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VIII Міжнародної науково-практичної конференції

ХІМІЧНА ТЕХНОЛОГІЯ: НАУКА, ЕКОНОМІКА ТА ВИРОБНИЦТВО

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Збірник містить наукові праці учасників VIII Міжнародної науково-практичної конференції «Хімічна технологія: наука, економіка та виробництво», що складаються з узагальнених матеріалів науково-дослідних робіт науковців різних галузей виробництв та наукових закладів України.

У збірнику висвітлюються актуальні питання спеціальної хімічної технології і виробництва боєприпасів, утилізації відходів виробництв різних галузей, енергозбереження, моделювання технологічних процесів, соціально-економічні аспекти виробництва та природокористування в умовах війни.

Збірник корисний робітникам хімічної промисловості, науковим співробітникам, аспірантам і студентам спеціальностей хіміко-технологічного та соціально-економічного профілів, фахівцям інформаційних технологій виробництва.

Наукові праці учасників конференції подаються в авторській редакції.

VIRAL GENE THERAPIES. AAV VIRUS VECTORS IN CURING GENETIC DISEASES: OVERVIEW, HISTORY AND MODERN RESEARCH.

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Gene therapy has become an increasingly popular topic due to ongoing scientific research and clinical trials, which show promise in treating a wide range of diseases. This approach aims to cure genetic conditions previously deemed incurable by introducing new genetic material into targeted cells. Gene therapy now is considered also for many non–life-threatening conditions, including those adversely affecting a patient's quality of life. [1] Viral vectors have been employed for the treatment of diseases such as metabolic, cardiovascular, muscular, hematologic, ophthalmologic, and infectious diseases and different types of cancer. [11] Gene therapy has been especially successful in the treatment of combined immunodeficiency syndromes, showing lasting and remarkable therapeutic benefits.[4][5]

The first clinical trial of gene therapy was conducted in 1990 using a retroviral vector to treat severe combined immunodeficiency (SCID). Since then, numerous advancements have been made. Notably, China became the first country to approve a gene therapy, *Gendicine*, for treating head and neck cancer in 2003.[2] Despite their success, early setbacks raised significant safety concerns of gene interventions. In 1999, a clinical trial at the University of Pennsylvania resulted in the death of Jesse Gelsinger, an 18-year-old patient, due to a massive immune response to an adenoviral vector (Ad5) used to deliver the ornithine decarboxylase gene.[3] Similarly, a 2000 trial led to cancer development in four patients following retroviral gene therapy[6]. These incidents prompted stricter regulations for all gene therapy trials and changed how we view gene therapies.

While non-viral vectors in gene therapies are considered safer, they currently lack efficiency in gene delivery. Notably, the majority of these gene therapy trials, almost 70%, are based on viral vectors [10].Viral vector expression cassettes can be engineered with elements to enhance target specificity and increase transgene expression. [7] These features have been explored and refined to develop efficient methods for delivering genes of interest into mammalian cells. Five main classes of viral vectors have been tested for clinical applications: retroviruses (RV), adenoviruses (AV), adeno-associated viruses (AAV), lentiviruses (LV), and herpes simplex viruses (HSV) (Table 1).[8] The 5 main classes of viral vectors can be categorized in 2 groups according to whether their genomes integrate into host cellular chromatin (on retroviruses and LV) or persist in the cell nucleus predominantly as extrachromosomal episomes (AAV, AV, and herpes viruses). [11]

Table 1. Comparison of different viral vector types [9]

Vector	Toxicity	Persistence of express.	Capacity (kb)	Postmitotic cells	Virion size	Difficulty of production[a]
rAAV	No	>1 year	≤5[b]	Yes	20 nm	Medium
Ad	Some	>1 year[c]	≤37	Yes	80 nm	Difficult
MoMLV	No	Persistent	≤8	No	100 nm	Easy
Lentiviral	No	Persistent	≤9	Yes	100 nm	Easy
HSV-1	Some	Months	≤150[d]	Yes	150 nm	Difficult

The undesired properties of some viral vectors, including their immunogenic profiles or their propensity to cause cancer have resulted in serious clinical adverse events and, until recently, limited their current use in the clinic to certain applications, for example vaccines and oncolytic strategies.[13] While LV vectors are now preferred for ex vivo gene correction, AAV has emerged as the preferred vector for in vivo gene transfer due to its favorable safety profile compared to other vectors, ability to transduce a variety of tissues, and availability of a large number of viral capsids with different tropism.[12]

This paper focuses on AAV (adeno-associated virus), the most widely used viral vector in gene therapy research. AAV was discovered over 50 years ago and has since become one of the leading gene delivery vectors in clinical development, it's a versatile vector technology that can be engineered for very specific functionality in gene therapy applications, so that is essential to understand biology and structure of AAVs.

