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Viral vectors for gen therapy

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Abstract— Viruses have evolved to become highly efficient at nucleic acid delivery to specific cell types while avoiding immunosurveillance by an infected host. Because of these characteristics, viruses are appealing gene delivery vehicles, or vectors, for gene therapy. Several viruses, including retrovirus, adenovirus, AAV, and herpes simplex virus, have been mutated for lab use in gene therapy applications. Because each of these vector systems has its own set of advantages and limitations, it is best suited to certain applications. Retroviral vectors can permanently integrate into the infected cell's genome, although transduction requires mitotic cell division. Adenoviral vectors may successfully carry genes to a wide range of dividing and nondividing cell types, but in vivo gene expression is generally limited by the immune clearance of infected cells. Herpes simplex virus can transport enormous quantities of foreign DNA; yet, cytotoxicity and transgenic expression maintenance remain challenges. AAV can infect both non-dividing and dividing cells, although it has a limited DNA capacity. On the other hand, Chimeric viral-vector systems, which combine favourable traits of two or more viral systems, are also being investigated. Although viral-mediated gene delivery has shown to be the most effective method of gene transfer, nonviral methods are also being researched. Many of these nonviral technologies combine elements of viral vectors to improve gene delivery or expression efficiency.

Keywords: Therapy, immunotherapy, gene therapy, adeno-associated virus, adenovirus, retrovirus, herpes simplex virus, viral vector.

1-Introduction

The major goal of gene therapy is to introduce a functional gene into a targetcell and restore protein production that is absent or deficient due to a geneticdisorder. Although the basic principle of gene therapy is quite simple, successesrely considerably on the development of the gene transfer vectors.[4].Over the years, several gene transfer vehicles have been developed thatcan roughly be divided into two categories: synthetic and virus-based gene delivery systems. Synthetic gene delivery systems depend on the direct delivery of genetic information into a target cell and include direct injection of naked DNAand encapsulation of DNA with cationic lipids (liposomes). Although these delivery systems exhibit low toxicity, gene transfer, in general, is inefficient and often transient[5].

Viral delivery systems are based on replicating viruses that can deliver genetic information into the host cell. In general, genomes of replicating viruses contain coding regions and cis-acting regulatory elements. Thecoding sequences enclose the genetic information of the viral structural and regulatory proteins and

are required for the propagation of infectious viruses, whereascis-acting sequences are essential for packaging viral genomes and integration into the host cell[3, 6].

To generate a replication-defective viral vector, the coding regions of the virus are replaced by the genetic information of a therapeuticgene, leaving the cis-acting sequences intact. When the viral vector is introducedinto producer cells providing the structural viral proteins in trans, the production ofnonreplicating virus particles containing the genetic information of a therapeutic gene is established[4]. The ability to generate replication-defective viral vectors is the backbone of developing virus-based gene delivery vehicles[7].

Currently available viral vectors for gene therapy are based on a variety of viruses and can be divided into two categories: integrating and nonintegrating vectors. Adeno-associated virus and retrovirus vectors (including lentivirus and foamy virus) can integrate their viral genome into the host cell's chromosomal DNA, allowing for lifelong gene expression[4]. Vectorsbased on adenovirus (Ad) and herpes simplex virus type 1 (HSV-1) represent thenonintegrating vectors. These vectors deliver their genomes into the nucleus of thetarget cell, where they remain optional[8].

In this review, we give an overview of the development of vectors derived fromviruses, discussing their specific properties and problems. Furthermore, we give abrief overview of clinical studies using viral vectors[4].

2. Main Body

2.1. Gene Therapy Delivery Systems

In a biological context, an unprotected nucleic acid (RNA or DNA) does not last long. Furthermore, the nucleic acid cannot enter the cytoplasm (where RNA may perform its role) or the nucleus (where DNA is transcribed and the cell genome can be transformed) on its own. As a result, one of the most difficult aspects of gene therapy is delivering an effector into cells. Viral vectors are one of the most promising methods for delivering gene therapy[2].

