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Review

Virus-mediated gene delivery for human gene therapy

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ABSTRACT

After over 20 years from the first application of gene transfer in humans, gene therapy is now a mature discipline, which has progressively overcome several of the hurdles that prevented clinical success in the early stages of application. So far, the vast majority of gene therapy clinical trials have exploited viral vectors as very efficient nucleic acid delivery vehicles both in vivo and ex vivo. Here we summarize the current status of viral gene transfer for clinical applications, with special emphasis on the molecular properties of the major classes of viral vectors and the information so far obtained from gene therapy clinical trials.

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1. Genes as medicines

The concept to use genes as drugs for human therapy, originally conceived around 1970, was the logical consequence of at least two major advances occurring at the time, namely the exponential growth in the knowledge of human gene function and the impact of their mutations, and the progressive development of more effective technologies for DNA delivery into mammalian cells [1]. Initially, gene therapy was synonymous for supplying a missing cellular function by transferring a normal copy of an otherwise mutated gene into the relevant target cells. This concept applies little to the current advancement of gene therapy. While replacement gene therapy is obviously at the basis of the vast majority of gene therapy clinical trials for inherited disorders, these represent less than 8% of all clinical trials so far conducted. In most other instances, protein-coding cDNAs are used to modulate cell behavior (Table 1).

Key examples of this application include, among others, the block of cancer cell proliferation by interfering with cell cycle regulatory proteins; immune cell activation by transferring genes coding for co-stimulatory proteins into cancer cells; the secretion of growth factors and cytokines coding for neurotrophic factors in Parkinson's or Alzheimer's diseases, and the production of angiogenic factors in peripheral or cardiac ischemia. For an extensive review of these applications, cf. ref.: [2].

Even more notably, besides protein-coding nucleic acids, the spectrum of gene therapy applications is enormously increased by the possibility to use small nucleic acids (DNAs or RNAs) with regulatory function. These molecules now belong to one of at least six possible

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Table 1
Spectrum of therapeutic nucleic acids used by gene therapy and examples of their clinical applications. For an extensive description, cf. ref. [2].

Category	Type of nucleic acid		Examples of clinical application
Protein-coding DNA sequences	Proteins substituting missing or mutated cellular proteins		Replacement therapy for Duchenne muscular dystrophy, lysosomal storage disorders, hemophilia and several other inherited disorders
	Proteins modulating cellular functions		Costimulatory proteins (e.g. B7, ICAM-1, LFA-3) to activate cytotoxic T lymphocytes; HIV-1 RevM10 mutant to block HIV-1 replication
	Secreted growth factors and cytokines		Neurotrophic factors in Parkinson's and Alzheimer's diseases; VEGF in myocardial and peripheral ischemia
	Proteins regulating cell survival and apoptosis		HSV-1 thymidine kinase prodrug gene therapy to induce cell death in neuroblastoma; Bcl-2 for amyotrophic lateral sclerosis
	Antigens for vaccination		Antitumor and antiviral vaccination
	Antibodies and intracellular antibodies		Intracellular antibodies against HIV-1 integrase, Rev or reverse transcriptase to block viral replication; intracellular antibodies against antiapoptotic proteins in cancer
	T-cell receptor (TCR) subunits		Modified T-cell receptor subunit genes to retarget immune response towards tumor and viral antigens
Non-coding nucleic acids	Oligonucleotides and modified oligonucleotides	Phosphorothioate oligonucleotides 2′-Ribose modified oligonucleotides Locked nucleic acids (LNA) and ethylene-bridged nucleic acids (ENA) Morpholinos (PMO) Peptide nucleic acids (PNA)	Oligonucleotides blocking gene expression (e.g. to inhibit viral replication or to inhibit expression of a proapoptotic protein in cancer); oligonucleotides modulating pre-mRNA splicing (e.g. to induce exon skipping in Duchenne muscular dystrophy)
	Catalytic RNAs and DNAs Small regulatory RNAs	Ribozymes and DNAzymes siRNAs and shRNAs	Targeting pathological alleles in dominant inherited disorders; targeting viral mRNAs to inhibit viral infection
		MicroRNAs	Modulating cell function (e.g. stimulation of myocardial cell proliferation after myocardial infarction)
	Long antisense RNAs		Inhibition of viral gene expression (e.g. inhibition of HIV-1 replication)
	Decoys		Sequestering a factor essential for viral replication (e.g. Rev protein in the course of HIV-1 infection)
	Aptamers		Sequestering a relevant growth factor (e.g., VEGF in the treatment of age-related macular degeneration)

