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Review

Foamy virus: an available vector for gene transfer in neural cells and other nondividing cells

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Foamy viruses (FVs) are classified to a subfamily of retrovirus presented in a wide range of hosts. None of FVs has been found to associate with any diseases in their native hosts. Such a unique biological character of FVs makes it suitable for the development of vectors for gene transfer. However, it is still controversial whether foamy virus vectors (FV vectors) can be applied to the central nervous system (CNS). In this review, we summarize the studies of FV vectors, which have been used for transduction of neural cells and other nondividing cells. We further discuss the potential mechanisms underlying the infection and propose that FVs can be used to develop transfer vectors for gene therapy in neurological disorders. *Journal of NeuroVirology* (2010) **16,** 419–426.

Keywords: central nervous system; foamy virus; gene therapy; neural cells; nondividing cells

Introduction

Gene therapy has been proposed to be a potentially alternative method to conventional pharmacological therapy for a wide rang of diseases including nervous system disorders. It is believed that gene therapy can overcome the disadvantage of the traditional therapy such as common side effects and low efficacy.

A critical prerequisite of gene therapy is to obtain safe gene carriers that can efficiently introduce specific gene into target cells. Two main classes of gene delivery systems are available: nonviral and viral vectors. Most nonviral vectors, such as cationic lipids, polymers, dendrimer, and peptides (Meredith *et al*, 2009), are designed to overcome the problems of safety and inflammatory response (Marchand *et al*, 2009; Shi *et al*, 2000). However, nonviral vectors have low efficiency due to intracellular and/or extracellular barriers that prevent the introduction and/or integration of exogenous genes and their long-term expression. Thus it is of great interest to generate recombinant vectors that can overcome the shortage of nonvirus vectors.

Viral vectors have been developed as therapeutic gene transfer carrier for the nervous system (Glorioso *et al*, 2003) and other systems. Retroviral vectors are the most commonly used vehicles; however, not all of retroviruses are suitable for gene delivery. For instance, human T-lymphotropic virus (HTLV) vector is very efficient and safe; but its transduction is cell cycle dependent, which is not suitable for nondividing cells, particularly for neural cells (Roe *et al*, 1993). On the other hand, although human immunodeficiency virus (HIV) vectors are able to transduce nondividing cells and integrate their proviral DNA into host genome, the potential applications of these vectors for gene therapy based on a pathogenic virus are limited by safety concerns

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and remain a public health issue (Lewis *et al*, 1994). Thus more efficient and safer vectors are urgently needed for gene therapy.

Foamy viruses (FVs) are classified into the subfamily *Spumaretrovirinae* and contain structural genes *gag*, *pol*, and *env*, and regulatory genes *bel-1*, *bel-2*, and *bel-3* (Flugel *et al*, 1987, 1991; Maurer *et al*, 1988), as well as dual promoters (an internal promoter [IP] presented in *env* and the other promoter located in 5′ long terminal repeat [LTR]) (Löchelt *et al*, 1995). With such unique structure, nonpathogenic nature, and the large packaging capacity, FVs have been developed into an effective vector system. FVs can change the infected cells into foam-like appearance and produce numerous vacuoles (Achong *et al*, 1971) *in vitro*. Rethwilm reported that foamy virus vectors (FV vectors) could transduce CD34-positive stem cells more efficiently than gammaretroviral and lentiviral vectors (Rethwilm, 2007). Recently the potentiality of FV vectors *in vitro* and in some animal models has been evaluated by reviewing their history (Trobridge, 2009). However, it is still controversial whether foamy viral vectors could transduce nondividing cells. And this issue determines whether they can be applied to the central nervous system (CNS). In this review, we will focus on the ability of FVs to transduce neural cells and other nondividing cells. We will also discuss the potential mechanisms underlying FVs infection and the advantages and disadvantages of FV vectors.

Transduction of neural cells and other nondividing cells by FVs

Currently, several studies have suggested that FVs can effectively transfect neural cells and other nondividing cells, and may be further developed as a tool for gene therapy in brain diseases (Table 1).