During evolution, viruses developed various mechanisms of entry into cells, prolonged preservation of their genetic material inside cells, and suppression or modification of protective mechanisms in the host organism, reverse genetics (methods for modifying genomes and the creation of recombinant viruses) has been evolving[9]. Recombinant forms of the majority of viruses may be created, and most of them, at least theoretically, have been explored as vectors for gene therapy, although only a small number of viral delivery methods are employed. In this review, we discuss the latest trends in gene therapy development, in particular various aspects and perspectives of clinical development of viral delivery systems[2, 9].

More often, the possibility of gene therapy or the possibility of affecting the dynamics of tumour progression bychanging signal pathways are discussed (proofofconcept). The number of articles describing oncolytic viruses is about 20fold less (330 per year) and has not grownsince 2012. This may be explained by the development ofnew approaches in immunotherapy of malignanciesaimed at overcoming immune system tolerance[10].Another popular gene therapy approach is the development of ex vivo T-lymphocyte modification to treat human immunodeficiency virus (HIV) infection. The number of publications on this topic peaked in 2005 and has remained rather stable since then. As a result, there is a growing interest in gene therapy, as well as an extension of the areas where it can be used. Furthermore, in recent years, the technologies utilized to deliver gene therapy have altered dramatically[11].

Up to now, several gene therapy delivery techniques have been developed. Viruses are thought to be the most promising gene therapy delivery method for clinical usage. Many data concerning different viral-

based vectorshave accumulated (Fig. 1). The choice of the safest andmost efficient delivery system depends on the task thatneeds to be solved by a new gene therapy approach[2].

2.2. Properties of Vectors for Gene Therapy

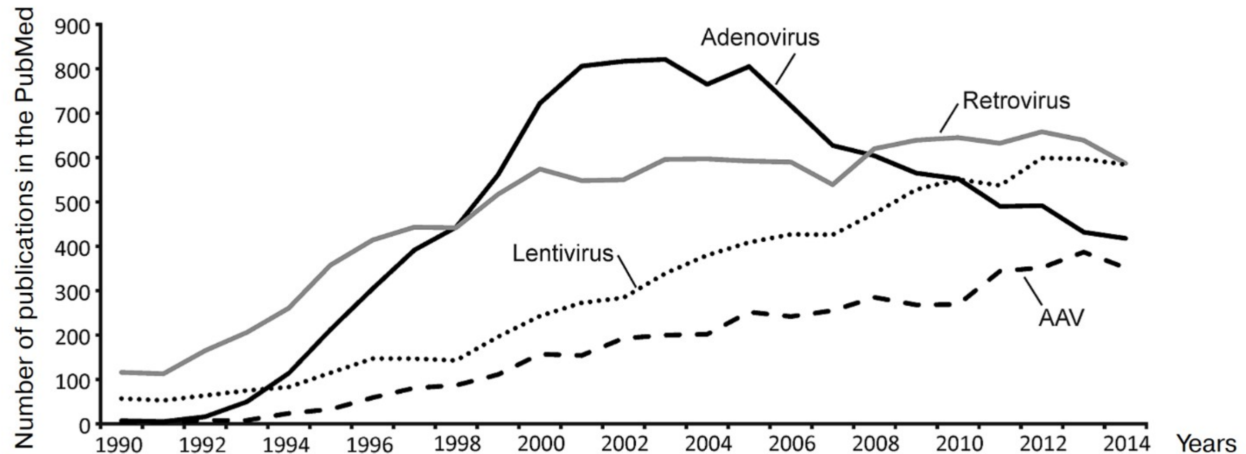

Fig 1. Dynamics of publications on different viral vectors in gene therapy from 1990 to 2014 publications in the PubMed data base (y axis) according to year (x axis). AAV, adeno associated viruses[2]

