ADENOVIRAL VECTORS:

NOVEL TREATMENTS AND POTENTIAL CURES

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Overview

Vectors are an integral component of gene therapeutics. Serving as molecular vehicles designed to introduce specific genes into the genome of an organism, they attract the scientific research community with promises of treatments and cures for genetic diseases.

An infectious agent causing acute respiratory and other diseases (Ryan, 1994), the adenovirus (Ad) has become a highly promising viral vector in current medical technology. Named after the adenoid tissue in which they were first discovered, adenoviruses consist of linear, double-stranded DNA and associated proteins, all within an icosahedral capsid – a twenty-sided polyhedron. This capsid is made up of 252 mostly identical protein molecules, arranged to form 20 triangular faces with a glycoprotein spike, also known as a penton fibre, at each of the 12 vertices (Campbell *et al.,* 1999) (Fig. 1 & Fig. 2). Of the almost 100 different serotypes of adenoviruses, 47 are known to infect humans (Ryan, 1994) and are identified as Ad1 through Ad47.

Used to deliver genes to mammalian cells, Ads are among the most common vectors used in gene therapy, second only to retroviruses (Vorburger & Hunt, 2002). Aside from applications in gene therapy, Ad vectors are used in the development of recombinant viral vaccines and in situations where high-level expression of transgene products is desired (Graham, 2000). The popularity of Ads for use as vectors can be partially attributed to its large and easily manipulated 36 kilobases (kb) double-stranded DNA genome, the stability of the viral particle itself, its efficiency in replication, and its ability to transduce a variety of different cell types (Hitt *et al.,* 1994).

Various methods exist for inserting foreign genes into the Ad genome, most of which involve either the Ad5 or Ad2 genome and are distinguished, in part, by which genome regions are deleted and by where the foreign DNA is inserted. The replication cycle of the normal, or 'wild-type' adenovirus is typically divided into three stages – immediate early, early, and late. Immediate early replication pertains to the genetic region of the viral genome referred to as E1A. This region plays an essential role in regulating the genes of the early replication stage: genes E1B, E2A, E2B, E3, and E4. Gene products encoded by the E3 region are involved in host immune response during infection (Tollefson *et al.,* 1991; Wold & Gooding, 1991). Different construction procedures result in an assortment of Ad vectors, each with its own advantages, limitations, and ideal uses. Basic descriptions of two such types of Ad vectors are given here.

First-Generation and Helper Dependent vectors: an introduction

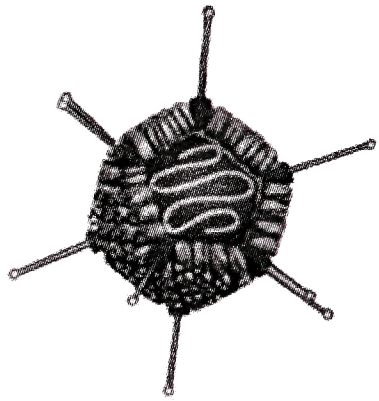
The two divisions of Ad vectors that merit the most attention are first-generation (FG) vectors and helper-dependent (HD) vectors.

Most commonly used are the FG vectors. Typically, these vectors have substantial E1 region deletions and may contain other deletions in the E3 region. Foreign DNA can be inserted into both the E1 and E3 regions, to a maximum of 8.3 kb (Bett *et al.*, 1994). Because the characteristic E1 deletion removes essential segments of the wild-type Ad genome, the virus is rendered replication-defective and propagation of the vectors can occur only in cells that express the lacking E1 proteins *in trans* (Latin, 'in transit'). One example of an E1-complementing cell type is the 293 cell line, derived from human embryonic kidney cells and developed in the 1970s by McMaster University's own Dr. F. L. Graham and colleagues (Graham *et al.*, 1977).

Figure 1: **Structure of the Adenovirus**

The double-stranded DNA of the adenovirus is enclosed within a 20-sided icosahedral capsid. Note the glycoprotein spikes at each vertex; there are 12 in total. In contrast to many other viruses such as the Human Immunod-eficiency Virus (HIV), adenoviruses do not possess a viral envelope.

