[Frontiers in Bioscience 13, 2653-2659, January 1, 2008]

Cancer gene therapy using adeno-associated virus vectors

Keerang Park[1], Wun-Jae Kim[2], Young-Hwa Cho[1], Young-Ill Lee[3], Heuiran Lee [4], Sunjoo Jeong [5], Eui-Sic Cho[6], Soo-Ik Chang[7], Sung-Kwon Moon[8], Bong-Su Kang[2], Yeun-Ju, Kim[1], Sung-Ha Cho[1]

[1]Department of Biotechnology, Juseong Gene Therapy R&D Center, Juseong College, [2]College of Medicine and Institute for Tumor Research, Chungbuk National University, [3]School of Engineering, University of Suwon, [4]Department of Microbiology, Research Institute for Biomacromolecules, University of Ulsan College of Medicine, [5]Department of Molecular Biology, BK21 Graduate Program for RNA Biology, Institute of Nanosensor and Biotechnology, Dankook University, [6]School of Dentistry, Chonbuk National University, [7]Department of Biochemistry, Chungbuk National University, [8]Department of Food and Biotechnology, Chungju National University, Korea

TABLE OF CONTENTS

1. ABSTRACT

Gene therapy has offered highly possible promises for treatment of cancers, as many potential therapeutic genes involved in regulation of molecular processes may be introduced by gene transfer, which can arrest angiogenesis, tumor growth, invasion, metastasis, and/or can stimulate the immune response against tumors. Therefore, viral and non-viral gene delivery systems have been developed to establish an ideal delivery vector for cancer gene therapy over the past several years. Among the currently developed virus vectors, the adeno-associated virus (AAV) vector is considered as one of those that are closest to the ideal vector mainly for genetic diseases due to the following prominent features; the lack of pathogenicity and toxicity, ability to infect dividing and non-dividing cells of various tissue origins, a very low host immune response and long-term expression. Particularly, the most important attribute of AAV vectors is their safety profile in clinical trials ranging from CF to Parkinson's disease. Although adenovirus and several other oncolytic viruses have been more frequently used to develop cancer gene therapy, AAV also has many critical properties to be exploited for a cancer gene delivery vector. In this review, we will briefly summarize the basic biology of AAV and then mainly focus on recent progresses on AAV vector development and AAV-mediated therapeutic vectors for cancer gene therapy.

2. INTRODUCTION

In 2005, the American Cancer Society estimated that the new cancer cases were about 1,372,910 and the dead were about 570,280 in the United States. In Korea, malignant cancers have been the leading death cause for the past several years although many conventional therapies, such as radiotherapy, chemotherapy, surgery, thermotherapy and biotherapy have been applied for treating various cancers. These fearful statistics demonstrate the necessity of newer therapeutic modalities for achieving successful cancer treatment and cure. Therefore, cancer gene therapy including oncology has been the center of gene therapy to improve the anti-cancer efficacy as well as to solve the problems of the conventional cancer therapies such as drug-resistance, side effects, toxicities etc., although gene therapy was initially developed for treatment of genetic diseases. Since the first trial treating melanoma with the retroviral therapeutic vector in 1990, more than 1,192 gene therapy clinical trials have been conducted worldwide, and more than 67% of them are for treating various cancers (1). As expected, the first gene therapy potential drug, Gendicine of the recombinant human p53 adenovirus vector was developed by SiBiono Gene Tech Co., Ltd, and was released from China in 2004. They recently reported that the clinical trials of treating 4,000 patients with 50 different cancers resulted in certain degree of success. Gendicine was effective when administrated alone, showed synergic

Table 1. The genome size, homology to AAV2 and target tissues of AAV1 - AAV8 (7)

Serotype	Origin (genome size, homology to AAV2)	Target tissues
AAV1	Simian sources (4,718 nucleotides, 80%)	Skeletal muscle, Liver cells
AAV2	Human clinical specimens (4,681 nucleotides, 100%)	Different types of cells in the CNS, ubiquitous
AAV3	Human clinical specimens (4,722 nucleotides, 82%)	Megakaryocytes
AAV4	Simian sources (4,767 nucleotides, 75%)	Rat retina
AAV5	Human clinical specimens (4,642 nucleotides, 55%)	Apical airway cells, Liver cells
AAV6	Recombinant of AAV2(5') and AAV1(3') (4,683 nucleotides, 82%)	Apical airway cells
AAV7	Rhesus monkey (4,721 nucleotides, 84%)	Skeletal muscle
AAV8	Rhesus monkey (4,393 nucleotides, 84%)	Liver cells, Skeletal and cardiac muscles