To be effective, a sufficient amount of a therapeutic gene must be delivered into the target tissue without causing significant harm. Each viral vector system possesses a unique set of features that influence its applicability for certain gene therapy applications[12]. For some diseases, long-term expression from a tiny percentage of cells might suffice (for example, hereditary abnormalities), but for others, significant but temporary gene expression may be required. Gene therapies aimed at interfering with viral infection or inhibiting cancer cell proliferation by reactivating inactivated tumour suppressor genes, for example, may necessitate gene transfer into a substantial proportion of aberrant cells. A phenomenon known as the bystander effect may help gene transfer schemes based on the delivery of tumour-specific toxins or the conversion of prodrugs into toxins. This allows the gene product or the transformed product to be used[12]. Other gene transfer strategies for cancer-based on the induction of immune responses to tumour antigens or the interruption of the tumour vascular supply may require intermediate levels of gene transfer in a cell-type-specific subset of the cells within, or from, a tumour. Finally, oncolytic viruses do not contain transgenes but are genetically engineered to allow tumour-specific viral replication resulting in cell lysis, and spread to neighbouring malignant cells. All of these approaches are in or near clinical trials[13, 14].

Regulated gene expression will be required for several types of gene therapy. Exogenous insulin expression will need to be closely regulated in the case of diabetes, based on rapid changes in glucose concentrations and metabolic disturbances[15]. In this case, proper post-translational processing that is responsive to these metabolic cues will be required. In some circumstances, such as anaemia, the hematocrit may be controlled by turning on or off the erythropoietin gene by oral medications (for example, tetracycline derivatives) that regulate a specific trans activator that activates or represses a specific promoter[15].

Because viral gene expression is responsible for the majority of the clinical and immunological repercussions of viral infection, gene transduction by recombinant vectors is frequently well tolerated.

