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Applications of Semliki Forest Virus Expression System for AIDS Vaccine Development and Gene Therapy

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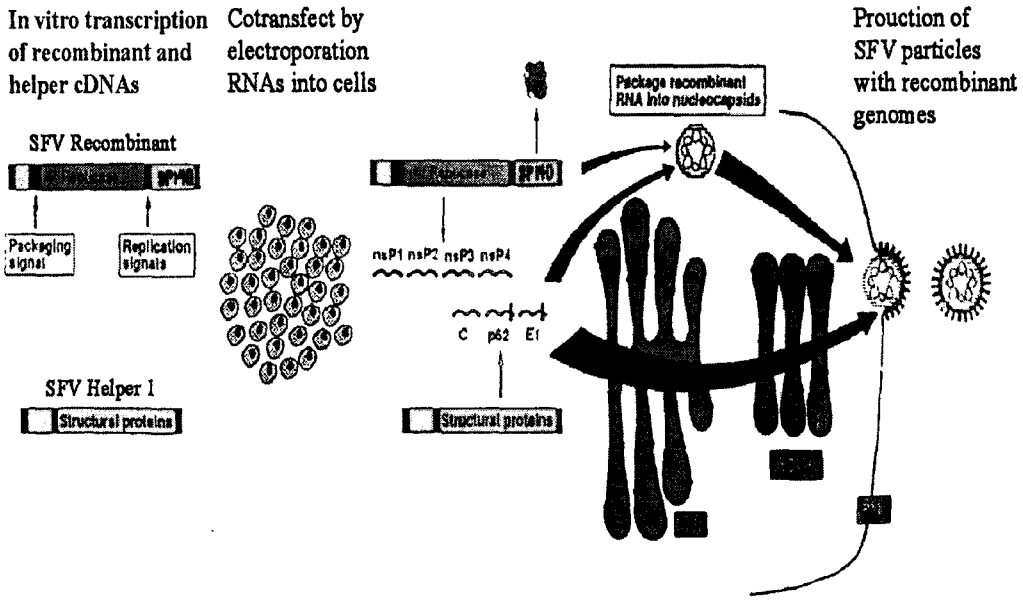
The Semliki Forest virus (SFV) contains a single stranded RNA virus and belongs to alphavirus. The alphavirus have 26 currently recognized members. Complete or partial sequences of the RNA genomes of more than 10 alphaviruses have been reported, and these alphaviruses all share a minimum amino acid sequence identity of about 45% in the more divergent structural proteins and about 60% in the nonstructural proteins. The SFV genomic RNA is 11,442 nucleotides in length exclusive of the 5'-terminal cap and a 3'-terminal poly(A) tract. It is divided into two major regions: a nonstructural domain encoding the nonstructural proteins or replicase proteins, which forms the 5'-terminal two-thirds of the RNA, and a structural domain encoding the three structural proteins of the virus, which forms 3'-terminal one-third. A SFV replication-deficient expression system is currently available. Full-length cDNA clones have created based on an attenuated laboratory strain of SFV. The structural genes have been deleted, which allows for the insertion of foreign DNA downstream of the 26S subgenomic promoter. Infectious virus particles are made by co-transfection *in vitro* transcribed RNAs from the replicon and helper plasmid. The helper plasmid contains the structural genes needed for viral assembly but lacks the signal sequences needed for packaging, therefore only recombinant RNA particles are packaged creating viral particles for one round of infection. SFV expression vectors have been used in a range of mammalian cell lines to express foreign constructs. I adopted this SFV expression vector to make immature and mature human immunodeficiency virus (HIV) for a new AIDS vaccine candidate and to generate a multiple gene transfer vector for gene therapy purposes.

AIDS was first described in 1981 as an outbreak in a homosexual community. Serological and epidemiological studies have shown that the disease has spread to almost all countries in the world. But the classical vaccine strategies based on attenuated live virus or whole inactivated virus have severe limitations. Therefore, most efforts to develop an efficient vaccine have focused on newer vaccine approaches. In this study we have cloned and expressed the structural proteins (Gag and Env proteins) from primary HIV-1 isolate in mammalian cells by the SFV-based expression system, to broaden the host range of target cells for the particles and to make the system suitable also for human cells. Semliki Forest virus has been adapted as an expression system to achieve high-level expression of the heterologous proteins. Replication-competent RNAs(replicons) were transcribed in vitro and introduced into BHK-21 cells by means of eletroporation. Cotransfection of BHK-

21 cells by SFV-helper constructs containing the structural protein genes resulted in the infectious recombinant viral particles that could be used to infect the mammalian cells. We could find that amphotropic envelope virus-like particles carrying the genes of gp160, Gag and Gag-pr proteins. These proteins produced in mammalian cells were detected by immunocytochemistry and Western blot analysis. The Gag and Env protein genes were cloned in a SFV replicon on the same plasmic with separate subgenomic promoters. The RNA transcripts containing the two genes were transfected into mammalian cells and VLPs composed with these two proteins were produced. The VLPs composed of the HIV-1 structural proteins were confirmed with Western blot analysis. In advance, we put the third gene of proteinase in connection to the end of Gag and Env genes with a separate promoter. This plasmid construct contained the genes of three functional proteins derived from HIV-1 primary isolate. Three different proteins were expressed in a mammalian cell at the same time successfully by the transfection with a single step RNA transcript made from this plasmid. The expression of these three proteins was confirmed by immunocytochemistry.



The gene delivery of multiple tumor suppressors can provide an efficient tumor therapy in the case of malignant human gliomas containing multiple genetic alteration and/or inactivation. As such, the current study presents a new delivery system that can express three anti-tumor genes using SFV vector in the expectation of combined or synergistic effects of angiogenesis inhibition by angiostatin and apoptosis induction by p53 and PTEN. Recombinant SFV containing three anti-tunor genes was found to efficiently transduce and express each anti-tumor gene in glioblastoma cells. In addition, rSFV also resulted in a more effective induction of apoptosis *in vitro* and inhibition of tumor growth in nude mice *in vivo* when compared with other rSFVs containing one or two anti-tumor genes. Accordingly, the current results demonstrate that a triple anti-tumor gene transfer using a SFV vector would appear to be a powerful strategy for cancer gene therapy.