Transduction of the G_1/S-arrested cells

Although FV vectors have shown a wide range of host, controversy still exists whether FV vectors could be used to transduce the G_1/S-arrested cells or G_0-arrested cells. The integration pattern (integration or unintegration) of foamy virus genome in G_1/S-arrested cells also remains debatable. It has been demonstrated that FVs can transfect G_1/S-arrested MRC5 cells when these cells were treated with aphidicolin prior to and during the infection, suggesting that the preintegration complex (PIC) can enter the nucleus of G_1/S-arrested cells (Saïb *et al*, 1997). However, FVs cannot transfect MRC5 cells at G_0-arrested stage, indicating that foamy virus genome cannot import the nucleus of G_0-arrested cells. In addition, both one-LTR and two-LTR circles were present in aphidicolin-treated cells, suggesting that foamy virus PIC can enter the nucleus in the absence of mitosis (Saïb *et al*, 1997). In contrast, Trobridge *et al* reported that FV vectors were not capable of transducing human fibroblasts arrested by aphidicolin (G_1/S phase) but efficiently transduced G_0 fibroblasts that were later stimulated to divide, and a partial cell cycle including mitosis but not DNA synthesis was required. They proposed the persistence of transduction in quiescent cells as a possible explanation (Trobridge *et al*, 2004). In addition, the different observations may be due to the fact that FV vectors enter the nucleus by distinct mechanisms, as both the viral genome and Gag proteins accumulate near the centrosome (Saïb *et al*, 1997). Most recently Lo *et al* (Lo *et al*, 2010) confirmed that the foamy virus genome was found in the nuclei of cells arrested in the G_1/S phase and further provided evidence that the foamy virus genome remained unintegrated in G_1/S phase-arrested cells. It is known that lentiviruses can productively infect postmitotic cells or cells arrested in the G_1/S phase because the PIC traverses an intact nuclear membrane (Snyder *et al*, 1997; Suzuki *et al*, 2007). Furthermore, the observation that foamy virus genome and Gag can enter the nuclei of growth-arrested cells indicates an ability for the foamy virus PIC to cross an intact nuclear membrane similar to that of lentiviruses (Lo *et al*, 2010). However, it is suggested that a role for cellular proteins may have been down-regulated due to the lack of foamy virus genome integration in G_1/S phase-arrested cells, in viral DNA integration into the host genome (Lo *et al*, 2010).

Transduction of the human neuron cells (NT2.N)

Most retroviral vectors are limited in their clinical applications in nondividing and/or terminally differentiated cells because these viral vectors can replicate only in the dividing cells (Miller *et al*, 1993; Vile *et al*, 1995). Interestingly, Mergia *et al* demonstrated that NT2.N neurons, postmitotic human neurons, were transduced with either simian foamy virus-1 (SFV-1) or murine leukemia virus (MuLV) vector at the same multiplicity of infection (MOI) in the presence of a mitotic inhibitor. The transduction rate was 30% to 50% cells in SFV-1 vector particles, but was <1% in MuLV vector. This result not only provides the evidence that SFV-1 can efficiently transduce nondividing cells, but also confirms that the SFV-1 vector can express heterologous genes independent of the cell cycle.

Transduction of the cultured rat hippocampal neurons

To determine whether FVs can be used to transduce neuron, Liu *et al* introduced glutamic acid decarboxylase (GAD) gene into hippocampal neurons using FV vectors (rdvGAD67) and detected significant up-regulation of intracellular and extracellular γ-aminobutyric acid (GABA) (Liu *et al*, 2005).

