Viral vectors and gene therapy

INTRODUCTION

Somatic gene therapy is defined as the transfer of a heterologous gene with the purpose of correcting a genetic defect or providing a new therapeutic function to the target cell, and thus curing a disease or alleviating associated symptoms. The rationale of somatic gene therapy is the correction of diseases at the most fundamental level: the genetic code. Ideally, this goal should be achieved by correcting a defective gene in the human genome. However, correcting the actual genetic defect (i.e., a premature STOP codon in the coding sequence for a certain gene) is not possible because: (a) genetic tools to alter DNA of living cells in such a way are not available (b) often, genetic mutations in a specific gene are heterogeneous (see example for OTC deficiency below). At present, gene transfer technology may provide:

1. Expression of a functional copy of the gene of interest (this is only effective when the genetic defect is of recessive nature).
2. Addition of a new function by transferring an exogenous gene (Example: an antisense RNA against a virus).
3. Inhibition of the unfavorable action of a gene by introducing a counteracting gene (Example: delivery of anti-inflammatory mediators in rheumatoid arthritis).

Gene therapy is most commonly associated with genetic deficiencies. But the spectrum of potential applications of gene therapy goes well beyond that:

- Genetic deficiency
- Viral infection (Example: human immunodeficiency virus)
- Autoimmunity (example: rheumatoid arthritis)
- Cancer
- Diseases in which several genes and the environment interact, such as diabetes, coronary artery disease.

An example of a genetic defect: Ornithine transcarbamylase (OTC) deficiency

OTC is the main enzyme responsible for elimination of ammonium from the blood. Mutations in the ornithine transcarbamylase (OTC) gene leads to OTC deficiency, characterized by elevated levels of ammonium in blood. Ammonium is highly toxic to neurons. OTC deficiency leads to varying degrees of mental retardation and it may also result in early death. The gene encoding OTC is present on the X chromosome. Thus, inactivation of the OTC has more severe consequences in men than in women (women may have a defective copy and a normal copy, and in that case the severity may vary widely).

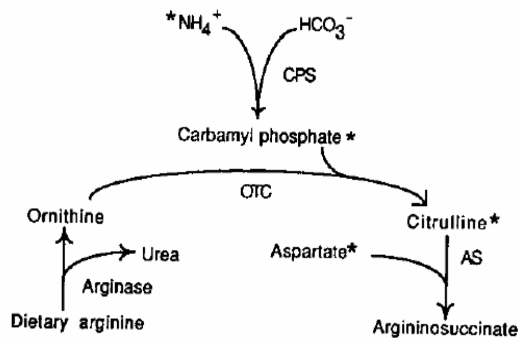
Gene therapy for OTC deficiency has been attempted in mice with retroviral vectors and in humans with adenovirus vectors. Important considerations about this tipe of disease with regard to gene therapy are:

- The site of gene delivery
- How long expression remains after treatment
- How much enzymatic activity is needed

A recent clinical trial for correction of OTC deficiency produced the first human death by a gene therapy strategy. Detail information about this unfortunate episode can be found at http://www.biospace.com/articles/010300.cfm. A pdf file containing such information can also be downloaded from this course's web site (gene_therapy_fatality.pdf). Other examples of genetic defects: phelnylketonuria (PKU), hemophilia (blood coagulation factors

VIII or IX), sickle cell anemia, adenosine deaminase deficiency (ADA), muscular dystrophy and cystic fibrosis.

Replication-defective and –competent viruses/vectors.
Terminology:
- Viral vector, gene transfer vector, gene therapy vector are identical terms.
- Replication-competent: term used for a virus that is able to replicate and spread in normal cells.
- Replication-defective: a virus which cannot replicate in normal cells beyond the first cycle of infection.
- Attenuated virus: replication competent virus with a reduced ability to cause disease.



