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Original article

Multiply attenuated, self-inactivating lentiviral vectors efficiently transduce human coronary artery cells in vitro and rat arteries in vivo

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Abstract

Endothelial cells (ECs) in normal vessels are poorly transducible by retroviral vectors, which require cell division for gene transduction. Among retroviruses, lentiviruses have the unique ability to integrate their genome into the chromatin of nondividing cells. Here we show that multiply attenuated, self-inactivating, lentiviral vectors transduce both proliferating and growth-arrested human umbilical vein ECs (HUVECs), human coronary artery ECs (HCAECs), and human coronary artery smooth muscle cells (HCASMCs), with high efficacy. Lentiviral vectors containing the enhanced green fluorescence protein (EGFP) transgene driven by either the cytomegalovirus or the elongation factor-1α promoter, but not the phosphoglycerate kinase promoter, directed high-level EGFP expression in endothelial and smooth muscle cells. The endothelium-specific Tie2 promoter also directed transgene expression in ECs. Re-insertion of *cis*-acting sequences from *pol* of human immunodeficiency virus type 1 (HIV-1) into the vectors improved transgene expression. A lentiviral vector containing the vascular endothelial growth factor transgene promoted EC proliferation and sprouting in vitro. In vivo gene transfer was studied by lumenal infusion of vector containing solutions into rat carotid arteries. Lentivirus-mediated EGFP gene transfer was observed in ~5% of ECs. Lentiviral vectors containing the LacZ transgene achieved detectable β-galactosidase activity in rat arteries, albeit at a lower level compared with adenoviral vectors. This difference was mainly due to the lower concentration of lentiviral vector preparations. Lentivirus-mediated gene transfer was associated with minimal neointimal hyperplasia and scant inflammatory cell infiltrates in the media and adventitia. These observations indicate that lentiviral vectors may be useful for genetic modifications of vascular cells in vitro and in vivo.

Keywords: Lentivirus; Gene transfer; Endothelial cells; Vascular smooth muscle cells

1. Introduction

Gene transfer is both a powerful tool for the investigation of vascular biology and a promising approach to vascular diseases [1]. Gene transfer vectors currently used in vascular gene therapy trials include plasmid DNA vectors, DNA–liposome complexes, and adenoviral vectors [2,3]. However, plasmid DNA vectors are limited by poor DNA uptake, while adenoviral vectors are limited by tissue inflammation and short-lived transgene expression. Immune responses to viral gene products are mainly responsible for the short duration of transgene expression using adenoviral vectors [4]. In addition, both naked plasmid DNA and adenovirus DNA are intrinsically unstable due to the lack of chromosomal integration. In contrast, retroviral vectors integrate into the host cell chromatin, thus providing a potential for permanent genetic modifications of target cells. This is an attractive feature for gene therapy approaches to chronic pathophysiological conditions such as arteriosclerosis, venous bypass grafts, and vascular prostheses, which likely require expression of a protective gene for extended periods of time, ideally for a lifetime. Additional advantages of retroviral vectors include the lack of viral open reading frames, thus avoiding immune responses to viral

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gene products, and a large capacity to accommodate exogenous DNA sequences. For these reasons, vectors derived from oncoretroviruses such as the murine leukemia virus (MLV) have long been favored in gene therapy protocols. MLV vectors have been utilized in experimental gene therapy for post-angioplasty neointimal hyperplasia [5], as well as for genetic engineering of endothelial cells (ECs) seeded on the luminal surface of vascular prostheses [6]. In these models, MLV vectors could be used because the cellular target consisted of proliferating cells. In fact, MLV vectors do not efficiently transduce nondividing cells because they require the breakdown of the nuclear envelope that accompanies mitosis for the nuclear import of the preintegration complex [7]. This is a significant limitation for cardiovascular applications because ECs proliferate at low rates in uninjured blood vessels.