Table 1 Transduction of various types of neural cells and other nondividing cells by FVs

Virus	Transduced cells	Simple viewpoint	Authors	Year	Literature source
Human foamy virus (HFV)	MRC5 cells blocked in G1/S phase	Foamy virus genome can enter the nucleus of G_1/S-arrested cells	Saib *et al*	1997	*J Virol*
Simian foamy virus	NT2.N	The SFV vectors can transduce human neurons	Mergia *et al*	2001	*Virology*
HFV	Human fibroblasts	Foamy viral transduction intermediates persist in quiescent cells	Trobridge *et al*	2004	*J Virol*
HFV	Cultured rat hippocompal neurons	HFV vectors can introduce GAD into rat hippocompal neurons with high efficiency	Liu *et al*	2005	*Intervirology*
HFV	Cultured astrocytes	Astrocytes can be efficiently transfected by HFV vectors and GAD can be long-term expressed	Liu *et al*	2007	*Mol Ther*
HFV	Dorscal root ganglion (DRG) neurons	Neuropathic pain can be reduced with the high expression of GAD67 in DRG neurons transduced by HFV vectors	Liu *et al*	2008	*Neurosci Lett*
Foamy virus	CNS and brain tissues	Foamy viruses have much higher efficiency for transducing brain tissues *in vivo*	Caprariello *et al*	2009	*Gene Ther*
HFV	HSF cell lines	The foamy viral genome remains unintegrated in the nuclei of G1/S phase-arrested cells	Lo *et al*	2010	*J Virol*

Transduction of the dorsal root ganglion (DRG) neurons

Furthermore, following the infection of DRG neurons with FVs carrying GAD67 gene, the neuropathic pain was reduced in the spinal cord injury rat model, accompanied with increased GAD67 mRNA level (Liu *et al*, 2008).

Transduction of the cultured astrocytes

In addition to neurons, there are large numbers of astrocytes in CNS, which could be used as target cells for gene therapy due to their strong ability for survival and integration (Lundberg *et al*, 1996; Snyder *et al*, 1997; Ridet *et al*, 2003). Liu *et al* reported that more than 50% of astrocytes expressed the green fluorescent protein (GFP) reporter protein, correlating well with that ~50% cells expressed GAD gene *in vitro* (Liu *et al*, 2007). These data demonstrated that FV vectors can efficiently introduce target gene into astrocytes, with great clinical potential for treatment of neurological diseases.

Transduction of brain tissues in vivo

To investigate whether FV vectors can be used for the treatment in CNS *in vivo*, Caprariello *et al* injected FVs or lentiviruses with the target gene into the corpus callosum of adult rat and found that the volume of brain parenchyma transduced by FVs with the target gene was significantly larger than that of lentiviruses 1 week after the injection (Caprariello *et al*, 2009). These data showed that FV vectors can be used to deliver genes in CNS.

Possible mechanisms for the transduction of neural cells and other nondividing cells

The underlying mechanisms of foamy viral transduction in neural cells and nondividing cell are still unclear. The foamy virus life cycle is unique in that single-stranded viral RNA is converted into double-stranded DNA within virion-producing cells and can transcribe DNA independent of target cell nucleotide pools and can then use DNA as the functional genome (Yu *et al*, 1996a). The special character may allow the foamy virus genome to be persistent as a PIC that can enter the nucleus in the absence of mitosis. Based on the life cycle and the structure of FVs (Figure 1), the following possible mechanisms may account for the transuction of neural cells and other nondividing cells by FVs.

I. The replication of FVs is similar to hepadnavirus, e.g., hepatitis B virus (Yu *et al*, 1996; Moebes *et al*, 1997; Delelis *et al*, 2004), but differs from other retroviruses. The reverse transcription of foamy virus genome takes place during viral particle formation. There is about

