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Review

Lentiviral gene delivery to plasmolipin-expressing cells using *Mus caroli* endogenous retrovirus envelope protein

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ABSTRACT

Gene therapy is a promising method for treating malignant diseases. One of the main problems is target delivery of therapeutic genes. Here we show that lentiviral vector particles pseudotyped with *Mus caroli* endogenous retrovirus (McERV) envelope protein can be used for selective transduction of PLLP-expressing cells. As a therapeutic gene in McERV-pseudotyped vector particles we used *miniSOG* encoding the cytotoxic FMN-binding protein, which can generate reactive oxygen species under illumination. Significant cytotoxic effect (up to 80% of dead cells in population) was observed in PLLP-expressing cells transduced with McERV-pseudotyped vector particles and subjected to illumination. We demonstrated that the McERV-pseudotyped HIV-1 based lentiviral vector particles are an effective tool for selective photoinduced destruction of PLLP-expressing cells.

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Contents

Abbreviations: FMN, Flavin mononucleotide; GB, Glioblastoma; McERV, *Mus caroli* endogenous retrovirus; mSOG, mini Singlet Oxygen Generator; NB, Neuroblastoma; PLLP, Plasmolipin; ROS, Reactive oxygen species; VSV, Vesicular stomatitis virus.

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1. Introduction

The existing therapeutic approaches against malignant diseases, such as thermotherapy, chemotherapy, and radiotherapy, often have severe side effects. For now, surgery remains the most effective treatment, but not every tumor is suitable for surgery [1]. Some tumors are resistant to known therapeutic approaches [2,3], so alternative therapy is needed. One of the options is gene therapy, which is now considered a promising area of cancer therapy. Generally, the vectors used in this therapy are classified into viral and nonviral vectors [4,5]. Viral vectors such as adenoviral vectors, retroviral vectors, and lentiviral vectors are efficient delivery agents that provide high level of gene transfer and expression. An important aim is vector target specificity.

In our work, we used modular LeGO vector system, based on lentiviral vectors [6]. In this system the lentiviral envelope protein can be replaced with other viral glycoproteins. Here we used the envelope protein of recently cloned *Mus caroli* endogenous retrovirus (McERV) [7]. Analysis revealed that plasmolipin (PLLP), a major component of myelin that is also expressed on some epithelial cells, is the receptor for McERV. PLLP was first described in the early 1980s and was isolated from canine and bovine kidney plasma membrane, thus originally referred to as plasma membrane proteolipid protein [8,9]. Addition of PLLP to lipid bilayers showed that this protein was capable of forming cation-specific ion channels. Unlike most retroviral receptors, PLLP is tissue-specific. Plasmolipin is found in brain, peripheral nerve and kidney [10–12].

In this paper we show that McERV is a suitable envelope protein for targeting cancer cells that express PLLP. The transduction efficiency of McERV-pseudotyped, lentiviral-vector particles encoding green fluorescent protein (eGFP) was determined for several cell lines, including neuroblastoma (NB) and glioblastoma (GB), and also cancer cells from different origin (leukemia and lymphoma cells). Lentiviral vectors pseudotyped with surface glycoprotein of vesicular stomatitis virus (VSV-G), which uses low-density lipoprotein receptor (LDL-R) for cell entry [13], were used as control. A phototoxin gene was chosen as a model therapeutic gene, because its toxicity can be induced specifically in transduced cells. Genetically encoded phototoxins generate reactive oxygen species (ROS) upon light illumination [14]. The first discovered phototoxin was KillerRed, red fluorescent protein that is induced by green-orange (~520–590 nm) light [15]. Recently a protein that efficiently generates ROS on exposure to blue light was developed. This protein called miniSOG (mini Singlet Oxygen Generator) or mSOG was engineered by site-specific mutagenesis from Arabidopsis phototropin 2, which naturally binds FMN but does not generate ROS. mSOG absorbs blue light with maximum at 448 nm and a shoulder at 473 nm and fluorescents green (peaks at 500 and 528 nm, fluorescence quantum yield 0.37) [16]. mSOG shows high phototoxicity in mammalian cells *in vitro* [17,18]. We used mSOG in all experiments to induce phototoxicity.