efficacy when combined with conventional therapies, and effectively inhibited tumor recurrence when used after surgeries (2). In cancer gene therapy, therapeutic genes are introduced by gene delivery systems of viral vectors or nonviral vectors (liposome, naked DNAs, etc.). Both delivery systems have pros and cons to be exploited for development of an ideal gene delivery vector. Among several viral gene delivery systems such as retrovirus, adenovirus, lentivirus, etc., AAV is regarded as one of the best gene delivery systems if the size of therapeutic gene is small enough for rAAV to be packaged efficiently (3). It is non-pathogenic, very limited in immune responses and able to transduce both dividing and non-dividing cells such as endothelial cells, skeletal muscles, cardiac myocytes, neurons, lungs, hepatocytes, renal cells and various cancer cells. AAV vector also shows long-term persistent transgene expression, although there is concern that two major obstacles of AAV vectors, the relatively poor transduction efficiency of AAV on certain cell types and the presence of pre-existing immunity to AAV in humans might limit its clinical applications for certain diseases or for some patients (3). Among AAV serotypes, AAV serotype 2 (AAV2) vectors are being mostly used for clinical trials of cystic fibrosis, hemophilia, Canavan's disease (4) and several AAV serotypes including AAV2 are recently being tested for cancer-specific tropism (4, 5). Here, we briefly review the biology of AAV, recent progress on development of AAV vectors, and the current status of AAV-mediated cancer gene therapy.

3. BIOLOGY OF AAV

AAV belongs to the family of Parvoviridae, a group of viruses among the smallest of single-stranded and non-enveloped DNA viruses. All serotypes of AAV are dependent either on a helper virus such as adenovirus, herpes simplex virus, or on the genes of helper virus for replication and productive infection. Therefore, AAV are replication-deficient viruses and classified as dependoviruses. In the absence of helper virus, AAV can be integrated into the human chromosome 19 (AAVS1 site) as a provirus for latency (6). The viral particle consists of icosahedral symmetry with a diameter of 18 - 26 nm, molecular weight of 5.5 - 6.2 million Daltons (Da) and genome size ranging 4,393 to 4,767 nucleotides. The identified AAV serotypes have very similar capsid morphologies and genome lengths as shown in Table 1.

The atomic structure, life cycle and genomic structure of AAV2 have been well determined among AAV serotypes. AAV2 particle carrying the genome of 4,681 nucleotides is composed of three different capsid proteins (VP1, VP2 and VP3 in a ratio of 1:1:8) and the physical structure of AAV2 has been precisely determined by several groups. The unique characteristics in the atomic structure of AAV2 are groups of three-fold peaks and interstrand loops placed between two neighboring subunits. The positively charged groups located on one side of each peak are believed to be attached to the primary cellular receptor, heparin sulfate proteoglycan (HSPG). AAV2 is also shown to bind to two different co-receptors, fibroblast growth factor receptor 1 (FGFR1) and alphavbeta5 integrin. FGFR1 seems to enhance the virus binding to the cells, whereas alphavbeta5 integrin might be involved in endocytotic process. After endocytosis, the AAV2 viral particles are released from endosome in low pH that is needed to induce conformational changes of viral proteins, which is critical for a successful endosomal release and nuclear entry. Some of the AAV genomes are integrated into the human chromosome 19 (10q13-qter) with assistance of Rep68 and Rep78, while other virus genomes remain as episome in nuclei.

The genome of AAV contains two open reading frames (ORF) responsible for *rep* and *cap*, and is flanked by palindromic inverted terminal repeat elements (ITR). The *rep* gene positioned in the left ORF encodes the four Rep proteins (Rep78, Rep68, Rep52, and Rep40) that play important roles in several steps of the viral life cycle such as viral DNA replication, transcriptional control, site-specific integration, accumulation of single-stranded genomes and viral packaging. On the other hands, the *cap* gene located in the right ORF produces three different capsid proteins of VP1, VP2 and VP3 with the ratio of 1:1:8. These structural proteins are generated from a single gene, but their translation is initiated at different start codons. As a result, these structural proteins differ in size such as 87, 73 and 62 kDa, respectively, and they have the identical C-termini, but possess unique N-termini. The ITRs at both ends of the AAV genome have at least three essential functions such as primer for a new DNA strand synthesis, a Rep binding site (RBS) for Rep78 and Rep68 containing the helicase and the strand-specific endonuclease activities, and a terminal resolution site (TRS) that is identical to a sequence in human chromosome 19 and required for the site-specific integration of the viral genome. In addition, the most attribute of recombinant AAV production is that only these 145 base ITRs are required in *cis* to produce recombinant AAV and all other viral sequences can be supplied in *trans* (7, 8).