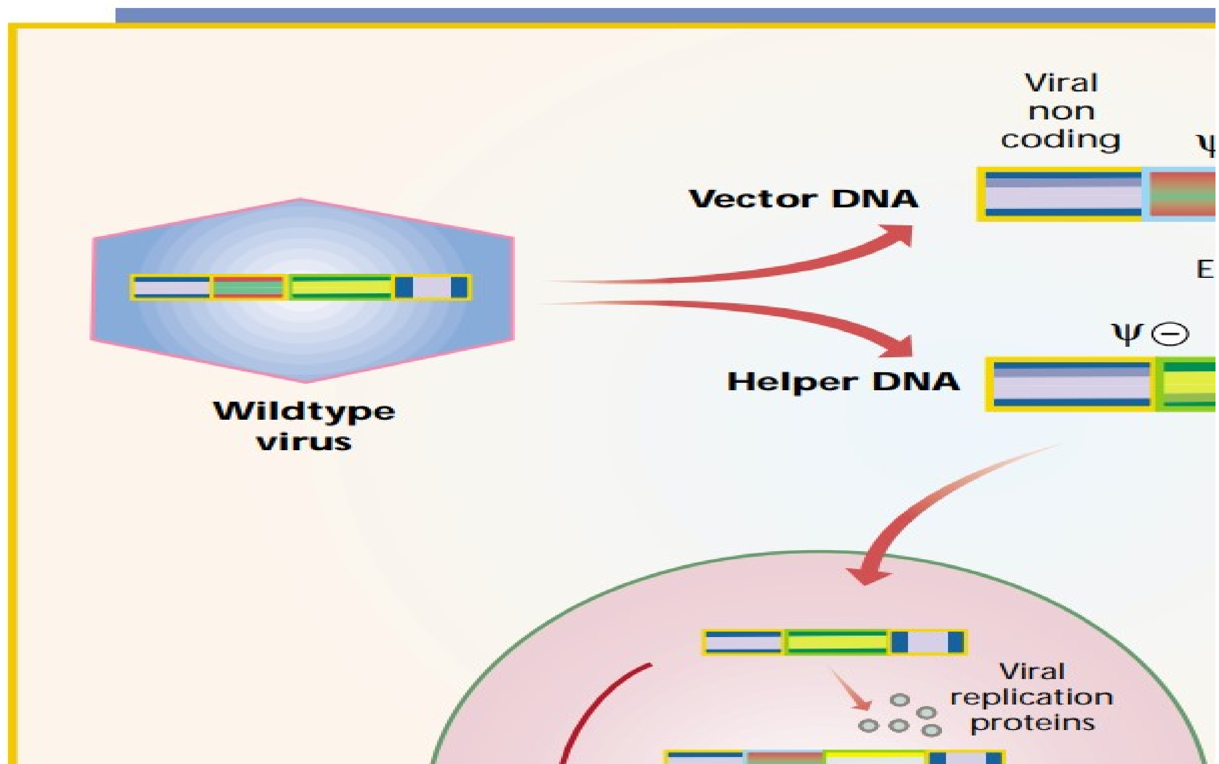

Fig2. Generic strategy for engineering a virus into a vector. The helper DNA contains genes essential for viral replication placed in a heterologous/unrelated DNA context that can be delivered as a plasmid, helper virus or stably inserted into the host chromosomal DNA of the packaging cell. The helper DNA can be delivered as a single molecule or in some cases split into different DNA molecules for safety reasons (see text). The helper DNA lacks the packaging domain (ψ) so it itself or its RNA cannot be packaged into a viral particle. The helper DNA of some vectors also lacks additional transfer functions, to increase safety[3].

Acute toxicity from the infusion of foreign materials, as well as cellular damage, may be detected by using gene transfer vectors[13].

Immune responses directed against the transduced cells, humoral immuneresponses against the therapeutic gene product and the potential for insertional mutagenesis by certain integrating vectors[3]. The possibility of eliciting an immunological reaction (or autoimmunity) to a gene product never seen by the recipient's immune system is unknown, even with pure vector preparations. Because of the presentation in the setting of class I vs class II, gene transduction into antigen-presenting cells (APCs) may also break tolerance to its product[3]. Because most research has used inbred animal strains with non-species-specific transgene products, it's been challenging to create reliable predictions of these immune responses in humans. Polymorphic variation in host immune-relevant genes, the transgene product, the vector utilized for gene transfer, and the target organ are all likely to influence immune responses[8].

Currently used vectors integrate randomly. Integration is a mutagenic process that disrupts and activates the transcription of biological genes, including oncogenes[3]. Despite this, most transduced cells tolerate integration well, and it is vital to ensure the recipient's stability of the newly inserted genetic material. However, human efforts to date have relied on vectors that only integrate into a limited percentage of cells inside a target tissue[3]. The dangers of viral integration may need to be reevaluated when more effective vectors are developed that may target a wider range of cells, including stem cells with self-

renewal and huge clonal expansion capabilities. The unintentional introduction of vector sequences into germ cells is another unwanted possible outcome. Despite the low likelihood that this would occur with an integrating vector, any such germline occurrence poses significant ethical and safety concerns[16].
The ability to infect selectively a specific target cell or tissue, such that after parenteral administration, tissue-specific uptake occurs without widespread tissue dissemination of a therapeutic gene that is toxic or antigenic when expressed from the "wrong" tissue, is one desirable vector property that could mitigate some of these potential risks. Although attempts to accomplish tissue-specific targeting continue to garner a lot of attention, there hasn't been much success in practice[17]. The use of tissue-specific promoters to induce transduced gene expression and the alteration of recombinant viral particles' surface recognition elements to alter their cell-recognition capabilities are two current approaches to avoiding some of the drawbacks of promiscuous transduction, The effort to design vectors that can incorporate into predefined places within the genome is a difficult task. This would prevent accidental integration with potentially dangerous sites that may cause the negative outcomes mentioned above[3].