2. Materials and methods

2.1. Genetic constructs

Coding sequence of mSOG was amplified from plasmid pDARP-mSOG [19] using the following set of primers: 5′-cgcggatcccgccaccatggaaagagcttttgtg (up) and 5′- ccggaatccgctagccatccagctgc (down). The amplified DNA was digested with *Bam*HI and *Eco*RI endonucleases (restriction sites are underlined in primer structures) and inserted into the LeGO-iPuro2 lentiviral vector digested with the same enzymes. *Escherichia coli* XL-1 blue strain (Stratagene) was transformed with the ligation mixture and clones containing the insert encoding miniSOG were selected. Generated plasmid LeGO-mSOG-iPuro2 was sequenced to confirm correct coding sequence.

2.2. Cell culture

Human embryonic kidney cell line HEK-293, human GB cell lines U-251 MG, U-87 MG, LN-18, A172 and mice fibroblast cell line SC-1 were cultivated in DMEM (Dulbecco's modified Eagle's medium; Gibco) with 10% FBS (fetal bovine serum; Gibco), 4 mM L-glutamine (Gibco), 10 U/ml penicillin and 10 μg/ml streptomycin (Gibco). Human NB cell lines Lan-1, Kelly, SK-N-AS, SK-N-BE, SK-N-SH, SH-SY-5Y, IMR-32 and human histiocytic lymphoma cell line U937 were cultivated in RPMI 1640 medium (Gibco) with 10% FBS (fetal bovine serum; Gibco), 4 mM L-glutamine (Gibco), 10 U/ml penicillin (Gibco), 10 μg/ml streptomycin (Gibco). Human acute myeloblastic leukemia cell line Kasumi-1 and acute T cell leukemia cell line Jurkat were cultivated in RPMI 1640 medium (Gibco) with 20% FBS (fetal bovine serum; Gibco), 4 mM L-glutamine (Gibco), 10 U/ml penicillin (Gibco), 10 μg/ml streptomycin (Gibco). Cells were grown at 37 °C and 5% CO_2.

2.3. Quantitative real-time PCR

RNA extraction was performed from $1*10^6$ cells using TRIzol reagent (Invitrogen) in accordance with the manufacturer's protocol. Approximately 1 μg of RNA was used for the synthesis of complementary (c)DNAs with RevertAid First Strand cDNA kit (Thermo Fisher Scientific). Real-time PCR was performed in triplicate using the Maxima SYBR Green qPCR Master Mix (Thermo Fisher Scientific) with the following three specific primer pairs:

For PLLP: sen-5′-CGCCACCGTTCTCTACATCA-3′; asen-5′-GGCGATCATCACCAAACACG-3′;
For human ACTB: sen-5′-ATGGATGATGATATCGCCGC-3′; asen-5′-CTTCTGACCCATGCCCAC-3′;
For mouse ACTB: mus sen: 5′-TCAAGATCATTGCTCCTCCTG-3′; mus asen: 5′-ACGCAGCTCAGTAACAGTCC-3′;

The expression levels of PLLP were normalized to that of the human ACTB or mouse ACTB (for SC-1 only).

2.4. Transfection, lentiviral transduction and flow cytometry

HEK293 packaging cells were seeded in 100 mm Petri dishes in the amount of $3.0–3.5 \times 10^6$ cells per dish 12–14 h prior to the transfection. DNA of the lentiviral vector LeGO-G2 containing the marker gene of enhanced green fluorescent protein (eGFP), LeGO-C2 containing marker gene of red fluorescent protein mCherry or LeGO-mSOG-iPuro2 containing the phototoxin gene and the plasmids directing the synthesis of the proteins required for the particles formation were introduced into HEK293 cells via calcium

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