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Gene transfer system derived from the caprine arthritis–encephalitis lentivirus

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Abstract

Lentiviruses are attractive candidates for therapeutic vectors, because of their ability to infect non-dividing target cells. Vectors based on HIV-1 efficiently transfer gene expression to a variety of dividing or quiescent cells, but are subject to reservations on safety grounds. Caprine arthritis encephalitis virus (CAEV) is a lentivirus inducing only minor pathology in its natural host and in related species after cross-species transmission. To test the CAEV potential as vector for gene transfer, a cassette expressing the green fluorescent protein (GFP) under control of a CMV promoter was inserted into the CAEV genome, producing the pK2EGFPH vector. When pseudotyped with vesicular stomatitis virus (VSV)-G envelope protein, this vector allowed efficient transfer of GFP expression in human cells (up to 86% of GFP-expressing cells into the TE671 cell line). Three vectors carrying different parts of the viral *gag*, *pol* and *env* genes were then developed, together with a CAEV packaging system. These vectors allowed delimitation of the minimal CAEV sequences necessary for an improvement of vector production compared to the previously described CAEV-based vectors [Mselli-Lakhal et al., 1998. Defect in RNA transport and packaging are responsible for low transduction efficiency of CAEV-based vectors. Arc. Virol. 143, 681–695]. While our previous vectors were produced in a helper/vector system, the present vectors are produced in a helper/free system. However, these vector titers remain lower than those obtained with other lentiviral vectors carrying equivalent packaging sequences. We discuss on possible reasons of such differences and possible improvements.
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Keywords: CAEV; Lentivirus; Vector; Gene; Transfer; Tool

1. Introduction

The capacity of lentiviruses to integrate their genome into the chromosome of non-dividing and terminally differentiated cells makes them attractive candidates as delivery vectors for therapeutic genes (Bukrinsky et al., 1993; Vigna and Naldini, 2000; Pandya et al., 2001). Lentiviruses are, however, complex retroviruses expressing a number of genes with the potential to disrupt cellular function, and are themselves responsible for major pathology (Amado and Chen, 1999; Buchschacher and Wong-Staal, 2000). Lentiviral vectors derived from human immunodeficiency virus type 1 (HIV-1) have been shown to transfer marker or therapeutic genes efficiently *in vivo* to targets including central nervous system (Naldini et al., 1996a,b; Blömer et al., 1997), retina (Myoshi et al., 1997), liver and skeletal muscle (Kafri et al., 1997; Park et al., 2000). Lentiviral vectors have been shown to be efficient for gene delivery into hematopoietic stem cells (Sutton et al., 1998, 1999) and cancer cells (Pang et al., 2001; Solly et al., 2003). For potential human therapy, it is preferable to avoid the use of lentiviral vectors derived from a virus causing severe and lethal disease like immunodeficiency.

Among the several species of animal lentiviruses, caprine arthritis–encephalitis virus (CAEV) has a relatively simple genome structure with only *vif*, *tat* and *rev* accessory genes in addition to the viral structural determinants (Saltarelli et al., 1990). CAEV is widespread in goat herds throughout the world (Crawford and Adams, 1981; Adams et al., 1983), but transmission to human had never been documented despite exposure to

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milk from infected animals, which is the major route of natural transmission from mother to offspring.

To be acceptable as a potential vector, a viral construct should be relatively easy to produce in adequate quantities, should express the transported gene at satisfactory levels in the selected target cells for a prolonged period of time, and should be incapable of independent propagation. To this end, constructs derived from HIV-1 carry only residual elements of viral genes indispensable for the production of vector particles (Gasmi et al., 1999; Kim et al., 1998), or have been rendered self-inactivating to make them non pathogenic (Zufferey et al., 1998; Miyoshi et al., 1998). Unfortunately, it is difficult to find a suitably animal model for testing before human use. Attempts to obtain efficient lentiviral vectors derived from animal lentiviruses have been described (Olsen, 1998). Other lentiviral vectors have since been developed from Simian immunodeficiency virus (SIV; Nègre and Cosset, 2002; Mangeot et al., 2002), feline immunodeffiency virus (FIV, Poeschla et al., 1998; Wang et al., 1999), equine infectious anemia virus (EIAV; O'Rourke et al., 2002), from Maedi visna virus (MVV, Berkowitz et al., 2001), and from caprine arthritis encephalitis virus (CAEV, Mselli-Lakhal et al., 1998).

