FELINE IMMUNODEFICIENCY VIRUS VECTORS IN GENE THERAPY

ABSTRACT

Viral mediated gene therapy is a promising area of research, but for full clinical application it is still necessary to evaluate the risks and benefits of its application in humans. Stable integration of virus genome in the host's genome is an advantage all members of Retroviridae family possesses, but only the lentiviruses members, like human immunodeficiency virus (HIV) can infect quiescent cells. The use of HIV in gene therapy clinical protocols, despite of its efficiency, has not been allowed yet due to safety concerns. In this context, the development of lentiviral vectors with less potential to human pathogenesis is the major reason why feline immunodeficiency virus (FIV) is being researched. This review aims to present relevant aspects related to lentiviral based systems and the potential use of FIV in gene therapy.

The Feline Immunodeficiency Virus (FIV) was isolated in 1986 and is classified as a lentivirus, based on its morphology, Mg²⁺-dependent reverse transcriptase activity and tendency to permanent infection. It is associated with lymphadenopathies of domestic cats, originating a syndrome similar to the Acquired Immunodeficiency Disease (AIDS) caused in humans by HIV with which it presents great similarity, concerning the genomic organization, replicative cycle, and ability to integrate the viral genome into the host genome and to infect quiescent cells (Pedersen et al. 1987, Olmsted et al. 1989a, b, Talbott et al. 1989). There have been some reports of in vitro productive infection of human cells (Ikeda et al. 1998, Johnston & Power 1999), but in general the wild type is pathogenic only to Felidae members.

The interest in FIV developed mainly due to safety concerns related to the possibility of infection following the use of retroviral gene therapy in clinical protocols. In this context, the use of vectors with less potential for human pathogenesis, derived from lentiviruses that are endemic to other species (Price et al. 2002), accounts for the use of FIV in retroviral gene therapy research.

Nevertheless, some modifications on the FIV genome are necessary to make real these expectations, due to the low activity of FIV promoters in human cells, the lack of some cellular factors needed for the nuclear transportation of mRNA, integration
and export in human cells, the absence of recognition of transactivator elements by the human cellular transcriptional apparatus and the inefficiency of FIV rev function in human cells (Sparger et al. 1992). The need for these modifications has turned the viral vector design scientists into artists. But how can they produce this art?

The wild FIV presents tropism for T and B lymphocytes, macrophages, microglia and astrocytes. The target cells are recognized by the surface chemokine receptor CXCR4, to which the virus binds through envelope glycoproteins, usually gp120 (Lombardi et al. 1994). In gene therapy, however, this tropism can be expanded by the addition of a new envelope, a procedure first described by Burns et al. (1993) and called pseudotyping. In that work, the virus chosen was the Vesicular Stomatitis Virus (VSV), which has a G glycoprotein on its envelope surface recognizing phospholipids in mammal cell membrane. The stability of the viral particles is also increased when G-VSV pseudotyping is used, diminishing the titer losses during viral manipulation (Burns et al. 1993, Ory et al. 1996, Reiser et al. 1996).

To modify FIV at the genomic level, it is important to know the function of the lentivirus genes. This will define the deletions that can be made without function loss (Miyazawa et al. 1994, Miller et al. 2000, Bukrinsky & Hauffer 1999). In order to do so, some premises must be respected, and they relate to the transient transfection procedure.

High titers of retroviruses (which includes lentiviruses) can be obtained by a transient three-plasmid expression system, based on the split of gene functions. Due to this fact, the Gag- Pol proteins, the envelope proteins and the genome itself are provided in trans, as the packaging vector, envelope vector and therapeutic vector, respectively (Pear et al. 1993, Soneoka et al. 1995). This system allows the production of helper-free viral stocks, which is one of the important safety concern involved in retroviral mediated gene therapy (for details, see Cornetta et al. 1991).

Another important characteristic of this system is that it produces viruses that can only transduce cells, since the only genetic material packaged is represented by the therapeutic vector. This specific vector presents psi, an encapsidation factor located downstream to LTR S' (Miyazawa et al. 1994, Browning et al. 2001). Experiments with HIV-based vectors comprehending sequential deletions in this region have shown that the psi encapsidation signal interferes with the virus packaging and the viral titer (Iwacuma et al. 1999).

The use of three plasmids is also a safety factor due to the low frequency of recombination events between the plasmids. At least two of those events are necessary to get the genes together again and restore the infectivity of the viral system (Chong et al. 1998, Patience et al. 1998). This is valid to all lentivirus-based vectors.

The procedure described above depends on the establishment of a packaging cell line (PCL) to be transfected. This choice must take into account molecular changes and the future application of the virions. Viral supernatants produced in a PCL that shares the same origin of the target cells usually present higher viral titers (Shinohara et al. 1999). The inactivation by the complement system is a controversial issue, particularly when intravenous vector administration is intended (Takeuchi et al. 1994, Cosset et al. 1995, McCormack et al. 2001). The use of a PEGylation system may bypass this problem (Croyce et al. 2004).

The use of feline packaging cells is not recommended because primates develop immune responses against alpha-1,3-galactosyl, substrate of an enzyme expressed in feline cells, called alpha-1,3-galactosyl transferase (Galili et al. 1987, Takeuchi et al. 1994). Other cell types, such as the human renal endothelium line 293T PCL, are thus recommended (Pear et al. 1993, Soneoka et al. 1995).