Image courtesy of the-scientist.com. (2000) (www.the-scientist.com/images/ yr2000/apr17/aden.jpg)



In contrast, HD vectors are able to contain significantly greater amounts of foreign DNA inserts than FG vectors, as HD vectors possess large genome deletions – typically almost the entire viral genome. It is known that Ad-based vectors can accept inserts of up to 105% of the wild-type genome (Parks *et al.,* 1996), and HD vectors take full advantage of this ability. Recalling that the wild-type Ad genome is 36 kb, HD vectors could allow for inserts of up to 37 kb (Hitt & Graham, 2000). As their name suggests, HD ('helper-dependent') vectors can only replicate in cells coinfected with a helper virus to provide the essential proteins that the HD vector can no longer create itself. Because virtually all their viral genes have been deleted, HD vectors elicit less of an immune response than their FG vector counterparts (Morsy *et al.*, 1998; Maoine *et al.*, 2001).

Advantages and limitations of First-Generation and Helper Dependent vectors

Recalling that FG vectors retain most of their original viral genes, they are a poor choice for long-term treatment as they are capable of eliciting a host immune response, leading to cellular immunity with repeated treatment (Kafri *et al.,* 1998). However, animal models have shown that short-term applications of Ad-based vaccines are sufficient to protect against various types of pathogen infection (Imler, 1995). Short-term gene therapy using FG vectors is also being studied in the field of cancer research, one example of which will be addressed later in this article.

More suitable for treatments of chronic conditions, such as Cystic Fibrosis, HD vectors are being examined for their potential in long-term gene therapy. Unfortunately, the HD vector system is

Figure 3: **Targeting of an Adenoviral Vector**

The knob domain (round structure) of the penton fibre is the normal binding molecule of the adenovirus, and interacts with the CAR expressed on the majority of healthy epithelial cells (light spheres). Replacement of the CAR domain with an antibody (y-shaped structure) will allow for targeting of the viral vector against specific cells, such as cancer cells (dark sphere).

Image courtesy of The American Association *for the Advancement of Science (2003).*

still plagued by several problems. Not only is the HD vector itself sometimes relatively unstable, but the helper virus used during propagation of the vectors can also contaminate the final preparation (Hitt & Graham, 2000). However, interest in these promising vectors is great and studies are underway to create more appropriate structural designs and production methods for HD vectors.

Problems facing adenovirus vectors

Since their development, there have been certain issues that are common to all Ad vectors, and which continue to prove challenging in varying degrees in the implementation of the different vector types.

One such challenge is that of the immune system. HD vectors were developed in part to minimize host immune response, but immunogenicity still remains a hindrance to both FG and HD vectors. The immune response can be stimulated by proteins for which the virus encodes and/or transgene immunogenicity (Morsy *et al.,* 1998), and presents the problems of transient duration of transgene expression and reduced efficacy in both initial and repeated administration (Hitt & Graham, 2000). Progress is being made in this area, however, and it should be noted that some studies have shown long-term expression with HD vectors (Morsy *et al.,* 1998; Morral *et al.*, 1999; Maione *et al.*, 2001).

Being a partial result of repeated administration of Ad vector treatment, the immune response can also be attenuated by reducing the frequency of patient exposure to the treatment. Prolonged *in vivo* transgene expression cannot occur with conventional Ad vectors, as they do not integrate with high frequency into the host genome (Hitt & Graham, 2000). So, even as a diseased cell is successfully treated by genetic therapy, the same treatment in other cells will be lost when those modified cells die and do not pass on their altered genomes to their daughter cells. In response to this challenge, the possibility of hybrid vectors is currently under investigation. These hybrid vectors combine the high transduction capabilities of Ad with an integration-competent virus, typically an adeno-associated virus or a retrovirus (Fisher *et al.*, 1996; Bilbao *et al.*, 1997).

