ADENOVIRUS VECTORS AND THEIR APPLICATION IN GENE THERAPY

ABSTRACT

Adenovirus vectors have been used for cancer and several heritable diseases gene therapy, due to their ability to infect a broad range of cells and tissues, among other suitable properties. Adenovirus manipulation involves insertion of foreign DNA into its genome, compensated by deletion of early regions E1 and E3. E1 deletion creates a defective vector able to grow only on complementing cell lines. E3 region is nonessential for replication and is also deleted in most vectors, to make room for exogenous DNA. One of the major obstacles to have a broad usage of adenoviral vectors in gene therapy is the host immune response against viral proteins, which arise due to a leaky viral gene expression in the target cell. This fact triggered a big effort from several research groups to create a new generation of these vectors. Introduction of additional deletions (E.g. in E2) was an attempt to further silence viral gene expression, being the most radical the so-called all deleted adenovirus vector. Strategies to modify the native tropism of adenovirus also have been developed, intended to allow a targeted gene delivery. We will review adenovirus vectors development, current and potential applications.

Key words: adenovirus, gene therapy, viral vectors.

Introduction

Human adenoviruses (Ads) were isolated for the first time in the fifties and cause a broad range of symptoms: respiratory disease, conjunctivitis and gastroenteritis (1). Some of these isolates were able to transform rodent cells in culture (2). In consequence, a great number of groups engaged in research to know these viruses in detail and to check if they could be a cause of human cancer. The conclusion was that in the natural host, human adenoviruses do not cause tumors (3), and as a consequence of this effort a deep knowledge of adenovirus was generated (4). One fundamental question was to study the adenovirus genes responsible for cell transformation, located in early region 1 (E1). To understand the role of these genes mutants were generated which needed a complementing cell line to support their replication. A cell line (HEK293) derived from human embryonic tissue immortalized with the left most part of the human adenovirus type 5 (which includes E1 region), became a key reagent for these studies, and for the future development of replication defective adenovirus vectors (5). Group C human adenovirus ability to infect a broad range of tissues and the stability of the particle, which aloud it to be highly concentrated, are important characteristics for an in vivo gene transfer vector. Finally, the safety credentials to adenovirus vectors were given by the long-term use of the enteric Ad4/Ad7 live vaccine, to cross-protect against respiratory adenoviruses (6).

Strategies for vector construction

The pioneer strategy to develop a functional adenovirus vector involved transfection of defective viral genomes, plus the transgene connected to a viral DNA fragment, in to the complementing cells (7, 8). This permits homologous recombination and recovery of the quimeric virus by screening the viral
plagues formed. The *in vitro* ligation of DNA fragments previous to transfection was also used in those experiments, including approaches to express antigens like the hepatitis B surface antigen (9).

Adenovirus genomes can circulate during infection, these genomes can be cloned and virus is recovered upon transfection into cells (10). The inverted terminal repeats, which flank adenovirus linear genome, appear linked in these circles and the DNA around them will be looked at as the genome to be packaged. Since the capacity of the capsid to incorporate DNA is limited, those cloned genomes can be converted in non-packagable sized plasmid vectors. These could be made packageable genomes upon recombination with shuttle vectors carrying genes of interest, after co-transfection in HEK293 cells. Such kind of modifications improved the efficiency, and lowered wild type contamination of Ad vector preparations (11).

Some limiting factors of the above approach are the low efficiency of plaque formation in cell culture, and the long time it takes for screening. A way to accelerate this process is to work with plasmid homologous recombination in appropriate bacterial strains, amplify the plasmid containing the whole Ad vector genome, and then transfet it in HEK293 cells. Several researchers achieved this goal and produced efficient systems for Ad vectors construction (12, 13, 14). Another efficient method for constructing recombinant adenovirus vector was developed based on an *in vitro* ligation, taking advantage of unique sites for the rare cutting restriction enzymes I-CeuI, Sall, and P1-SceI (15). In this case the plasmid containing the Ad vector genome is also amplified in bacteria, but avoiding the recombination step. The cloning in cosmids is also an alternative strategy to deal with the 36-38 Kb genome of Ad vectors. In this case cos sites are positioned flanking the Ad vector genome by ligation of DNA fragments, followed by packaging with a phage I *in vitro* system and growth in bacteria (16).

Adenoviral vectors lacking E1 and E3 regions are called the first generation. They still being in broad usage, but attempts to overcome the leaky expression of structural genes, lead to the new generations of vector and complementing cell lines. In terms of construction strategy they are not very distinct, but one type of vector with no viral genes, the "all deleted" has special characteristics. The strategy involves flanking of the exogenous DNA (around 28 Kb) by the intact inverted terminal repeats (ITRs). A helper virus with a deletion in the 5′ITR, making its packing signal partially defective, gives the necessary functions to generate virus particles. Since the recognizing and packaging of the all deleted vector genome is more efficient, sequential cycles of replication enrich the virus particles with vector in a cell containing both genomes (17, 18). The smaller genome gives the vector particles lower density and a way to purify them in CsCl gradients. Variations, taking advantage of the CRE recombinase/loxP system, were developed to excise the packaging signal from the helper or the genes from the vector. This was achieved by growing/transfecting constructs with *loxP* sites properly positioned, into 293 cells expressing CRE recombinase (19, 20).