The present study reports an improvement in the transduction efficiency of the previously described CAEV-based vectors (Mselli-Lakhal et al., 1998). A better knowledge of the residual viral elements necessary for packaging of vector RNA into virions has allowed the development of CAEV-based vectors with improved transfer efficiency of green fluorescent protein (GFP) to various recipient cells. Producing the vectors in a vesicular stomatitis virus VSV-G pseudotyped system allowed infection of human host cells, which are refractory to infection by native CAEV. VSV-G envelope protein conferred additional stability to the vector particles and facilitated the vector stock concentration.

2. Materials and methods

2.1. Cells and viruses

Goat synovial membrane cells (GSM) were derived from an explant of carpal synovial membrane from a goat embryo as previously described (Mselli-Lakhal et al., 2000), and were grown in Eagle's minimum essential medium (MEM, Gibco-BRL, France), supplemented with 10% fetal bovine serum (FBS, Gibco-BRL, France). The large T-immortalized goat embryo fibroblast cell line, TIGEF (Da Silva Teixeira et al., 1997), was obtained by transfection of GSM cells with a SV40 plasmid deleted from its replication-origin (pMK16-SV40-ori).

Human epithelial cell lines TE671, derived from a human rhabdomyosarcoma (ATCC 184B5), A431, derived from a human epidermoid carcinoma (ATCC: CRL-155) and Hela derived from human cervical carcinoma (ATCC: CCL2) were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS. THP-1 cells were derived from the peripheral blood of a 1-year-old boy with monocytic leukemia (ATCC: TIB-201) and were cultured in non-adherent conditions in RPMI 1640 supplemented with 10% FBS. Differentiation into adherent macrophages was induced by addition of 20 ng/ml of PMA (Phorbol 12-myristate13-acetate, Sigma, France) to the medium.

2.2. Plasmid and vectors

As a proof of principle, we constructed a vector containing the GFP gene under control of the cytomegalovirus (CMV) early gene promoter from pTR-UF5 (Clonetech, Ozyme France). The CMV-GFP cassette was inserted into a Sma I site, 16 nt from the end of the *tat* ORF of pK9Kb (Turelli et al., 1996). This last construct was then supplemented by insertion of a *Hin*dIII-missing fragment containing both the end of the *env* ORF and part of the second exon of *rev* and the 3′ LTR. The resulting vector, called pK2EGFPH, was transfected using the calcium phosphate method into TIGEF cell line. For infection of human cells, VSV-G glycoprotein was incorporated into the viral envelope. TIGEF cells were co-transfected with the pK2EGFPH plasmid and pHCMV-G, a construct kindly provided by Dr. J. Burns (Burns et al., 1993), which produces VSV-G protein from a CMV promoter.

The pGPE-GFP, pGP-GFP and pGE-GFP were derived from CAEV-pBSCA genome. To construct the pGPE-GFP vector, a CMV-GFP cassette was derived from the pTR-UF5 plasmid by *Eco*RI digestion and ligated to *Eco*RI digested CAEV-pBSCA DNA. To construct the pGP-GFP vector, the same CMV-GFP cassette was derived from the pTR-UF5 plasmid by *Eco*RI/ *Sal*I digestion and ligated to *Eco*RI/ *Xho*I digested CAEV-pBSCA DNA. To construct the pGE-GFP vector, a PCR amplified fragment was introduced into NdeI digested CAEV-pBSCA plasmid.

Construction of the pBSCAδE5 has been previously described (Mselli-Lakhal et al., 2000). The CAEV LTR was first replaced by a CMV promoter. The CMV promoter was PCR amplified and sub-cloned into the pGEM-T plamid. The CAEV LTR was removed from pBSCAδE5 by Apa I digestion. The fragment corresponding to the CMV promoter was purified from pGEM-T vector by ApaI digestion and introduced into the ApaI site of pBSCAδE5 plasmid to generate pBSδECMV. To remove the Ψ signal, sequential deletions were introduced in the CAEV leader sequence using Bal 31 enzyme. To do this a fragment containing CAEV LTR and leader sequence situated between the end of the CAEV LTR and gag ATG was introduced into the pUC18 plasmid. Digestion with Bal31 was performed for 5, 10 and 15 mn starting from gag ATG to generate sequential deletions, which were characterized by sequencing after subcloning into M13 plasmid. One clone containing a 50 pb deletion between the splice donor (SD) and the end of the leader sequence was introduced into pBSδECMV by a PstI/ XbaI digestion to generate the pBSCA∂Ψ∂E construct.

2.3. Production and titration of vector particles

To produce VSV-G pseudotyped pK2EGFPH particles, pHCMV-G plasmid DNA was cotransfected with pK2EGFPH into the TIGEF cells using the calcium phosphate method as previously described (Mselli-Lakhal et al., 2000). The pGPE-GFP, pGP-GFP and pGE-GFP vectors were similarly produced

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