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Advances in Studies on Recombinant Adeno-associated Virus Vector

Yan Qiu, Bin Yang, Xi Lan, Xue-Ri Li, Xiang-Ping Yin and Ji-Xing Liu
State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Animal Virology of Ministry of Agriculture, Key Laboratory of Epizootic Diseases of Grazing Animal of Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China

Corresponding Author: Ji Xing Liu, State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Animal Virology of the Ministry of Agriculture, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou 730046, China Tel: +86-931-8342685 Fax: +86-931-8340977

ABSTRACT

Recombinant Adeno-associated Virus (rAAV), as a common tool of gene therapy has many merits such as, nonpathogenicity, long-term expression, low immunogenicity, as well as its large-scale infectivity to many kinds of cells. As a result, scientists pay high emphasis on the rAAV. There is no doubt that the rAAV vectors will make great contribution to human health. This study reviewed the progress of the rAAV vector in recent years. It mainly states the characteristics, application, production and security of rAAV.

Key words: Recombinant Adeno-associated Virus (rAAV), vector, gene therapy, advances

INTRODUCTION

Adeno-associated Virus (AAV) is a member of Parvoviridae family. As it can not produce cytase, its replication depends on the help of other virus (helper virus), such as, adenovirus and herpes virus, so it was classified as dependovirus (Schlehofer *et al*., 1986; Berns, 1990). Although, at least 14 serologically distinct AAVs and multiple variants have been identified and isolated from humans or primates (Samulski *et al*., 1982; Rutledge *et al*., 1998; Gao *et al*., 2002, 2004; Chiorini *et al*., 1997, 1999; Grimm and Kay, 2003; Bantel-Schaal *et al*., 1999; Buning *et al*., 2008), the best constructed recombinant AAV is AAV serotype 2 (AAV2) (Hildinger and Auricchio, 2004; Verma and Weitzman, 2005). Recombinant Adeno-associated Virus (rAAV) vectors are widely used in gene therapy, because of their nonpathogenicity, long-term expression, low immunogenicity, as well as its large-scale infectivity to many cells.

PRODUCTION OF rAAV VECTOR

AAV virions are very small (20-25 nm). The genome of AAV virus is a linear single-stranded DNA (ssDNA), which is approximately 5 kb. They contain two Open Reading Frames (ORF) and the Inverted Terminal Repeats (ITR) (Carter and Samulski, 2000). The ITRs are retained in recombinant adeno-associated virus (rAAV) vectors which play important role in replication, packaging and integration (Verma and Weitzman, 2005). AAV are extensively used in gene therapy, for instance, in conventional the viral Rep (replication protein) and Cap (capsid protein) ORFs are replaced by the gene of interest, so the recombinant virus particles are produced by providing the packaging cell with the essential Rep and Cap functions in trans as well as the necessary viral helper functions (Hildinger and Auricchio, 2004).

In laboratory, rAAV is usually produced in transient cell as follows: Three kinds of plasmids are co-transfected into the host cells; (1) A vector plasmid contains the transgene expression cassette flanked by the viral ITRs, (2) A AAV helper plasmid encodes the two AAV-specific ORFs, rep and cap and (3) Another AAV helper plasmid acts as the role of adenovirus for AAV replication (Buning *et al*., 2008). Therefore, after the transcription and translation of Rep and VP proteins the vector plasmids are encapsidated into preformed AAV capsids (Dubielzig *et al*., 1999; King *et al*., 2001). Some researchers used insect cells to produce rAAV particles in large-scale and they demonstrated that rAAV particles produced in the insect cells yield per cell were increased five-fold compared to mammalian cells (Urabe *et al*., 2002). The density gradient centrifugation (CsCl or iodixanol) and column chromatography are widely used to purify rAAV particles from cell lysate (Zolotukhin *et al*., 1999). The most efficient purification methods of empty capsids from genome containing capsids is CsCl density gradients (Zolotukhin *et al*., 1999; Scallan *et al*., 2006).

CHARACTERISTICS OF TRANSFECTION AND EXPRESSION

The rAAV vectors have been shown to mediate long-term gene expression *in vivo* (Clark *et al*., 1997; Flannery *et al*., 1997; Flotte *et al*., 1993; Koeberl *et al*., 1997; Snyder *et al*., 1997, 2011; Xiao *et al*., 1996; Herzog *et al*., 1999), so, those rAAV gene drugs based on this merit are good candidates for chronic disease treatment. It has been proved that rAAV vectors could transfect various kinds of cells. The size of target gene construct in rAAV vectors is finite, the smaller gene constructs more effectively and a gene larger than 4,800 bp is not suitable for rAAV construction. Previously, researchers argued that the cap could cause the body's immune response which could reduce the transfection efficiency. So, we can improve the transfection by eliminating the immune response stimulated by cap. Transfecting into a host cell successfully or not depends on three points: (1) rAAV vectors successfully attached to and interacted with target cells, then, nucleic acid enter into the cell, (2) The nucleic acid can effectively converted into a double chain and (3) The transfected rAAV can conduct an adequately expression. The tissue tropism differs with the serotypes of AAV. For instance, rAAV-1 can express more effectively than rAAV-2 in muscles, islet, heart, vascular endothelial, central nervous system and liver cells, in addition, rAAV-3 is more suitable for cochlear hair cells and rAAV-4, rAAV-5, rAAV-6, rAAV-7, rAAV-8 are, respectively more active in brain tissue; central nervous, eyes, joint and liver cells; muscles, heart and tracheal epithelial, muscle, muscle, pancreas, heart and liver (Gao *et al*., 2002; Snyder *et al*., 2011; Wang *et al*., 2005; Kawamoto *et al*., 2005; Mingozzi *et al*., 2002; Louboutin *et al*., 2005).