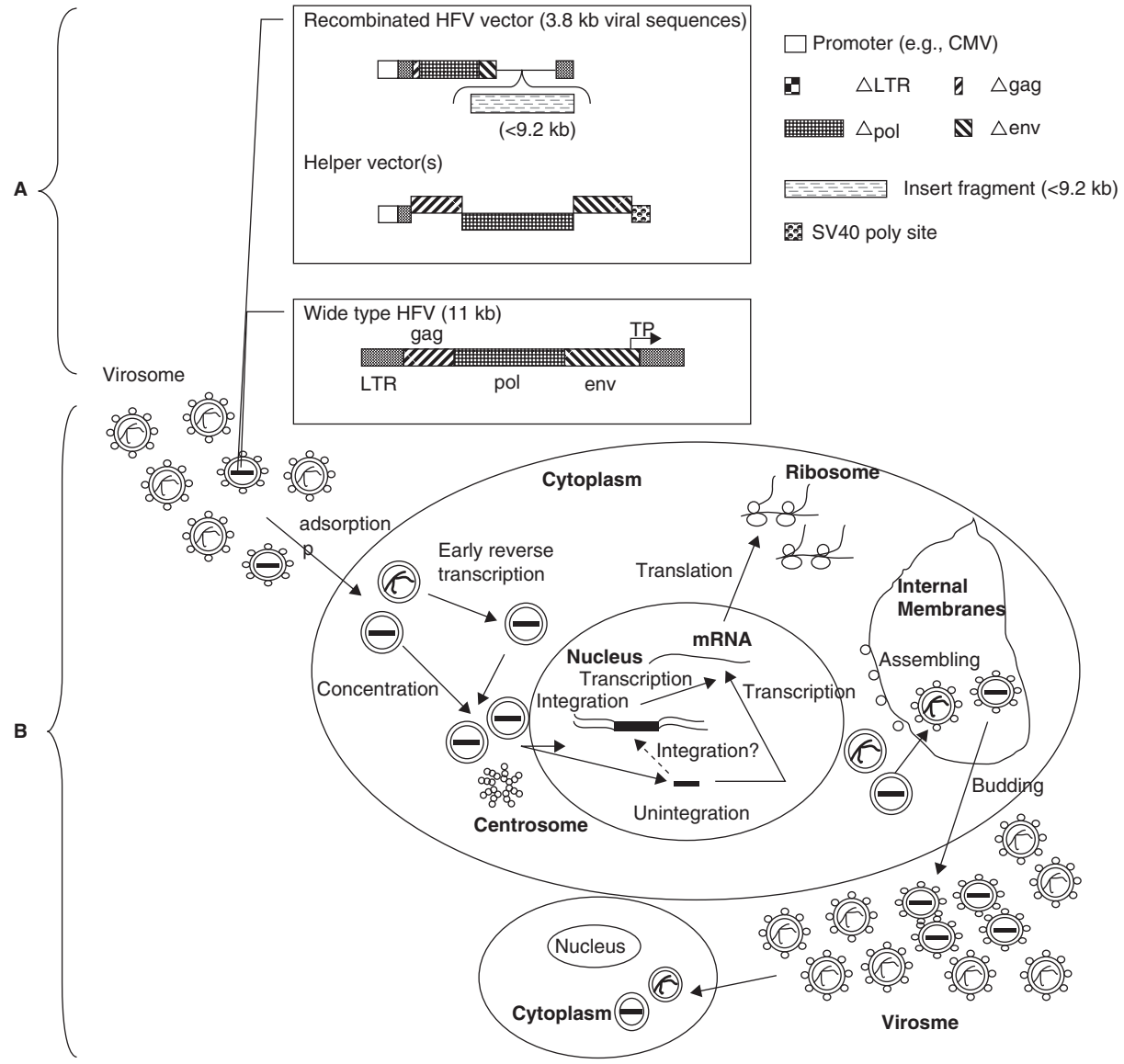

Figure 1 A proposed model for the transduction of nondividing cells by FVs. (**A**) The map of the wild-type HFV cDNA (about 11 kb) and the recombinant HFV vector with minimal viral sequences (3.8 kb) containing a 9.2-kb transgene cassette. In addition, the helper plasmid(s) is/are constructed to express the packaging signal by a single vector/or by several vectors, respectively. (**B**) About 20% of the extracellular and intracellular infectious viral particles contain viral full-length cDNA genomic via the early and late reverse transcription, which is beneficial for viral integration. After entering cells, FVs concentrate around the centrosomes, and then enter the nucleus. The microtubules network may play an important role in importing the virus into the nucleus. The expression of foamy viral proteins is different from others, especially in Pol protein, which comes from a spliced mRNA directly rather than from Gag-Pol fusion protein. After the assembly, the packaged viral particles bud from the internal membranes to infect other cells, and this life cycle may be more suitable for the survival of the virus.