2.3.Type of Viral Vectors

The capacity to distribute exogenous nucleic acids to cells of different organs is essential to the idea of gene therapy. Although some success in the absorption of "naked" DNA has been noted, effective transport and lasting expression have only been shown in a few number of tissues, such as muscle. To improve the efficiency and stability of gene delivery, numerous laboratories are evaluating both viral and nonviral vectors for gene transfer[16]. Viruses have evolved to be exceedingly effective at delivering nucleic acids to particular cell types while evading immunosurveillance. If the pathogenicity of a specific virus, such as adenovirus, can be eliminated while the efficiency of gene transfer and expression is retained, the gene may be well suited for gene therapy[3]. Nonviral vectors such as liposomes are nonpathogenic but are also less efficient in the transfer of nucleic acid to the nucleus of cells. Each vector system has strengths and shortcomings that must be addressed in order for the vector to be suitable for broad gene therapy applications. Here is a description of each vector system and how it has been modified for use in gene transfer[16].

2.3.1. Adenoviral Vectors

Adenoviruses were among the first viruses to be properly investigated, and their potential in gene therapy was initially suggested more than 20 years ago. Adenoviruses are non-enveloped viruses having a genome of around 35 kb of double-stranded DNA. Adenoviruses are attenuated before being utilised as vectors for gene therapy by deleting a genomic segment coding for early proteins[2]. By removing a varied number of genes, different levels of attenuation may be achieved: only one E1B gene (first-generation vectors), the majority of early genes (second-generation vectors), andeven full deletion of all genetic information of an adenovirus (so-called gutless vectors)[18].
Viruses with smalldeletions can be propagated in cultured cells with geneticdefects allowing virus reproduction. Production of gutlessvectors requires special producer cell lines. The large sizeof the genome and the possibility to delete amajor part ofit provide high coding capacity for these vectors: 12 kbcan be inserted in early generation vectors, and up to30 kb in gutless vectors[6]. It is vital to highlight that the adenoviral genome does not integrate into the host cell's DNA, making the vectors relatively harmless. On theother hand, the viral life cycle is not adapted for the increasedduration of transgene expression.Originally, adenoviruses were intended to be employed for a wide range of therapeutic applications, ranging from treatment to regenerative medicine[19]. It was later shown that even

genetically inactive adenoviral particles contain highly immunogenic capsids. Systemic application of adenoviral vectorsis complicated by the fact that many viral componentsbind nonspecifically to blood components (proteins ofthe coagulation cascade, complement proteins, erythrocytes, platelets), leading to inactivation of the virus[20]. Moreover, systemic administration of high doses ofadenovirus can lead to systemic inflammatory response,which can be lethal in some extreme cases. Thus,adenoviruses can be used as a gene therapy delivery systeminapplications when local administration is possible andan immune response is required, i.e. in the therapy of malignant tumors or in development of vaccines[18].

Adenovirus serotypes. The most widespread serotype,adenovirus C5, was also used as a backbone for the majority of adenoviral vectors[2]. Even a single administration ofsuch vectors will lead toa high probability of the development of a secondary immune response. To overcomethis limitation, simian adenoviruses have been isolatedand studied. Humans do not have antibodies to simianadenoviruses; therefore, their structural proteins can beused to create chimeric adenoviruses[2, 21].

2.3.2. Retroviruses and Lentiviruses

Retroviruses are RNA viruses. Replication of retroviruses has an obligatory step of RNA copying into DNA(reverse transcription) and integration into the genome ofa host cell. Early studies of the possible use of retroviruses for gene therapy began in the 1980. Before thediscovery of CRISPRCas systems (see below), retroviruses were the only possible way to modify a patient'sgenome[2]. Naturally, retroviral vectors were first applied tocuring monogenic disorders caused by a defect in a particular gene. The first gene therapy clinical trials usingretroviruses started in the early 1990s were aimed at the treatment of severe combinedimmunodeficiency caused bythe lack of adenosinedeaminase[21].

