CLASSIFICATION OF VECTORS FOR GENE DELIVERY IN EUKARYOTIC CELLS

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Introduction. A eukaryotic vector (EV) is a small DNA molecule capable of autonomic replication in animal cells. In addition to nucleotide sequences (NS) that ensure replication, EV should contain genes being used as selectable markers. It has one or more unique restriction sites to insert DNA being cloned. Since the direct cloning of recombinant DNA in animal cells would be an expensive and inefficient procedure, the EV is utilized to express already cloned heterologous NS in cells, while the cloning process itself is carried out in bacteria. For expression in eukaryotic cells, recombinant DNA is placed under the control of particular regulatory elements, which are recognized by the fermentative system of the host. The aim of this article is to outline and classify different vectors that can be applied for the delivery of genes.

Materials and methods. Analytical overview of the latest publications in the field of EV for gene delivery.

Results and discussion. As the result of the literature review a certain classification of vectors was created and will be discussed below.

Retroviral vectors are the ones being used most often. For clinical purposes, they are usually constructed on the basis of Moloney mouse retrovirus. They are freed from all viral genes and show a relatively long and safe period of expression. Packaged cell lines are constantly being exposed to viral genes that are necessary for replication and packaging of the genome. The vector sequences with therapeutic genes are flanked by the structural sequence of virus and packaging signal. After that the NS, required for packaging the vector DNA into a virion, flanks the expression gene. The main advantage of this vector is its low immunogenicity. In fact, the DNA integrates into the host cell genome and stably replicates within genomic DNA instead of remaining as an independent plasmid replicon in the cytoplasm. On the other hand, lower throughput of insertion, titers (than in other viral vectors), replication efficiency in self-inactivating (SIN) vectors [1] and risk of insertional mutagenesis, causing disruption of host gene function can be a huge problem while working with these vectors.

Adenoviruses is a family of DNA viruses that contains one double-stranded DNA molecule and has no lipid envelope. It can infect most types of cells (dividing and non-dividing) and replicate in the nucleus of an infected cell as episomal elements and have a high transduction efficiency. The insert size for target gene is 20,000 base pairs, which is quite a large capacity for recombinant adenoviral vectors. For example, after direct intracranial injection of recombinant adenovirus, its ability to infect neurons, astrocytes, microglia and ependymocytes was discovered [2].

Recombinant Adeno-associated virus is one of the most promising delivery vector for gene therapy and neurobiology due to its non-pathogenic properties, low immunogenicity to the host, tropism to most cells and tissues, high transduction efficiency and long-term expression. The only drawback it has is the small capacity of the vector (up to 5 thousand base pairs) [5].

Due to the ability of Lentiviruses, especially the human immunodeficiency virus (HIV), to include a relatively large amount of genetic material (up to 8 thousand base pairs) and to infect dividing and non-dividing cells, they can also be promising vectors for gene delivery *in vivo* [3]. They have a relatively low frequency of target gene insertion but can provide a long-term expression period of the transgene and induce a minimal immune response of the host organism. However, some authors consider these vectors to be unsuitable for *in vivo* delivery because of the increased risk of insertional mutagenesis.

Herpes simplex virus (HSV) is distinguished by its large size, big DNA capacity and efficient transduction of many tissues, including the central neural system. The virus itself includes approximately 80 genes. IE3 locus is often replaced during vector development. Other genes may be excluded too, which allows researchers to increase the volume of the vector or to suppress several genes of interest. The disadvantages of HSV vectors are short-term expression of cloned genes, toxicity to target cells, low transduction efficiency and the ability to infect only non-dividing cells [7]. Preclinical studies of this vector type are currently underway. Clinical trials in this area are likely to begin soon [6].

It has been shown that naked plasmid DNA participates in gene expression in the brainstem when intramuscularly injected into the tongue or when injected intraventricularly. Moreover, infusion of naked plasmid DNA into the area of spinal cord damage induces transgene expression in neurons and astrocytes. Such plasmid DNA is an attractive non-viral gene vector because of its simplicity, ease of production in bacteria and manipulation with standard recombinant DNA techniques. It also shows very little dissemination and transfection at distant sites after birth and can be re-injected into mammals (including primates) many times without eliciting an immune response. Furthermore, long-term expression of a foreign gene from naked plasmid DNA is possible even without chromosomal integration. In such a case the target cell needs to be postmitotic (as in muscle) or slowly mitotic (as in hepatocytes) and the immune response against the foreign protein is not formed. One of the important limitations of these methods is the problem of safe transfer of genes to specific organs in combination with their therapeutic effectiveness, which leads to stable gene expression [8].