Elimination of ammonium by OTC

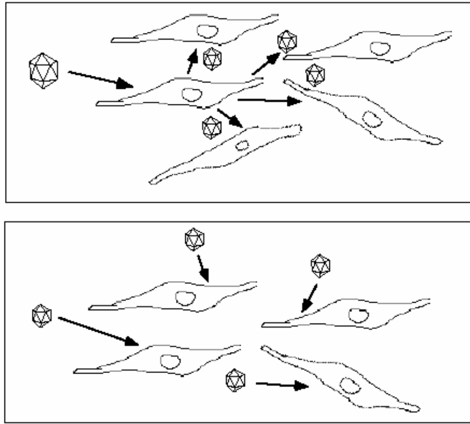
- When referring to defective viruses, we normally mean those which have been genetically altered not to replicate. However, there are "naturally" defective viruses, too (for example, endogenous retroviruses).

Replication-competent viruses have the ability to spread from cell to cell (leading to virus amplification). For the most part, this is an undesired feature in a gene therapy vector, because it could lead to uncontrolled viral replication. Even if the vector is attenuated, multiple rounds of replication may cause mutations and reversal to pathogenesis.

ADENOVIRAL VECTORS

Replication-competent Ad. Vectors

Replication-competent vectors are generally proposed in the context of selective replication in tumor cells. An example of this is ONYX-015 (Bischoff et al., *Science* 1996; 274:373). It was found the in the absence of the E1B-55Kd protein, adenovirus caused very rapid apoptosis of infected, 53(+) cells, and this resulted in dramatically reduced virus progeny and no subsequent spread. Apoptosis was mainly the result of the ability of EIA to inactivate p300 (inactivation of p300 results in inactivation of mdm-2, and lack of control over p53 proapoptotic activities). In p53(-) cells, however, deletion of E1B 55kd has no consequences in terms of apoptosis, and viral replication is similar to that of wild-type virus, resulting in massive killing of cells (cells die when virus progeny is about to be released).



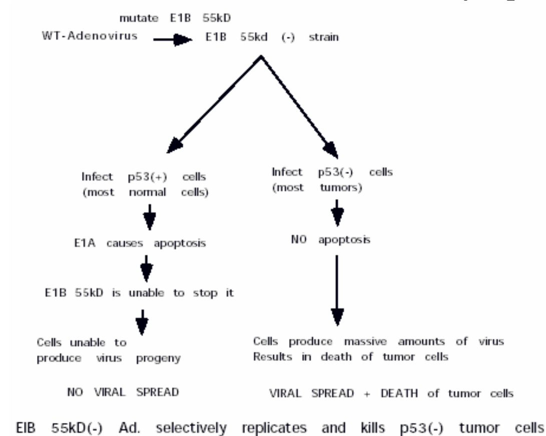
Replication competent (above) and defective (below) viruses.

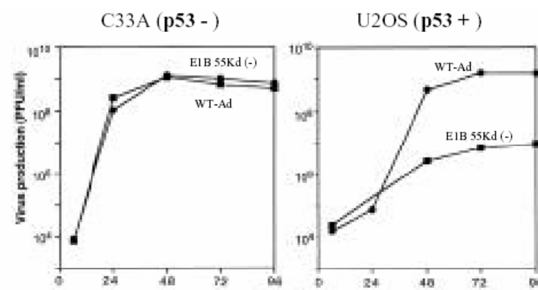
Replication-defective Ad. Vectors

Generation of replication-defective viruses is accomplished by deletion of certain essential genes. Such genes still need to be supplied for the production of the vector. Deleted genes can be provided *in trans* in several ways:

A. A helper virus

B. A DNA molecule (most commonly a plasmid, but also a cosmid)



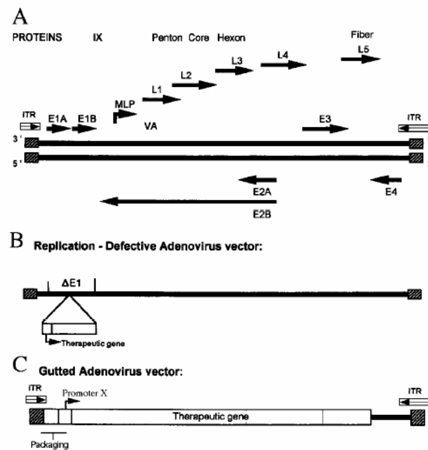


***Cis* and *trans*-acting elements.**

Replication-defective vectors always contain a "transfer construct". The transfer construct carries the gene to be transduced or "transgene". The transfer construct also carries the sequences which are necessary for the general functioning of the viral genome: packaging sequence, repeats for replication and, when needed, priming of reverse transcription (retroviruses). These are denominated *cis*-acting elements, because they need to be on the same piece of DNA (or RNA, if dealing with an RNA virus) as the viral genome and the gene of interest. *Trans*-acting elements are viral elements, which can be encoded on a different DNA molecule. For example, the viral structural proteins (for adenoviruses, those would be pentons, hexons, fiber, TP, etcetera) can be expressed from a different genetic element (a plasmid, a cosmid, a yeast artificial chromosome or a helper virus) than the viral genome (See Fig. 3).

E1-minus adenovirus vectors. In an early version of an adenovirus vector, the virus was

rendered defective by deletion of the E1 gene (E1A and E1B are the principal gene products **E1A** is an essential transcriptional activator for viral gene expression because in its absence there is no transcription of the rest of the early genes. **E1A** and **E1B** induce dramatic effects in the cell cycle and also may induce apoptosis, good additional reasons for eliminating E1 from the vector. These vectors must be produced in cells which express E1 *in trans*,



E1A(-) and gutless adenovirus vectors

such as 293 cells.. In normal cells, this virus is replication defective. However, in 293 cells, an E1(-) virus behaves as replication-competent. Although adenovirus vectors defective in E1 produced very low levels of viral antigens in the transduced cells, this resulted in **(a) toxicity to the host cell and (b) immune responses directed against viral antigens**. These immune responses cause immunological clearance of the transduced cells, limiting the efficacy of the strategy. In subsequent administrations of the vector, viral particles would be rapidly cleared due to a pre-existing immune response.

For the above reason, “gutless” or “gutted” adenoviral vectors were developed.
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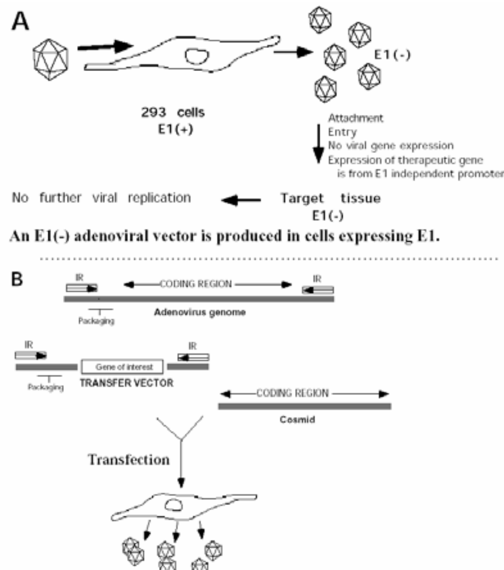
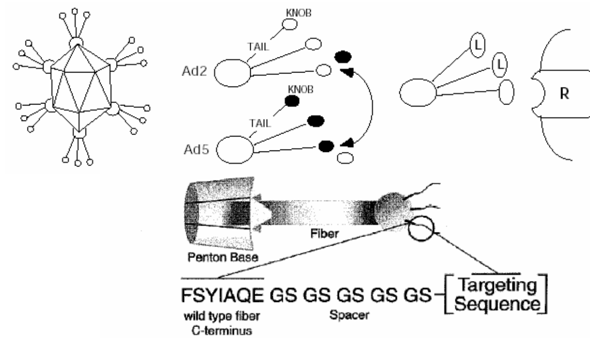
Adenovirus “gutted” or “gutless” vector. Adenoviral particles are highly immunogenic. Thus, when administering a second dose of the viral vector to animals or humans, a vigorous immune response by the host is able to neutralize the viral particles before they can transduce target cells. To address the issue of immunogenicity, “gutless” adenoviral vectors were constructed. In these vectors, no structural genes are contained in the transfer element. All structural genes required for generation of viral particles are provided *in trans* by either (a) a helper adenovirus, which is made E1(-) or (b) a large piece of DNA encoding all structural genes, sucha s a cosmid. The helper virus is rendered defective by deleting E1. Production of vector is accomplished by transfecting the gutless vector DNA into 293 cells, and infecting these cells with the E1(-) mutant, which will drive expression of all needed structural genes.