Retroviruses have a preferred site for genome integration, which is an essential trait. MLV proved to be a poor candidate in this regard since it is more likely to integrate into actively transcribed genomic loci near the start of a transcription unit, resulting in oncogene expression control dysregulation. Five out of twenty patients in the initial clinical study developed leucosis as a result of this integration[22].

2.3.3. Adeno Associated Viruses

Adenoassociated viruses (AAV) are small non-enveloped viruses with single-stranded DNA that belongto the family Parvoviridae. AAV are nonautonomousparvoviruses, which means that they are not able toreplicate in the absence ofadenovirus[2]. In nature, AAV infectshumans and stay inactive in the cell nucleus. Most viralgenomes do not integrate into the genome (they stay asepisomes). A small part of genomes (about 0.1%) can beintegrated into the host cell genome,with integrationoccurring specifically at a single site of chromosome[2]. AAV based vectors created up to now are not able to integrate into a genome and, thus, do not have a genotoxiceffect. One of the drawbacks related to such a life cycle isthat the number of AAV genomes in dividing cellsdecreases gradually, which, in turn, leads to a decrease of atransgene expression level. For this reason, AAV is thebest choice for transfection of slowly dividing cells, suchas myocytes, cardiomyocytes, etc.[18].

It should also bementioned that the AAV capsid is less immunogenic thanthose of adenoviruses or poxviruses. The severe systemicinflammatory response was not observed upon the use ofAAV; the virus is rather stable in blood, though animmune response was still observed. From 10 to 30% ofhumans are AAV seropositive. This does not excludethe possibility of a singlegene therapy application usingAAV, especially in the case of local administration of thevirus. Temporary immunosuppression or antibody trapsbased on empty AAV capsids can be used if needed [23].

To achieve a longer effect, several strategies can beused, such as the controlled release of small amounts of the vector from adapted carriers. Safety, facility of generation, and production made AAV based vectors, appearingonly in the mid1990s, as popular as adenovirus-based vectors, they are considered as the most perspective way of temporary gene expression for gene therapy[18].

2.3.4. Poxviruses

Viruses of the Poxviridae family are the largest andmost complex of viruses causing human disease. Theseviruses contain a double-stranded DNA genome of about180220 kb. One of the best-known representatives ofpoxviruses is the smallpox virus. The main tool for eradication of smallpox, achieved worldwide in 1980, was a livevaccine based on vaccinia virus – a poxvirus of unknownorigin (most likely isolated from horses). The use of this vaccine allowed the accumulation of much information concerning the side effects of the virus; moreover, it wasknown that the initial vaccinia virus was very reactogenic.New strains of the attenuated virus were found shortly before the end of the worldwide immunisationprogramme[2].

One of the more intriguing varieties, Modified Virus Ankara (MVA), was developed after 570 vaccinia virus passages in cell culture[24]. MVA lost around 15% of its genome as a result, including numerous genes involved in vivo pathogenesis and immune system suppression. MVA was utilized as a smallpox vaccine, and it was given to more than 120,000 people. As a result, the safety of this vector was clinically tested on a wide population. MVA is already being employed in the creation of more than 50 vaccines for viral, bacterial, and parasite illnesses[24].

2.3.5. Other Viruses

Reverse genetics (genome modification and generation of genetically engineered virus) is now possible foralmost all human viruses and some viruses ofbirds and other animals. RNAcontaining viruses are notvery widely used in genetherapy. Their tiny genome is not well suited to large-scale changes and readily loses heterologous inserts[2]. Replication produces a huge number of mutations, making drug standardization more difficult. It's tough to scale up production. The use of RNA viruses is limited to oncolytic therapy. Many non-pathogenic RNA viruses multiply efficiently in cancer cells but not in normal cells, and even unmodified viruses from various groups have a powerful oncolytic effect[25].There are several examples ofusing the herpes virus as abasis for gene therapy vectors. Being neurotropic, theseviruses are mainly used for the development of gene therapy approaches for the treatment of CNS dysfunctions[26, 27].