20% of the viral particles that contain viral full-length cDNA genome (Mergia *et al*, 2001), which can be used as the functional genome involved in the formation of PIC. Therefore, the nucleotide pools in the cells transduced by FVs may not play a critical role.

II. The Gag precursor of FVs is principally cleaved near the C-terminus and processed into mature product. The nucleocapsid protein produced by *gag* gene lacks the domain that is rich in cysteine and histidine (Schiephake *et al*, 1994); however, it has three domains that are rich in glycine and arginine (GR-box). The nuclear location signal exists in GR-box and the Gag protein is an important component or element of the PIC (Saïb *et al*, 1997).

III. The viral protein translation of FVs is significantly different from other retroviruses. The

pol gene products of other retroviruses, including reverse transcriptase, are generated as Gag-Pol fusion protein, and then cleaved to functional enzymes during the process of viral budding or release. But the translation of *pol* gene of FVs comes directly from a spliced mRNA (Yu *et al*, 1996; Enssle *et al*, 1996). Due to this characteristic, protease-reverse-transcriptase-integrase proteins derived from Pol are activated early, when viruses assemble. The early activation of these enzymes may facilitate the formation of the PIC.

IV. Incoming FVs accumulate around the centrioles in G_1/S-phase–arrested cells, and this phenomenon can be completely abolished by colchicine. It is suggested that the centrioles play an important role in the import of foamy viral genome into nucleus in the presence of the nuclear membrane.

V. The release pattern of foamy viral particles in the infected cells is different from other viruses. FVs bud from the intracellular membranes (Morozov *et al*, 1997), which makes them more suitable for survival (Patton *et al*, 2005).

VI. A Tas (transactivator of spumavirus) dependent enhancer element is located in the 3′ end of *gag* gene, and in the upper reach of *pol* gene (Yu *et al*, 1996b; Campbell 1996). In addition, a TATA box is located in the upper reach of reverse transcription domain. These suggest that an IP within the *pol* gene mediates transcription of reverse transcription domain.

The advantages of vectors derived from FVs

FVs belong to *Spumaretrovirinae* subfamily (Khan *et al*, 2009), and they are safer and more efficient gene delivery vehicle, compared to HIV and HTLV in the same family. There are many advantages to use FVs for gene therapy, particularly for the treatment of nervous system disorders (listed in Table 2):

VII. FVs can infect humans who are exposed to the infected animals or the tissues contaminated with such virus (Khan *et al*, 2009). Thus FV vectors can be used for transduction of different cells/tissues (Heneine *et al*, 1998; Russell *et al*, 1996) due to their ability to infect a wide range of host cells. The feature of broad host tropism makes FVs suitable for universal use in gene therapy.

VIII. A notable aspect is that almost all of the FV vectors are nonpathogenic to human (Caprariello *et al*, 2009), and no foamy viral transmission happens among people following gene therapy (Meiering *et al*, 2001; Liu *et al*, 2005). No clinical consequence (e.g., malignance) has been reported in those people infected voluntarily or nonvoluntarily by FVs (Meiering *et al*, 2001; Saïb *et al*, 1997), supporting that there is almost no risk for the recipients to develop malignancies.

IX. FV vectors can be used for transporting large fragments of target gene (Figure 1), because the genome of FVs is the largest among all retroviruses (11,021 bp) (Flugel *et al*, 1991; Trobridge *et al*, 2002). The FV vectors retaining the minimal *cis*-acting regions can carry 9.2-kb transgene cassette (Trobridge, 2002).