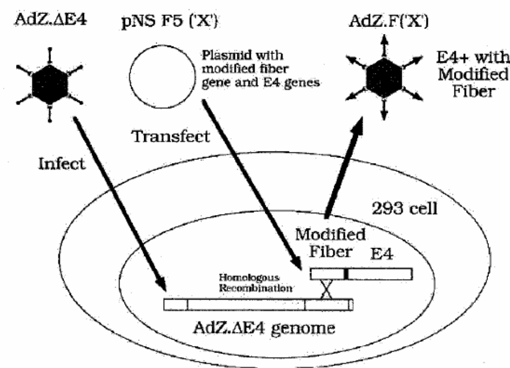
Production of E1(-) and gutless adenoviral vectors (upper and lower panels, respectively).



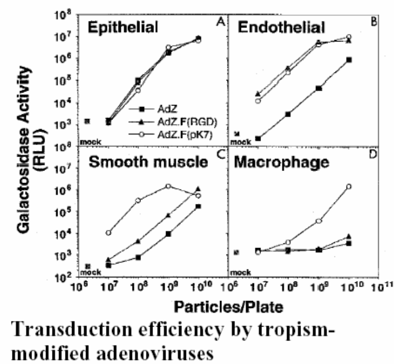
Vector Name	Target Receptor	Target Sequence
AdZ.F(RGD)	alpha-v integrins	ACDCRGDCFCG
AdZ.F(pK7)	heparan sulphate-containing receptors	KKKKKKK

Construction of chimeric fiber proteins

Infectivity of adenovirus vectors is initiated by binding of the fiber protein to the viral receptor, CAR (**c**oxackie and **a**denovirus **r**eceptor). Some cells express low levels (eg. Primary macrophages) and are therefore not very infectable. In addition, CAR is fairly ubiquitous. So when infecting with adeno vectors, some undesired tissues may acquire the vector, in addition to the target tissue. Following fiber-mediated attachment to cells, penton base binds via an RGD (arg-gly-asp) motif to $\alpha v \beta_3$ or $\alpha v \beta_5$ integrins. In order to develop a targeted adenovirus, it is therefore necessary both to ablate endogenous viral tropism and to introduce novel tropism. To restrict and manipulate cell and tissue tropism, chimeric fiber proteins were incorporated into an E1A(-) Ad vector (Adapted from Wickham et al., *J. Virol.* 71: 8221). Ligands were added by recombinant DNA techniques to the C-terminus of the Ad fiber protein. Ligands chosen were the RGD motif that binds to integrins, or a poly-lysine (KKKKKKK) that binds to heparin and heparan sulfate.



Construction of chimeric fibers



Transduction efficiency by tropism-modified adenoviruses

Advantages	**Disadvantages**
Good size	lack of integration
Infect non-dividing cells	highly antigenic
High progeny yields	
High expression	
Stable virions	

Questions on the reading materials:

The article "Gene Therapy's Wake up Call" by Vicky Browercan be found at http://www.biospace.com/articles/010300.cfm

1. Please comment on the dose of vector given to the patient.
2. How was this vector rendered defective? How did this deletion(s) impair virus replication?
3. Please speculate about the potential reasons for multi-organ failure and ultimately death of the patient. Would using a gutless vector help in this scenario?