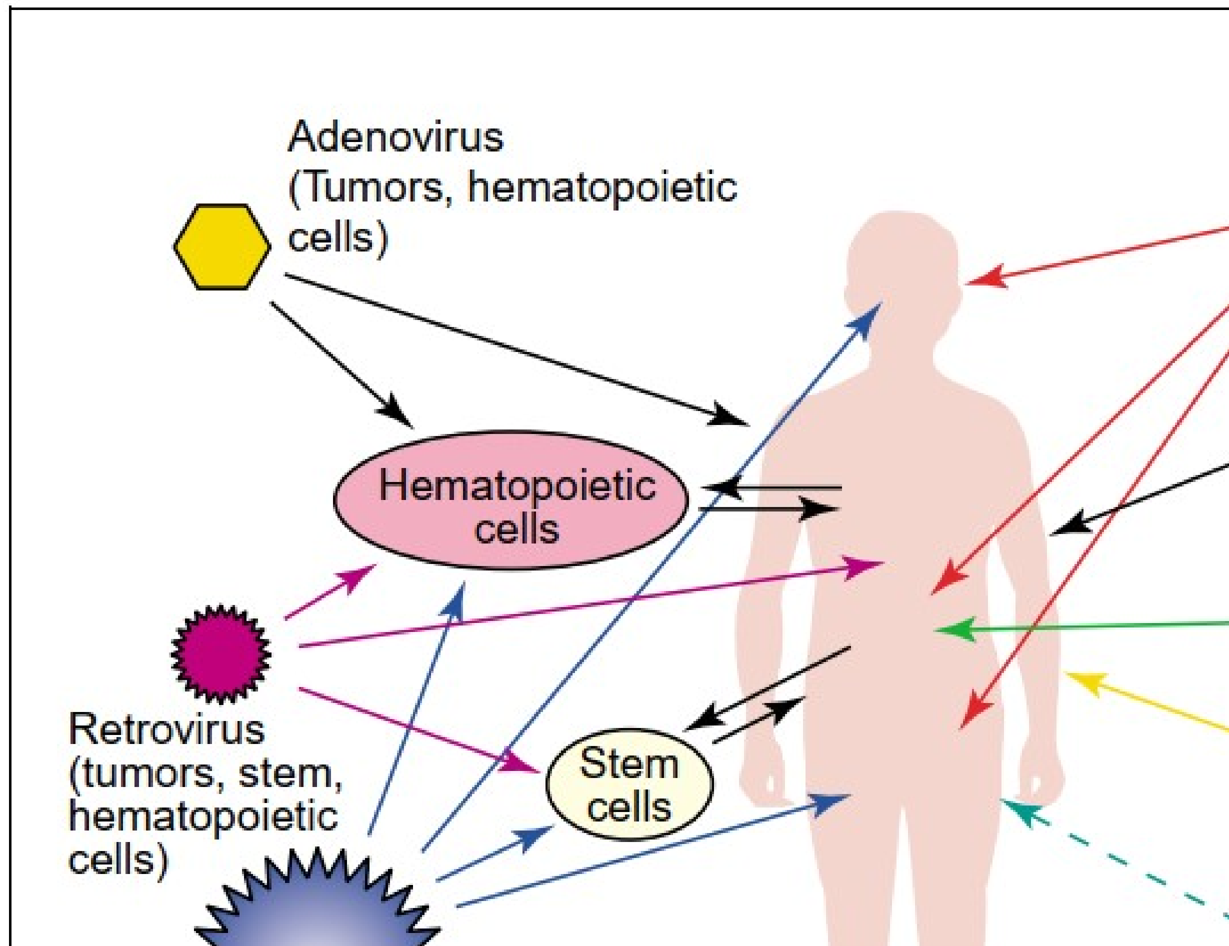

Fig 3. Clinical applications of viral vectors. Viral vectors are directly injected into specific tissues or administered systemically. Ex vivo applications are also possible[1].

