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Propagation kinetics of retrovirus transgene vector and replication-competent retrovirus in static and microcarrier cell culture systems using different medium exchange strategies

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Abstract

While retrovirus vector (RV) is the main virus vector used in human gene therapy trials, the biosafety issues that surround currently used RVs have become a matter of concern. Similar to the insertional mutagenesis in the therapeutic target cells, the generation of replication-competent retrovirus (RCR) must be minimized during the manufacture of the virus vector. This work investigated the kinetics of RV and RCR production in PA317-RCM1 producer cells in static and microcarrier cell culture systems. RCR in the progeny of transduced *Mus dunni* cells was detected by the PCR method and the titer of RCR was quantified by cell-based S+/L− assay. The specific rates of RCR production in microcarrier cultures were 271–462% higher than those in the static well-plate cultures. Increased medium exchange operations yielded higher specific rates of RCR production in both static and microcarrier cultures. The optimal medium exchange strategy was on an every 2-day schedule, yielding the highest RV/RCR ratio in static culture but not microcarrier culture. Results of this study presented the difficulty for gene therapy processes that together with the product RV also unwanted RCR produced in two different cell culture systems.

Keywords: Retrovirus vector; Replication-competent retrovirus; Medium exchange; Cell culture; Microcarrier

1. Introduction

Viral vectors derived from retrovirus (including lentivirus), adenovirus, adeno-associated virus, herpes simplex virus and other animal viruses have been widely adopted for ex vivo or in vivo gene delivery for treating patients with inherited or acquired diseases [1]. These gene-delivered viral vectors can be classified into two types: (i) integrating vectors that can integrate the transfer gene into the host chromosome to be expressed over the long term and (ii) non-integrating vectors that deliver the transfer gene to cells to be expressed transiently [1,2]. Of these viral vectors, retroviral vectors (RVs) are integrating vectors, which can deliver a therapeutic gene directly into the host cell chromosome. Although the integrating vectors deliver the gene with a permanent expression, the RV-mediated random integration process can induce tumorgenesis in the target cells. The fact that some patients treated with RV(s) for severe combined immunodeficiency syndrome developed leukemia has caused great concern regarding the safety of RV-based gene therapy [3].

Apart from the insertion mutagenesis caused by the retrovirus vector, the other important safety issue related to the products of the retrovirus vector concerns the presence of a replication-competent retrovirus (RCR). RCR is generated primarily by the homologous recombination of the retroviral element in the RV and the envelope sequences of the RV packaging cells [4]. Any of the retrovirus vector preparations must exclude RCRs since the infectious competent

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RCR may be replicable in patients with potentially deleterious effects [5,6]. In primates, the pathogenic RCR from gene therapy RVs has been shown to generate T cell lymphoma [7,8]. Various molecular designs have been made to suppress the generation of RCR, including designing multiple split packaging cells [4,9,10] and pseudotyping envelope using other viruses [10,11]. However, the appearance of the RCR in RV preparations cannot be completely prevented [12]. RCR can be generated from the RVs and the packaging cells in any step during the manufacture, from the development of the master cell bank to the production of RVs [13].

PA317 cells are the amphotropic packaging cells most widely used to generate RVs for human gene therapies [12]. We previously reported that the microcarrier cultivation of the PA317-RCM1 producer cells [14] on two solid microcarriers (Cytodex 1 and Cytodex 3) yielded high-titer RV as compared to Porous microcarriers such as Cytopore and Cultisphere [15]. This work further examines the optimal culture conditions that maximize the highest RV/RCR ratio in Cytodex 1 microcarrier and static well-plate cultures. RV and RCR production kinetics were quantitatively analyzed to compare the differences between PA317-RCM1 producer cells between microcarrier and static cultures. Different frequencies of medium exchange were also investigated to determine the optimal conditions for improving the production of RV but suppressing the generation of RCR in a cell culture. This study is the first to reveal the optimal medium exchange conditions for maximizing the RV/RCR ratio in the culture. Results of this study on the importance of the culture factors provides valuable information on the optimization of the cell culture process for producing RVs for gene therapy treatments.