classes, namely DNA oligonucleotides, small catalytic RNAs and DNAs (ribozymes and DNAzymes respectively), small regulatory RNAs (siRNAs and microRNAs), long antisense RNAs, decoy RNAs and DNAs and RNAs binding to other molecules thanks to their tridimensional structure (aptamers) (Table 1). Notably, DNA oligonucleotides must be administered to the cells from the outside. In contrast, all RNA therapeutics, similar to protein-coding cDNAs, can also be synthesized inside the cells by transferring their coding DNA sequences.

2. Viral vectors: the perfect biological nanoparticles

In most instances, the efficiency of gene transfer continues to represent the most relevant obstacle limiting the clinical success of gene therapy. Given the broad spectrum of characteristics and mechanisms of action displayed by both coding and non-coding nucleic acids, it is evident that no perfect universal system for their delivery exists. In all cases, however, the apolar and hydrophobic membranes of mammalian

Fig. 1. Schematic representation of the organization of the viral genomes of gammaretrovirus, lentivirus (HIV-1), AAV and adenovirus (left side), and of the corresponding vectors generated from these viruses (right side). *Gammaretrovirus and HIV-1*: the common retroviral genes (*gag*, *pol* and *env*) are shown in dark green; the HIV-1 accessory genes in lighter green; the long terminal repeats (LTRs) are boxed; the localization of genetic elements relevant for vector production (primer binding site, PBS, packaging signal, ψ, 5′ and 3′ splice sites, 5′ and 3′ ss, polypurine tract, PPT, central poly-purine tract, cPPT and Rev-responsive element, RRE) is indicated; on the vector side, the localization of the therapeutic gene is shown in orange and that of the remaining portions of the viral genes (*gag*, *pol*, *env*) with the respective initial letters (g, e, p) boxed in green; RSV: Rous Sarcoma Virus promoter, used to express the retroviral mRNA in the packaging cells; RU5: portions of the LTR left intact in SIN vectors. *AAV*: the viral genes (*rep* and *cap*) are shown in light green; the localization of promoter is shown by arrow; ITR: inverted terminal repeat; poly A: polyadenylation site on the vector side; P: promoter. *Adenovirus*: viral genes present in the both the wt virus and the vectors are shown in dark blue, with arrows indicating the direction of transcription; the genes removed in first generation adenoviral vectors (E1A, E1B, E3) are shown in light blue; the localization of the therapeutic gene and its promoter (P) is shown in orange; ITR: inverted terminal repeat; ψ: packaging signal. *Herpes simplex virus 1*: The HSV-1 genome consists of a linear, double-stranded DNA molecules of 152 kb containing more than 80 genes. The genome is composed of unique long (U_L) and unique short (U_S) segments which are flanked by inverted repeats. These are designated as TR_L and IR_L (terminal and internal repeat of the long segment, respectively) and TR_S and IR_S (terminal and internal repeat of the short segment). The repeats surrounding U_L are designated ab and b′a′, while those surrounding U_S are designated a′c′ and ca. There are two different origins of replication, oriL in the long segment and oriS in the short segment. OriS is duplicated, along with ICP4, because they are found in the inverted repeats surrounding the long segment. Approximately half of the genes are essential for viral replication in cell culture (listed on top); the other half are non essential for viral replication in cultured cells (bottom). Genes in blue are non-essential genes that are mutated in the replication-competent (attenuated) viruses so far developed and described in the text; genes in red are immediate early (IE) genes that are mutated in the replication-defective viruses. The genome contains three pac signals (shown in yellow) that assist in packaging the viral genome DNA into virions.