2.4. Combining Properties of More Than One Virus

There have been recent efforts to blend properties of vectors to obtain combinational properties of both vectors. One active area is the combination of adenovirus and AAV vectors. Double-stranded AAV genomes in adenoviral capsids containing a combination of AAV and adenoviral ITRs have been shown to transduce and integrate into cells[28]. An alternative method involves inserting AAV vectors into gutless adenoviruses and modifying the vector to express Preprotein transiently to achieve site specific integration and avoid insertional mutagenesis[29]. The importance of this approach is still unclear because the chromosome 19 site-specific integration may occur in the middle of a gene and occurs only in about 50–70% of the integration events, which would only decrease the already low risk of insertional mutagenesis by two-fold. This risk must be balanced by the potential toxicity of the Rep protein[30].

It has been claimed that retroviral genomes housed within an adenovirus can integrate with the absence of retroviral integrase activity. Before its worth to the gene therapy community can be realized, this system must be further characterized. Site-specific bacteriophage integrases are being developed for site-specific integration into chromosomal DNA[31]. Nonetheless, integrating the features of several viral vectors, possibly even non-viral delivery systems, will almost certainly play a role in future gene therapy attempts[31].

2.5. Closing Remarks

The viral vectors discussed in this article are not all-inclusive, but they do reflect those that are currently being tested in clinical trials or are in advanced preclinical development. Though the vectors detailed here have made and will continue to make significant contributions to clinical gene therapy applications, many other viral and non-viral vectors, some of which have yet to be fully exploited or even found, will likely supplement the existing arsenal. SV-40, -viruses and hepatitis viruses are among the other viral vectors being developed[32], negative-strand RNA viruses (for example, influenza and Ebola) and Epstein-Barr virus(EBV)[32].

No any vector system is likely to be ideal for all possible gene therapy applications. A "perfect" vector, on the other hand, will be supplied via non-invasive delivery pathways, target the desired number of cells inside the target tissue, and express a therapeutic amount of transgene product with the desired regulation for a predetermined period for a given application. Though more gene therapy achievements are inevitable soon, the true potential of gene therapy will not be realized until present vectors are refined or new vectors with the features outlined above are produced[17].

3. Conclusion

For some applications, such as vaccines, nonviral systems may constitute
adequate treatments. For other applications. problems with this approach remain in the term of efficacy and duration of expression. A major impetus to continue work on nonviral systems is the widespread, but presently largely unproven, belief that such systems will be safer than viral-based vectors.

Rapid progress has undoubtedly been achieved in the application of viral vectors for gene therapy. Cancer applications have dominated and will most likely continue to do so. Work on ADA, which was a superb early model in retrospect, progressed the research of monogenic disease applications. However, as emphasized throughout, many problems remain in treating more common diseases: control of long-term expression; the biology of the host cell response, in terms of specific and nonspecific effects and of humoral and cellular immunity; the ability to repeat dose; and the efficacy of growth in culture of some ex vivo target cell type, as well as gene transfer to these cells.

Perhaps the most effective vector for addressing at least some of these challenges would be one that could integrate its nucleic acid into any cell type, including nondividing cells. In terms of gene transfer expression, this vector would be as effective as a virus,as well as the supposed safety profile of lipids or molecular conjugates. Howbest to generate such a vector remains unknown. One approach is to removeundesirable features from a particular virus to improve its safety withoutcompromising itsefficacy. The alternative is to add factor, perhaps fromviruses, to lipids or molecular conjugates to improve their efficacy withoutcompromising their safety. This latter approach ruins that we reproduce theevolution of viruses from first principles. This goal is challengingin the absence ofbiological selection; hence, for the foreseeable future, the viralvector will continue to provide an attractive alternative and will rightly remainthe subject of intense widespread research.

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