Another way to use the efficient adenoviral infection machine to transfer genes is link DNA conjugates to the virus particle (21). An evolution of this concept was to use the essential component of the virion responsible for interaction with the cell surface. It is composed by the proteins penton, penton base and fiber. Isolated penton or penton base are able to form a structure called dodecahedron to which the fiber interacts by its N-terminal region. To achieve gene transfer, the fiber N-terminal region was linked to a polylisine tail, which is able to condense DNA. The whole structure enters the cell efficiently carrying DNA (22). Adenovirus genome stays transiently in episomal form in the cell nucleus, thus integration is necessary to have a permanent expression. An interesting strategy was devised to overcome this barrier, by making adenovirus a vehicle of a retrovirus vector unit. The cells infected *in vivo* by this adenovirus vector, became producers of retrovirus vector that infected and transferred the gene of interest to the neighbor cells in the target tissue (23).

**Persistence of expression**

The transient expression of a transgene by adenovirus vectors *in vivo* was shown to be due not only to the episomal state of its genome in the target cell. Comparison of expression in nude versus immune competent mice, also involved the immune response against viral proteins in the silencing of the transgene (24). The use of mutants for another adenovirus early gene (E2a), to construct adenovirus vectors already defective in E1 and E3, greatly extended the expression of the transgene (25, 26). These were called the second generation of adenoviral vectors. Deletions in E4 region could also improve the stability of expression, depending on the target tissue (27). A further development was the association of deletions of E1, E2a, E3 and E4 (except open reading frame 3), improving expression in the liver (28). The above mentioned all deleted vector was demonstrated to be useful for a stable expression in muscle, giving hope for muscular dystrophy treatment since it could harbor the dystrophin gene (29).

E3 region is involved in the immune response modulation of the host to adenovirus-infected cells (30). In the E3-deleted vectors this masking effect is absent and if it is inserted back, may contribute for a more stable expression. This was done for the whole E3 region and a sustained expression of the transgene was observed, with no rejection of a second administration of vector, as usually happens for de E3 deleted vector (31). A similar result was observed when only the gene for
the E3 protein gp19K (which interacts with the class I MHC proteins preventing their maturation) was re-inserted in to an adenovirus vector (32).

Retargeting of adenovirus vectors

Human type C adenoviruses (Ad5 and Ad2) are manipulated as vectors for gene therapy; being the broad range of cell types they could infect an amenable property. Their receptor is a ubiquitous protein termed CAR, for coxsackie-adenovirus receptor (33). CAR binds to the globular region of the fiber protein, also named knob, corresponding to its carboxy-terminus. For certain applications, however, it is necessary to restrict the vector infection to a given cell type. A way to do this is to make adenovirus, binding incompetent using an antibody directed against the globular region, and this strategy was used previously to the discovery of CAR (34). This virus could be then conjugated to a retargeting molecule, like asialoglycoprotein, folate, PEG or EGF, through a chemical or, antibody bridge (34, 35, 36, 37).

Modifications in the fiber gene are another way to interfere in adenovirus tropism. They can be achieved by exchanges of the entire gene or constructing quimeras of fiber N-terminal/C-terminal regions from serotypes that use different receptors (38, 39, 40). Less extensive modifications can also lead to interesting effects. One example is the attachment of a polysine tail to the fiber C-terminus, based on the enhanced ligation that sequences of positively charged amino acids generate to heparan-containing receptors (41). Several patient samples of acute myeloid leukemia cells, very resistant to gene transfer, were efficiently infected by this modified adenovirus vector (42).

Another region of the C-terminal region of the fiber that can be manipulated is the HI loop. It was demonstrated that small insertions in this loop do not interfere with the capsid assembly (43). One of the modifications was the insertion of the RGD motif, which mediates interaction with some integrins in the cell surface. This insertion gave the modified virus the property to enter cells via a CAR-independent mechanism and an alteration in the distribution of a systemically administered vector was observed (44, 45).

Applications of adenovirus vectors in gene therapy

Adenovirus vectors have been widely used in gene therapy, from initial experiments in cell culture to the clinical trials in humans. Despite a recent drawback, with a patient fatality after an attempt of gene transfer to liver (46), they still hold a hope for treatment of several diseases.

The characteristics of adenovirus vectors, make them specially suitable for cancer treatment, were temporary expression of a gene gives distinct properties to the cancer cells leading to their elimination. One strategy for cancer vector therapy is to express a suicide gene in association with a pro-drug, like Herpes Simplex Timidine kinase/ganciclovir or E. coli citosine deaminase/5-fluorocytosine. There are some examples of good results of these genes transferred in animal models by adenovirus, with an impressive reduction in tumor mass and even metastasis (47, 48, 49). The association of a concept to kill tumors by competent replication virus with the use of a suicide gene, further improves the efficacy of the adenovirus vector administered directly into the tumor (50). Immunologic intervention represents a promising strategy for cancer treatment, and adenovirus vectors are efficient for transfer cytokine genes into tumors. Interleukins 2 and more significantly 12, have been shown to produce regression and immunize the animals against a given tumor cell when transduced by means of adenovirus vectors (51, 52). Association of interleukins with suicide genes, costimulatory molecules and tumor suppressor genes, like p53, enhance antitumor action (53, 54, 55).

Several other diseases have been considered for treatment by means of adenovirus vectors transduced genes. Some examples are: cystic fibrosis (56), hemophilia (57), hypercholesterolemia (58) and muscular dystrophy (59). Finally, despite the obstacles research with adenovirus vectors for gene therapy goes on in a very accelerated pace, giving the perspective for broad clinical applications in near future.


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