AAVs are single-stranded DNA non-enveloped viruses whose viral genome is approximately 4.7 kilobases (kb) long and consists of two inverted terminal repeats (ITRs), in between which there are three genes, Rep (Replication), Cap (Capsid), and aap (Assembly) (Figure 1). These three genes give rise to at least nine gene products through the use of three promoters, alternative translation start sites, and differential splicing. The ITR plays a fundamental role in the life cycle of AAV by containing the replication of origin, packaging signals, and the ability to confer persistence to AAV genomes after infection. AAV belongs to the parvovirus family and is dependent on co-infection with other viruses, mainly adenoviruses or herpes simplex, in order to replicate. Initially Through the use of alternative splicing and start codons, the rep gene can produce four overlapping proteins (Rep78/Rep68 and Rep50/Rep42) that play crucial roles in viral replication, integration, transcriptional regulation, and assembly. The cap gene can produce three structural proteins (VP1, 2, and 3) that come together to form a ∼26 nm icosahedral particle. [14] Additionally, the assembly-activating protein (AAP) can help in capsid formation through an alternative reading frame. The only elements essential for viral packaging are the inverted terminal repeats, which have palindromic sequences forming a T-shaped hairpin structure with specific binding sites for Rep proteins. Therefore, rep and cap can work together in trans to aid in the assembly of virions and the creation of vectors carrying altered genetic material. The significant advantage of AAVs is that the resulting vector can transduce both dividing and nondividing cells, with stable transgene expression in the absence of the helper virus in postmitotic tissue.[16]

In gene therapies wild type AAV is not being used, instead a recombinant AAV (rAAV), which lacks viral DNA was engineered for therapeutic purposes. That way, in the absence of Rep proteins, ITR-flanked transgenes encoded within rAAV can form circular concatemers that persist as episomes and do not integrate in the human genome. [15] Additionally, various AAV serotypes have exhibited remarkably different expression patterns due to differences in cell entry and intracellular activities. For example, AAV9 has a preference for primary cell binding through galactose as a result of unique amino acid differences in its capsid sequence [18]. Moreover, different serotypes of AAVs can transduce genes to different organs or tissues (Table 2).

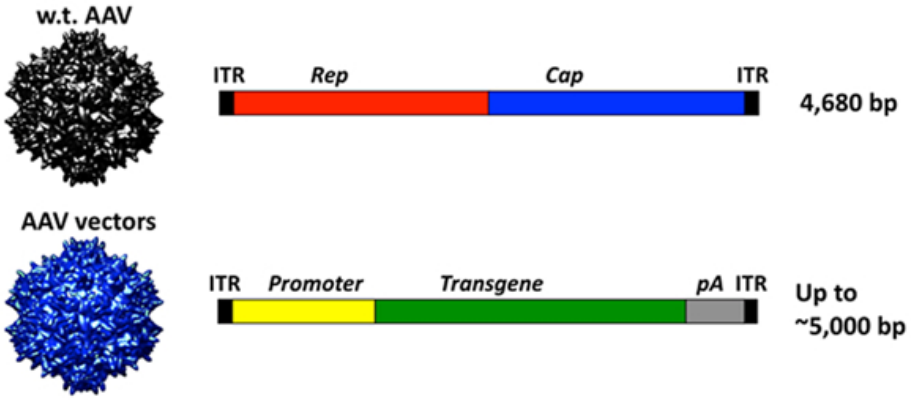

Figure 1. Schematic representation of the basic components of a genome of AAV and rAAV vectors. [18]

Table 2. Characterization of AAV natural serotypes. [19]