The interaction among retroviral vectors and endogenous retroviruses within the PCLs can result in the incorporation of endogenous retroviral transcripts in the recombinant virus (Patience et al. 1996). Chong et al. (1998) detected replication competent viruses (RCV) on the PCL culture supernant, produced during cell culture passages. To effectively solve these problems, it is important to combine the rescue assay with traditional molecular analyses, based on the detection of packaging and envelope vector sequences on transduced cell cultures. To reach it, a useful experimental approach is the use of culture medium conditioned by transduced cells to later transduce another cell culture, in order to verify the existence of functional RCV (Chen et al. 2001).

The issues discussed so far are related to the production of viruses, which include major safety concerns. Now it is important to detail topics related to transgene expression and the specific use of FIV
in gene therapy. The first question is: what should be changed in order to achieve adequate expression levels?

Since the LTRs of the feline immunodeficiency virus genome show low transcriptional activity in human cells (Curran et al. 2000), appropriate transgene expression is conditioned by the replacement of the LTR promoter by strong constitutive eucariotic promoters. It is also important to carefully analyze other regulatory elements, such as splice sites, because they seem to interfere with transgene expression and viral lter (Parolin et al. 1996, Cui et al. 1999). This should also be considered when choosing new strong transcription termination signals (Zaiss et al. 2002).

Many lentivirus-based constructions employ the Cytomegalovirus (CMV) promoter, which allows efficient replication in several human cell types (Sparger et al. 1992, Buchschacher & Wong-Staal 2000), although not in hematopoietic progenitor cells (Ramezani et al. 2000, Price et al. 2002). Scharfmann et al. (1991) and Kay et al. (2001) suggested that the inefficiency of CMV promoters in this cell type could be related to a silencing mechanism acting under this promoter in vivo. It was already shown, by Miyoshi et al. (1999) and An et al. (2000), that the CMV promoter is not a good choice for transgene expression in human hematopoietic stem cells. Alternatives with strong experimental support are the ERF1alpha promoter, from the human elongation factor (Zaiss et al. 2002) and the ubiquitin-C promoter (Lois et al. 2002). After these alterations, FIV vector is ready to be used in cell transduction experiments.

The first report of FIV use in gene therapy was made by Poeschla et al. in 1998. Their research showed efficient transduction in dividing, post-mitotic and quiescent cells, without a preference for feline cell infection. However, the authors used only established cell lineages, and the capacity of FIV vectors to transform others cell types remained unknown.

Wang et al. (1999) proposed an in vitro and in vivo correction of cystic fibrosis with a FIV-based vector, in a rabbit model. The in vitro expression of the transgene was maintained for at least three months and the in vivo experiments showed good transduction efficiency particularly within the respiratory system, including bronchioles, alveoli and progenitor cells.

Concerning optic diseases, it has been demonstrated promising results in trials with rats, rab-

bits and cats using reporter genes such as lac-Z and eGFP (Good et al. 2003, Loewen et al. 2003a, b, Cheng et al. 2004, Good et al. 2004). Most of the target cells for chronic retinal diseases are non-mitotic or have slow mitotic rates, which makes them a suitable target for lentiviral vectors. Loewen et al. (2003) have described the subretinal injection method in rodents, with long-term expression of the transgene (up to 16 months) in retinal pigment epithelium cells (RPCs). Less cell infiltration and less disruption of retinal structure, compared to the Adenovirus-based vector, was reported (Loewen et al. 2004), but in both cases the expression was limited to the RPCs. Glaucoma is a progressive optic neuropathy that could be a result of many different risk factors and diseases associated with high intraocular pressure. Loewen et al. (2002) effectively transduced trabecular meshwork cells - main keepers of the outflow regulation and intraocular pressure - without interfering on its function. Up now no results were obtained using FIV-based vectors carrying therapeutic genes, although there are many potential genes already been used with other vectors systems (Borrás et al. 2002).

To treat hemophilia A, Stein et al. (2001) used the intravenous administration of VSV-G pseudotyped FIV, carrying the functional factor VIII gene. The level of transgene expression achieved, even being lower than the normal factor VIII level, was enough to attenuate the disease phenotype. The maintenance of factor VIII gene expression as long as four months after the viral injection was an indicative of stable long term expression.

FIV was also employed in gene therapy of middle ear mucosal cells to treat chronic middle ear disease, without adverse effects in the inoculated rats (Djalilian et al. 2002). On the other hand, adverse effects were detected with retrovirus Moloney leukemia virus (Check 2002a, b, Kaiser 2003), and HIV-based vectors have not yet been tested in humans. FIV can thus be seen as an interesting alternative and should be compared to other retrovirus-based systems, in order to evaluate the benefits and risks associated to its use.

The application of gene therapy protocols has faced many more problems than those already foreseen when the idea was first developed almost two decades ago. Low gene transfer efficiency and the lack of long-term expression maintenance have been the main limitations. Much research effort is still needed, and the search for better viral vector
constructs must be encouraged, particularly concerning regulation of transgene expression, safety of the system and its adaptability to the human transcriptional machinery. FIV has met gene therapy. Will it be an efficient partnership? It depends on the establishment of an efficient collaboration among researchers from all fields: genetics, virology, cell biology, immunology and so on.

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