Among the *Retroviridae* family, the *Lentiviridae* subfamily is unique in that these viruses, including human immunodeficiency virus type 1 (HIV-1), productively infect nondividing cells [7]. Three components of the HIV pre-integration complex (the Gag proteins matrix, integrase and Vpr) have been implicated in the nuclear import of the viral genome during interphase [8]. Because only the early steps (attachment, entry, reverse transcription, and integration) of the lentivirus life cycle must be maintained in a lentiviral vector, and because these steps do not depend on viral protein synthesis, most *trans*-acting genes can be excluded from the transfer vector [9]. HIV-vectors pseudotyped with the vesicular stomatitis virus (VSV)-G glycoprotein transduce a variety of cells including neurons, hepatocytes, and retinal cells in vivo [10–12]. However, not all cell types are equally transducible by these vectors. Post-entry blocks to HIV-based transduction have been reported in hematopoietic stem cells and macrophages, but they were rescued by treatment with cytokines and fetal serum, respectively [13,14]. Although lentiviral vectors transduce nondividing hepatocytes in vitro, cell cycle activation is required for efficient in vivo gene transfer into the liver [15]. Moreover, adult cells may be less transducible than neonatal cells, as shown in retinal cells [12]. Together, these observations suggest that cell type-specific activation factors may be needed for efficient lentiviral transduction. It, therefore, is problematic to extrapolate previous data in human umbilical vein ECs (HUVECs) [16–18] to adult human coronary artery ECs (HCAECs), which show distinct biological features [19].

In the present study, we have evaluated lentivirus-mediated gene transfer into proliferating or growth-arrested HUVECs, HCAECs, and human coronary artery smooth muscle cells (HCASMCs). The efficiency of lentiviral vectors in these cells was optimized both by testing several transgene promoters and by reintroducing *cis*-acting sequences from *pol* of HIV-1 into the vector backbone. In vivo gene transfer was studied in rat carotid arteries. We used multiply attenuated, self-inactivating (SIN), lentiviral vectors of the third generation. Their safety profile was further enhanced by using a 4-plasmid conditional packaging system for vector production [20,21].

2. Methods

2.1. Transfer vector constructs

HIV-vectors were produced from the previously described SIN-18 vector, which contains a large deletion in the U3 region of the 3′ long terminal repeat (LTR) [20]. The SIN.CMV-EGFP-W vector contained the enhanced green fluorescent protein (EGFP) transgene driven by the human cytomegalovirus (CMV) immediate–early enhancer/promoter. The SIN.PGK-EGFP-W vector contained the murine phosphoglycerate kinase (PGK) promoter (nucleotides 424–930; a gift of Nicole Déglon, Lausanne, Switzerland) [21]. The SIN.EF1α-EGFP-W vector contained the human elongation factor-1α (EF1α) promoter (nucleotides 373–1561; a gift of Didier Trono, Geneva, Switzerland) [22]. The post-transcriptional regulatory element of woodchuck (W) hepatitis virus was inserted between the EGFP gene and the 3′ LTR [23]. A 118-bp sequence from *pol* of HIV-1 encompassing the central polypurine tract (cPPT) and termination sequences [16] was subcloned upstream of the transgene promoter to generate the SIN.cPPT.CMV-EGFP-W, SIN.cPPT.PGK-EGFP-W, and SIN.cPPT.EF1α-EGFP-W vectors, as well as the SIN.cPPT.CMV-LacZ-W vector containing the LacZ reporter gene. The SIN.cPPT.Tie2-EGFP-W vector contained the EC-specific mouse Tie2 (angiopoietin receptor) promoter (a gift of Curzio Rüegg, Lausanne, Switzerland) [24]. The SIN.cPPT.CMV-VEGF-W vector contained the CMV-driven human vascular endothelial growth factor (VEGF)-165 gene.

2.2. Packaging constructs

pMDLg/pRRE is a CMV-driven expression plasmid encoding the viral capsid, which contains the *gag* and *pol* coding sequences and a 374-bp Rev-responsive element (RRE)-containing sequence from HIV-1 downstream of the *pol* coding sequences [21]. RSV-Rev is a *rev* expressing plasmid in which the joined second and third exons of HIV-1 *rev* are under the control of the Rous sarcoma virus U3 promoter [21]. pMD.G is a CMV-driven expression plasmid that encodes the VSV-G envelope protein (gifts of Luigi Naldini, Turin, Italy). Plasmids were purified with endotoxin-free kits (Qiagen).

2.3. Lentiviral vectors

VSV-G-pseudotyped, HIV-1-based vector particles were produced by cotransfection of four plasmids (pMDLg/pRRE: 12 μg; pRSVrev: 3 μg; pMD.G: 5 μg, SIN vector: 20 μg) onto 293T cells [21]. Culture medium was replaced by serum-free SFM-II medium (Invitrogen) 15 h post-transfection. Thirty-two hours later, cell supernatants were harvested, filtered through a 0.45 μm filtration system, concentrated on Centricon Plus-80 Biomax MW 100,000 (Millipore, Le-Mont-sur-Lausanne, Switzerland), resuspended in PBS, and