APPLICATION OF rAAV VECTORS

Since, the first separation of rAAV in 1983, the human gene therapy has made a great progress. rAAV turns out to be one of the most popular virus vector, although there are still some obstacles in the application of gene therapy. Most of the clinical trials of AAV vector is based on serotype 2 and the majority are widely used in disorders caused by single gene chaos, such as, fiber cysts disease, muscle malnutrition disease and malignant tumor. In addition, rAAV-2 is also extensively used in the incubation period. In addition to rAAV-2, the other AV serotypes also show good transduction efficiency in different tissues.

The rAAV vectors showed extensive application in clinical treatments, and performed very well. Take three points for instance: (1) Application in chronic disease and genetic disease on the basis of long-term expression. Multiple AAV-cFVIII vectors shows a long-term therapy in the case of

dogs' hemophilia (Jiang *et al*., 2006), (2) Application in tissue localization treatments. In the study of (Bell *et al*., 2011), gene transfer vectors based on Adeno-Associated Virus 8 (AAV8) transduced predominantly hepatocytes near central veins and yielded lower transduction levels in hepatocytes in periportal regions. This transduction bias had important implications for gene therapy that aims to correct metabolic liver enzymes. In the research of Azat *et al*. (2011), they injected high-dose shRNA-AAV9 (short hairpin RNA-expressing AAV9) to neonates and efficiently silenced genes in cardiac and skeletal muscles without inducing liver toxicity and (3) Related rAAV vectors could kill cancer cells by expressing tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), so rAAV vectors are also applied in the treatment of tumor. In the present study of Lv *et al*. (2011), they demonstrated that AAV-mediated anti-DR5 (death receptor 5) mouse-human chimeric antibody expression significantly suppressed tumor cell growth by inducing apoptosis both *in vitro* and *in vivo*.

In general, rAAV vectors has been adopted extensively in the filed of medicine, such as, neurological, the treatment of cardiovascular diseases, the production of some medicine (Nonnenmacher and Weber, 2012) and so on. The research conducted by Noe *et al*. (2010) showed that rAAV1-NPY vector with the CBA promoter mediates powerful anticonvulsant effects and seems to be safe in rodents. And the study of Su *et al*. (2009) showed that AAV-mediated angiopoietin-1 and VEGF expression yielded a remarkable therapeutic effect.

SECURITY OF rAAV VECTOR

As rAAV could integrate to any chromosomes of host randomly, it pose a threat to its application in gene therapy. The rep protein generated by AAV have two main adverse influences to host, on the one hand, it could restrain the growth of cell, on the other, it helps rAAV to integrate to the 19th chromosome of human at a fixed position (Giraud *et al*., 1995; Weitzman *et al*., 1994). Although, the rep gene was knocked out in the construction of rAAV, it could integrate to chromosomes of host randomly. Miao and his colleagues proved this in liver cells transfection trial in 1998 (Miao *et al*., 1998). Nakai *et al*. (2005) discovered that parts of the integration region of rAAV in chromosome is in or near to 9 cancer gene sites. Although, in clinical practice we have not yet found any case of gene inactivation or tumor (Donsante *et al*., 2007; Bell *et al*., 2005), we still feel anxious about the safety of rAAV gene drugs.

The body conduct two main immune response against rAAV: Innate immune response and adaptive immune response. Although, the mechanism of innate immune response stimulated by rAAV is still unknown, the study of Zaiss *et al*. (2008) had proved the existence of innate immune response caused by rAAV. The capsid protein (Cap) and the target protein generated by rAAV should be responsible for adaptive immune response. The majority rAAV constructed nowadays do not contain the virus ORFs, but the ITR and target genes, so only the coat protein can cause the body's immune response.

CONCLUSION AND RECOMMENDATIONS

As the most promising gene therapy vectors in the 21st century, there are a lot of obstacles in the application to clinical therapy. The diversity of rAAV serotypes poses a threat to its security. First of all, the mass production and purification is very tough. What's more, the cytotoxic T-lymphocyte response caused by rAAV is also knotty. Ensuring the safety of rAAV is the premise of clinical therapy. Nowadays, the most studies focus on small animals, it is necessary to conduct trials on large animals and finally applied the rAAV gene drugs to clinical trials.

At the moment, we should make further efforts in discovering the following aspects: (1) The structure of rAAV capsid proteins, (2) the interaction mechanism between capsid proteins and host receptor, (3) the relationship between the rAAV random integration and the occurrence of cancer and (4) the tissue tropism of rAAV. However, there is no doubt that rAAV vectors will make great contribution to human health.

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