2. Materials and methods

2.1. Vectors and cells

The retrovirus vector was constructed from a bicistronic gene retroviral vector, pLNSIX, containing a SV40 promoter and the hammerhead ribozyme cDNA with a catalytic unit that targets the GUU site three nucleotides upstream of the *bcr-abl* fusion point, as presented in Fig. 1 [14,15]. The ribozyme-encoding retroviral vector, pRZI1, was first transfected to the ecotropic GP + E86 [9] cells and then the culture supernatant was employed to infect the amphotropic packing cell line, PA317 [12,16]. The cloned vector producer cell line PA317-RCM1 was formed using the selection medium, which contained 0.75 mg/mL G418. The stable ribozyme expression of the PA317-RCM1 cell line was verified.

NIH/3T3 cells, *Mus dunni* cells and PG-4 cells [17] were obtained from the American Type Culture Collection (CRL-1658, CRL-2017 and CRL-2032, respectively). The cloned PA317 and NIH/3T3 cells were grown in high-glucose Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS) supplemented with 1% penicillin/streptomycin (P/S) solution. *M. dunni* and PG-4 cells were grown in McCoy's 5A medium with 10% FBS and 1% P/S solution.

2.2. DNA isolation and PCR assay

The genomic DNA of transfected *M. dunni* cells was purified from 5×10^6 cells using a DNeasy^{TM} Tissue kit (Qiagen), following the manufacturer's instructions. In the PCR assay, the final concentration of each component was 1× HotstarTaq Master Mix (Qiagen), 0.5 μM of each primer

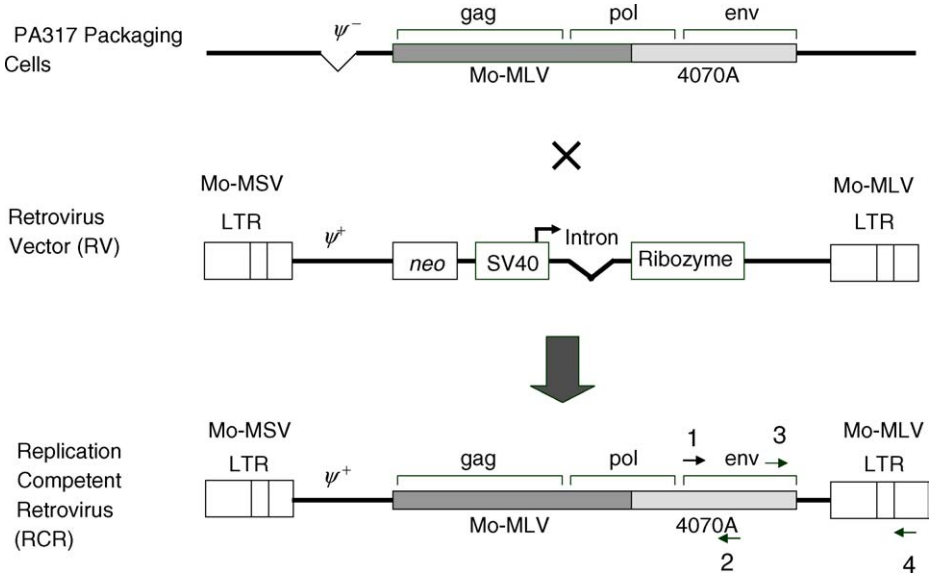

Fig. 1. Genetic constructs of amphotropic PA317 packaging cells, retroviral vector (RV) and replication-competent retrovirus (RCR). RCR is present because of the homologous recombination between the *gag*, the fused *pol* and *env* genes of PA317 packaging cells, and the RV as shown in this figure. The RCR construct includes two sets of primers employed to identify RCR using the PCR method. The primers are labeled 1–4.