Table 2 Comparison of foamy viruses, gammaretroviruses, and lentiviruses

	Foamy viruses	Gammaretroviruses	Lentiviruses
Pathogenicity	Have no pathogenicity	Have a certain degree of pathogenicity	Have the most serious pathogenicity
Safety	Have the highest security	Have relatively high risk of cancers	Have the highest risk of various diseases
Host tropism	A wider range of infection	A wide range of infection	A wider range of infection
Transduced cells	Dividing cells, nondividing cells, neural cells	Dividing cells	Dividing cells, terminally differentiated cells
Genome size	12–14 kb	Less than 10 kb	About 10 kb
Packaging capability	The ability to carry foreign gene is the largest, about 14 kb	The ability to carry foreign gene is less than 10 kb	The ability to carry foreign gene is about 10 kb
Promoter	A dual-promoter: U3-promoter and an internal promoter	Only one U3-promoter	Only one U3-promoter
Integration pattern	Inverse correlation between gene density and integration frequency	Irrelevant between gene density and integration frequency	Irrelevant between gene density and integration frequency
Integration site	Have little or no bias toward integration in or near the transcribed genes and oncogenes	Have bias toward integration near transcriptional start sites and within genes	Have bias toward integration near transcriptional start sites and within genes

Thus, FV vectors have great potential for delivery of large target gene.

X. FVs possess a dual-promoter: One is located in the U3 region of the LTR; and an IP located in the end of the *env* (Liu *et al*, 2005; Lochelt *et al*, 1993). The two promoters can coregulate the appropriate expression of therapeutic genes at the temporal and spatial levels in target cells.

XI. FVs have a distinct integration pattern: there is an inverse correlation between gene density and integration frequency. Moreover, FV vectors integrated significantly less near oncogenes by the analysis of integration site and statistical analysis (Bauer *et al*, 2008). These data indicate that FVs prefer inserting into nontranscribed areas of chromosome, and thus is an ideal viral vector for gene therapy.

XII. More recently, it is reported that integration sites increased gradually several weeks after injection, and most integration sites are in the regions of low gene density (Bauer *et al*, 2008). Based on this information, FV vectors may be safe and efficient with long efficacy carrier.

XIII. Finally it is simple and convenient to obtain FV particles, and FV particles are stable when subjected to different manipulations (Josephson *et al*, 2004).

Although the advantages of FV vectors stated above make such vectors as ideal carriers for gene therapy, the safety of retroviral vectors has been paid more and more attention. Especially the discovery of leukemia in 2 of 11 children receiving gene therapy using a retroviral vector has caused serious concern about the adverse effect of retroviral vectors in gene therapy applications (Hacein-Bey-Abina *et al*, 2003; Marshall, 2002, 2003). A possible reason is that the integration of viral gene may activate oncogenes and promote the development of severe diseases. However, the unique integration pattern and integration sites of FVs make them less likely to insert near oncogenes (Bauer *et al*, 2008). To date, no disease has been attributed to FVs in naturally or experimentally infected animals (Linial, 2000; Weiss, 1988). Animal caretakers or hunters infected with FVs remain healthy and human-to-human transmission of FVs has never been reported (Heneine *et al*, 1998; Schweizer *et al*, 1995, 1997; Wolfe *et al*, 2004). Therefore the FV vectors are safer compared with other retroviruses.

Nevertheless, it should be pointed out that FV vectors still have some disadvantages. It is still unclear whether integrated gene could mutate, which would make extremely serious threat to human beings. It cannot be ignored that the titers of retroviral vectors (including FVs) are frequently very low for gene therapy (Verma *et al*, 1997; Blau *et al*, 1997). Picard-Maureau1 *et al* have developed foamy virus–adenovirus hybrid vectors to solve this problem (Picard-Maureau *et al*, 2004).

Prospect

Identification of therapeutic genes and development of most suitable vectors are the major challenges in gene therapy field. With the accomplishment of the human genome project, identification of the functional genes is no longer a complex issue. However, choosing safe and effective vectors in the treatment of nervous diseases remains difficult. Given that FV vectors can efficiently transduce target cells without detectable side effects, there is no doubt that FVs represent a promising vector for gene transfer in neural cells and other nondividing cells.

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