-related acute lung injury [5], post-transfusion purpura [6], and various blood-borne infections [7,8]. Megakaryocytes, on the other hand, are considered attractive targets for gene therapy because they produce large numbers of platelets, which play an important role in hemostasis. In addition, the high biosynthetic capacity of the megakaryocyte-lineage progenitor cell makes it suitable for the delivery of therapeutic gene products at physiologically useful concentrations. Thus, the gene transduction of hematopoietic stem cells followed by stem cell differentiation along the megakaryocytic pathway represents a promising way of treating some disorders. However, non-cycling quiescent stem cells are transduced

Abbreviations: BFU-E, burst-forming units-erythroid; CFU-GM, colony-forming units-granulocyte-macrophage; CFU-Meg, colony-forming units-megakaryocyte; EPO, erythropoietin; GlyA, glycophorin A; GP2b, glycoprotein 2b; SCF, stem cell factor; TPO, thrombopoietin.

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poorly by retroviral vectors, which are the conventional vectors for exogenous gene transduction, because the breakdown of the nuclear membrane during mitosis is a prerequisite for efficient retroviral integration into the host chromatin [9,10]. Poor transduction efficiency in stem cells is also correlated with low-level expression of receptors for amphotropic retroviral vectors [11,12]. Furthermore, gene expression that is driven by promoter elements in the viral long terminal repeat of retroviral vectors is often only short-lived in transduced cells. On the other hand, adenovirus-associated vectors have been shown to efficiently transduce post-mitotic skeletal muscle and liver cells [13–15], although the efficiency of transgene transduction in hematopoietic stem cells is low [16], and the gene expression directed by these vectors in the hematopoietic lineage is detrimental in many instances.

In addition, as many genes are expressed in stage-specific manner during the course of hematopoietic development, it is necessary to establish a lineage-specific gene expression system in order to ensure the appropriate expression of transduced genes in hematopoietic stem cells. Previous attempts to create retroviral vectors for lineage-specific or cell-type-specific gene expression have focused on the incorporation of lineage-specific promoter elements into retroviral vectors. However, the insertion of an additional promoter with lineage specificity into a retroviral vector often results in promoter interference, which reduces the activity of the internal promoter and/or the long terminal repeat [17–19]. One potential solution to this problem is the use of self-inactivating (SIN) retroviral vectors, in which the promoter and the enhancer elements from the long terminal repeat are deleted after the integration of the provirus [20]. However, the incorporation of a cell-type-specific promoter into a retroviral SIN vector often leads to a severe reduction in viral titer [21].

More recently, attention has focused on generating vectors from lentiviruses such as the human immunodeficiency virus-1 (HIV-1). Unlike the retroviral SIN vectors, SIN-modified lentiviral vectors that contain an internal promoter appear to maintain a relatively high-titer [22–24]. Thus, several groups have developed lentiviral vectors for restricted gene expression in several hematopoietic cells, including red blood cells, T cells, and macrophages [25–28]. The recent discovery of lineage-specific regulatory elements in the human CD41 (glycoprotein 2b; GP2b) gene, the expression of which is restricted to megakaryocytes, offers a promising tool for the development of megakaryocyte-specific viral vectors [29].

In this study, we constructed an HIV-SIN vector that incorporated a 565-base pair fragment of the 5′-flanking promoter sequence of the GP2b gene, and we analyzed megakaryocyte lineage-specific gene expression following the transduction of human peripheral blood (PB) $CD34^+$ cells with this viral vector. Our results demonstrated that this vector is able to direct megakaryocyte lineage-specific expression of the green fluorescent protein (GFP) reporter gene in PB $CD34^+$ cells.

2. Materials and methods

2.1. Cells and cell lines

2.1.1. Human primary cell sources

Buffy coat PB $CD34^+$ cells were collected from volunteer blood donors after obtaining informed consent. All of the samples were processed within 24 h of collection.

2.1.2. Purification of $CD34^+$ cells

Mononuclear cells (MNCs) were isolated from PB using Ficoll-Paque™ PLUS (Amersham Pharmacia Biotech AB; http://www.amershambiosciences.com) density centrifugation. After washing twice with phosphate-buffered saline (PBS) and once with PBS containing 0.5% bovine serum albumin (BSA) and 5 mM EDTA (PBS–BSA), the MNCs were resuspended in PBS–BSA at a concentration of 10^8 cells per 300 μl.

For $CD34^+$ selection, MNCs isolated from PB were subjected to immunomagnetic separation using the MACS CD34 Progenitor Cell Isolation kit (Miltenyi Biotech, Auburn, CA; http://www.miltenyibiotec.com), as described previously [2,30]. Briefly, the MNCs were incubated with microbeads conjugated to mouse monoclonal anti-human CD34 antibodies and human IgG (FcR-blocking reagent) for 30 min at 6 °C. After washing with PBS–BSA, the labeled cells were filtered through a 30-μm nylon mesh and loaded onto a column installed in a magnetic field. Trapped cells were eluted after the column was removed from the magnet. The collected cells were applied to a second column, and the purification step was repeated twice.

2.1.3. Cell lines

Jurkat cells (human T-cell line, obtained from American Type Culture Collection) and CMK cells (human megakaryocytic cell line [31], provided by Dr. Yuzuru Kanakura of Osaka University) were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 50 U/ml penicillin, and 50 μg/ml streptomycin (GIBCO BRL, Grand Island, NY; http://invitrogen.com). The human kidney cell line 293T (obtained from American Type Culture Collection) was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin.

2.2. Vectors

2.2.1. Vector sources

The SIN-HIV vector pHIV CSCG and the packaging plasmid pCMVΔR8.2 were provided by Dr. Inder M. Verma of the Salk Institute in La Jolla, CA [24]. The vesicular stomatitis virus G (VSV-G) envelope plasmid pVSV-G was purchased from Sigma-Aldrich (Steinheim, Germany). The lentiviral vector pHIV GP2b was constructed by replacing the *Bam*HI-*Hind*III fragment that encodes the cytomegalovirus (CMV) promoter gene of pHIV CSCG with the 565-bp