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Virus-Mediated Gene Delivery Systems

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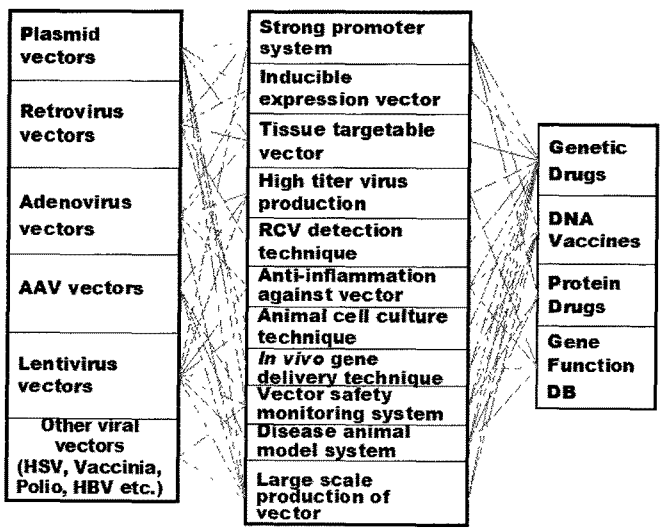
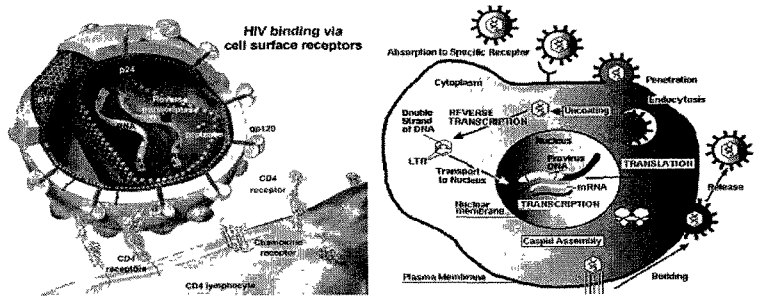
Over the years, a number of gene transfer vectors have been developed that can roughly be divided into two categories: synthetic and virus-based gene delivery systems. Synthetic gene delivery systems depend on direct delivery of genetic information into a target cell and include direct injection of naked DNA (plasmids) and encapsulation of the DNA with cationic lipids (liposomes). Although these delivery systems exhibit low toxicity, gene transfer in general is inefficient and often transient.

Virus-mediated gene delivery systems are based on life cycle of viruses that have the ability to deliver genetic information into the host cell. To generate a replication-defective viral vector, the coding regions of the virus are replaced by the genetic information of genes of interesting or siRNA expressing cassette, leaving the *cis*-acting sequences intact. When the viral vector is introduced into producer cells providing the structural viral proteins *in trans*, production of nonreplicating virus particles containing the genetic information of a therapeutic gene is established. The ability to generate replication defective viral vectors is the backbone of developing virus-based gene delivery vehicles.



Lentiviral Vectors

Currently the most commonly used viral vectors for gene delivery are based either on adenoviruses or retroviruses. Adenovirus vectors are generally used for gene delivery strategies that require the transduced gene to be active for only a short time. Gene delivery by adenoviruses is very efficient, but because the gene does not become integrated into the chromosomes of the target cell it tends to be lost over time. Also, there is a risk of inflammatory reaction to adenoviral vectors in some circumstances. For long-term gene expression, retrovirus vectors based on the murine leukaemia virus (MuLV) are preferred. When genes are delivered by derivatives of MLV they become integrated into the chromosomes of the target cell and are maintained for as long as the cell remains alive. Gene activity is easy to control and continues over long periods of time. Many clinical trials have been conducted with MLV-based systems and they have been shown to be well-tolerated with no adverse side effects. However, one major disadvantage of the oncoretrovirus-based vectors is their dependence on mitosis for transduction. A vector that offered efficient, safe delivery of genes to non-dividing cells and a long duration of gene expression could be expected to be widely used for curing inherited and degenerative diseases such as Gaucher's or Parkinson's disease. Since the discovery that lentiviruses could infect certain non-dividing cells this subgroup has been the focus of the development of a new type of retroviral vector that is able to transduce non-dividing cells.



Lentiviral vectors are a type of retrovirus that can infect both dividing and nondividing cells because their preintegration complex (cDNA-protein complex) can get through the intact membrane of the nucleus of the target cell. Lentiviruses can be used to provide highly effective gene therapy as lentiviruses can change the expression of their target cell's gene for up to six months. They can be used for nondividing or terminally differentiated cells such as neurons, macrophages, hematopoietic stem cells, retinal photoreceptors, and muscle and liver cells, cell types for which previous gene therapy methods could not be used. HIV is a very effective lentiviral vector because it has evolved to infect and express its genes in human helper T cells and other macrophages. The only cells lentiviruses cannot gain access to are quiescent cells (in the G0 state) because this blocks the reverse transcription

step. To understand how HIV is a good vector for gene therapy, we must understand the structure of HIV and how it functions and infects its host. Recent studies have shown the utility of VSV-G pseudotyped HIV vectors to transduce nondividing neurons and other cell types by direct injection *in vivo*.

Use of Non-Human Lentiviral Vectors

By using non-human lentiviruses, scientists hope to bypass the issue of host infection by the gene therapy vector. Researchers have developed non-human lentiviruses such as equine infectious anemia virus (EIAV) to be used as a lentiviral vector in humans. EIAV is a lentivirus that normally infects horses, donkeys, and mules. It has been shown to be able to infect mature macrophages, and thus has the potential to infect quiescent cells, and has relatively simple genome organization. The researchers constructed separate plasmids encoding EIAV proteins, a viral envelop, and an EIAV vector. They attempted to broaden the host range of the vector to human cells by using non-EIAV enhancer/promoter elements to drive expression and a non-EIAV envelope glycoprotein. Also, a biotechnology company in Korea, VectorCore A, has developed a new lentiviral vector system based on CAEV (caprine arthritis encephalitis virus).

In this presentation, I give an overview of the viral vector systems, discussing their specific properties and problems. Furthermore, I give a brief overview of application of lentiviral vector system in the area of gene silencing and expression *in vitro and in vivo*.