Another challenge of utmost importance is that of cell targeting. Ad vectors are capable of transducing a wide range of cell types – an attribute that, although advantageous under certain conditions, can prove to be highly unfavourable. This lack of target cell specificity is due in part to the presence of Ad receptors on many cell types, the primary receptor being the coxsackie-adenovirus receptor (CAR) (Hunt & Vorburger, 2002). Intravenous administration of Ad vector treatment results in the majority of the virions being taken up and expressed in the liver (Reynolds & Curiel, 1999) and this may not be appropriate for most applications. Although direct administration to target tissue is a viable option in some cases, accessibility of target tissue may be limited in many other cases. One possible solution is to modify the interactions between cellular Ad receptors and the Ad vector proteins involved in receptor binding (Fig. 3). Modification of Ad and Ad receptor interactions, aside from increasing the specificity of Ad vectors, may also have applications in broadening the range of tissues responsive to Ad vector treatment (Reynolds & Curiel, 1999).

Adenoviruses in cancer therapy

One very exciting possibility for Ad vectors is their use in cancer therapy. The Ad vectors previously discussed in this article have been replication-incompetent, as a result of major deletions in the E1 region. Recently, interest has turned to replication-competent Ad vectors for use in cancer therapy, as these vectors have the potential to selectively kill tumour cells and to amplify their oncolytic potential within the tumour mass (Ries & Korn, 2002). One of the first such vectors is the ONYX-015 vector. This adenovirus mutant lacks the E1b 55k gene, which normally functions to attenuate the virus-combating actions of a healthy cell's *p53* gene. (McCormick, 2000). The *p53* gene, which is defective in cancerous cells, is responsible for blocking cell division or causing the cell to self-destruct in response to DNA damage or viral DNA infection (Evans & Lahoti, 2000). Thus, ONYX-015 will be destroyed in healthy cells because it cannot defend itself against the products of the *p53* gene and, conversely, ONYX-015 will proliferate within *p53*-deficient cancerous cells, resulting in host cell lysis (Fig. 4).

Another application of Ad vectors in cancer therapy is their use to deliver and express immune modulating genes, such as those encoding cytokines, in hopes of stimulating a host immune response against tumour cells (Addison *et al.*, 1995; Stewart *et al.*, 1999).

Concluding remarks

Past and present Ad research encompasses a much greater expanse of information than could be addressed in the scope of this article, and it is hoped that the curious reader will continue to pursue his or her interest beyond the material discussed here. These are only a few of several Ad vector cancer treatment methods currently under investigation. In addition to applications in the field of cancer research, Ad vectors have also shown much potential in the treatment of genetic disorders such as cystic fibrosis (Goldman *et al.*, 1995), haemophilia (Kay *et al.*, 1994), and muscular dystrophy (Acsadi *et al.,* 1996). Although more research is required, the promise of novel treatments and cures remain, and it is hoped that this promise will be fulfilled in the near future■

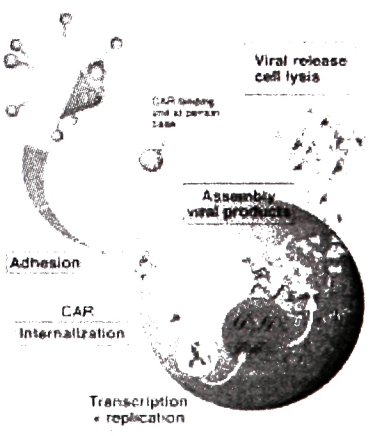


Figure 4: **Life Cycle of the Adenovirus**

Whereas replication-incompetent Ad vectors express the proteins coded for in their DNA but do not enter the lytic cycle, replication-competent Ad vectors cause host cell lysis as new viruses are released. It is hoped that selective internalization of mutated, replication-competent Ad vectors will result in destruction of cancerous cells without negatively affecting the healthy cells of the organism.

Image courtesy of The Oncologist *(2002).*