Last but not least, viral vectors can be encapsulated in liposomes (figure 1) made of cholesterol, phosphatidylcholine, phosphatidylglycerol, DOPE, DS, CHEMS, TMAG, DLPS, DOTAP that define the total charge of the liposomes. Encapsulation can be monolayer or bilayer. It helps improve transduction efficiency of vectors, avoid preexisting neutralizing antibodies and transduce cells that are otherwise difficult to transduce with a viral vector alone.

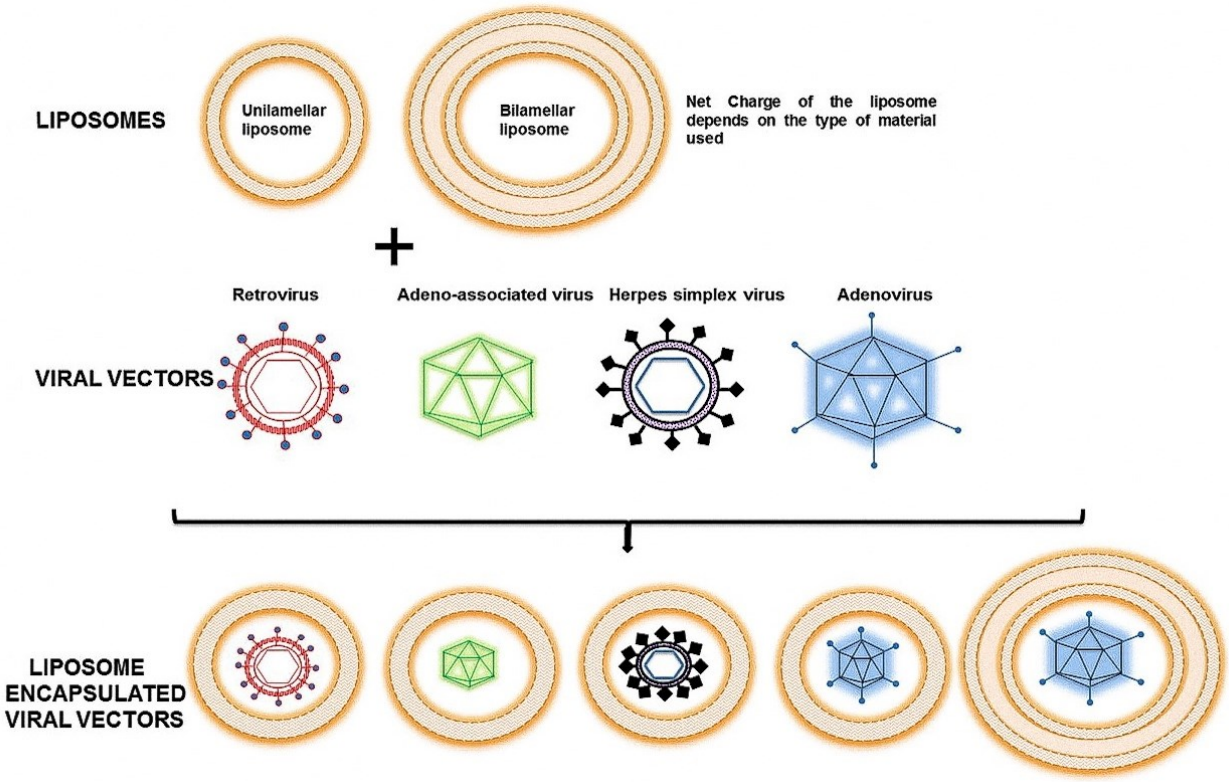

Figure 1. Liposomal encapsulation of viral vectors [9].

Conclusions. At the moment, no universal EV for the creation of recombinant DNA exists. All vector systems may have their own disadvantages and advantages specifics for each case. That means more studies in this field of science are required as it is the promising technique for curing a lot of diseases and cloning organisms.

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