Serotype	Origin	Primary Receptor	Secondary Receptor	Natural Tropism
AAV1	Non-human primate	Sialic acid	AAV receptor (AAVR)	Muscle, CNS, heart, liver, lungs
AAV2	Human	Heparan sulfate proteoglycan (HSPG)	Integrin, fibroblast growth factor receptor (FGFR), hepatocyte growth factor receptor (HGFR), laminin receptor (LamR)	Heart, CNS, liver, lungs, retina
AAV3	Non-human primate	HSPG	LamR, FGFR, HGFR, AAVR	Liver
AAV4	Non-human primate	Sialic acid	Unknown	Retina, lungs, kidney
AAV5	Human	Sialic acid	Platelet-derived growth factor receptor (PDGFR), AAVR	Retina, CNS, liver
AAV6	Human	HSPG, sialic acid	EGFR, AAVR	Heart, liver, muscle, retina
AAV7	Non-human primate	Unknown	Unknown	Liver

A vast majority of AAV studies were carried out with AAV serotype 2, the most prevalent strain found in the human population.[20]

The first step in AAV infection requires binding to cell surface glycan receptors[21]. This key step mediates cell surface attachment of virions and triggers subsequent cellular internalization and trafficking leading to transduction. Cellular uptake of AAV particles into endocytic vesicles is thought to be mediated by integrins and/or different transmembrane receptors.[22] After entering into the cell, rAAV travels to the nucleus in order to deliver its genetic material. Although intracellular transport strategies of rAAV are not yet fully understood and there is room for debate about our current understanding of those processes, researchers are developing new hypotheses and extending our knowledge.

AAV8	Non-human primate	Unknown	LamR, AAVR	Muscle, heart, CNS, liver
AAV9	Human	Galactose	LamR, AAVR	Heart, CNS, liver
AAV10	Non-human primate	Unknown	Unknown	Muscle, myoblast tissue
AAV11	Non-human primate	Unknown	Unknown	Muscle, myoblast tissue
AAV12	Non-human primate	Unknown	Unknown	Salivary glands, muscle

AAV vectors, although having ideal characteristics for applications as gene therapy agents, have certain limitations. The main point of consideration is its small packaging size, which greatly limits the number of genes it can carry, or, with large enough coding sequences, they just won't work effectively. An additional consideration relates to the biology of the single-stranded AAV-delivered transgenes. After delivery to the nucleus, the single-stranded transgene needs to be converted into a double-stranded transgene, which is considered a limiting step in the onset of transgene expression.[23] However, there are ways to introduce self-complementary AAV, which has a double-stranded genome of complemental strands, thereby bypassing that process. The downside is that while the expression of genes will be more rapid, the packaging capacity will drastically shrink. Moreover, some researchers say that self-complementary AAV tends to be more immunogenic.

The production of rAAV is still very expensive, despite years of research. Transfection of plasmid DNA into eukaryotic cells was the first way to produce rAAV and still remains a main protocol for clinical uses. The most traditional approach is a calcium phosphate plasmid precipitation on human embryonic kidney 293 cells (HEK293)7 or HEK293-T8 with equimolar amount of the vector construct plasmid (prAAV-promoter-transgene) and the helper plasmid that provide the AAV Rep and Cap functions as well as the Ad5 genes (VA RNAs, E2A, and E4OEF6). Cells are amplified in Corning cellstacks or roller bottles. The biggest challenge with that model is a lack of scalability, though new approaches can be used to deal with that problem.

The first commercially approved gene therapy product in the Western world was Glybera (alipogene tiparvovec), which is based on an adeno associated viral (AAV) vector for the treatment of the inherited metabolic disorder lipoprotein lipase (LPL) deficiency (LPLD).[24]

The versatility and safety profile of AAV vectors have firmly established their significance in the progression of gene therapy. With advancements in manufacturing technologies, it is anticipated that costs may decrease, thereby enhancing accessibility to these pioneering treatments. Future research will undoubtedly refine AAV-based therapies, broadening their therapeutic potential while addressing existing limitations. Consequently, pharmaceutical research and development has placed a great deal of emphasis on finding

safe and efficient gene delivery systems, and will want to bring about a change in the way we think about disease-state intervention.

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