4. AAV SEROTYPES

To date eight major primate serotypes (AAV1 - AAV8) and many other serotypes have been identified and the majority of them have been isolated as contaminants of adenoviral cultures. AAV2, 3 and 5 were isolated from human clinical specimens (AAV5 isolated from a condylomatous lesion), whereas AAV1 and 4 were identified from simian sources. AAV7 and 8 were cloned from rhesus monkey and AAV6 is considered as the recombination of AAV2 and AAV1. Most of them are highly homologous, whereas AAV4 and AAV5 are quite different from the other serotypes. Main differences are located on the capsid proteins, which result in distinct tropism of AAV4 and 5. Recently the primary attachment receptors for AAV4 and 5 have been identified as alpha2-3-O-linked or alpha2-3-N-linked sialic acids, respectively, and the co-receptor for AAV5 was found as the platelet-derived growth factor receptor (PDGFR). AAV2 and AAV3 are believed to share HSPG as the primary receptor, however, the co-receptor(s) for AAV4 and the receptors for AAV1 and AAV6-8 remain to be identified. As shown in Table 1, many in vivo studies have clearly demonstrated that the various AAV serotypes show distinct tissue or cell tropisms. When the tropism of AAV serotypes in human cancer cell lines has been tested, AAV2 was the most efficient serotype in most of the tested tumor cells (4, 5). Because of the widely expressed heparin sulfate in many different tissues, the HSPG binding explained the broad range of cell specificity of AAV2 infection. Therefore, AAV2 vector has been mostly employed for the current gene therapy research and has also been used for most of the clinical trials.

5. AAV VECTOR DEVELOPMENT

The key steps to achieve success for gene therapy are to develop an ideal gene delivery vector tailored for certain disease. Somia and Verma summarized the seven characteristics that an ideal delivery vector should have as follows; 1) simple to produce; 2) sustained production or regulated expression of the target gene; 3) no immunogenecity; 4) tissue specificity; 5) size capacity; 6) integration or replication and segregation; 7) infection of dividing and nondividing cells (9). To date many viral and nonviral gene delivery vectors have been developed and several viral vectors including retrovirus, adenovirus, AAV, lentivirus and herpes simplex virus are widely studied for gene therapy. Among these vectors, the AAV vector is one of those that are closest to the ideal vector although there are a few concerns about using AAV vectors, the limited capacity of therapeutic gene, the requirement to improve mass-production and purification, and the presence of pre-existing immunity to AAV in humans. Therefore, over the past years intensive studies have been carried out to improve gene delivery by a capsid modification for tissue-specific tropism as well as novel strategies to increase transgene expression. Three major approaches have been exploited to modify the surface of AAV particles and to improve therapeutic gene expression: receptor targeting, mixed capsid in the viral particle, or self complementary vectors.

5.1. Receptor targeting and mixed capsid vector

Two prominent strategies of chemical cross-linked bifunctional antibodies and capsid gene modifications have been used to improve AAV receptor targeting. Ponnazhagan et al employed the high affinity biotin-avidin interaction to crosslink purified targeting ligands to biotinylated rAAV2, which resulted in greatly enhanced transduction in the ligand-targeted cells (10). Recently, Stachler et al generated RGD-modified AAV1, which resulted in success of targeted gene delivery to newly formed capillaries in tumors when tumor bearing mice were intravenously administered with it (11). Many other approaches for genetic modification of AAV capsid proteins have been attempted based on the following strategies prior to complete determination of the atomic structure for AAV: 1) sequence alignment of AAV2 with other parvoviruses obtaining known crystal structure; 2) insertional mutageneses of the entire AAV2 capsid genome in a random manner; 3) identification of peptide regions in AAV2 capsid responsible for immune response by incubation of AAV2 neutralizing serum with AAV2 capsid peptide pools. However, some genetic modifications of capsid can interfere even with proper packaging of virus particle, or with the stability of the virion particle, which may result in loss of function. Therefore, a successful modification of AAV capsid requires an optimal three-dimensional fit of each inserted ligand. As a solution of these problems, Hauck et al systematically exchanged capsid domains between AAV1 and AAV2 to identify responsible regions on the AAV1 capsid for transduction in skeletal muscle (12). This approach demonstrated the importance of this type of strategy to develop a novel capsid for tissue-specific tropism. Furthermore, the amino acid sequences of AAV serotypes are highly homologous each other, it is possible to form a viral particle from capsid subunits of different serotypes to generate mixed capsid vectors. Rabinowitz et al mixed capsid genes of AAV1 - AAV5 to generate mixed capsid for a novel tissue-specific tropism and the other groups performed similar mixing experiments using newly identified AAV serotypes to develop novel serotypes with unique features of tropism (13-15).

5.2. Self-complementary vectors

When AAV with a single-stranded genome enters the cell, the second-strand synthesis is the rate-limiting step for efficient transduction, which often results in poor transduction. Lately, several groups reported that DNA of less than half the size of the wild type AAV genome can be packaged as a dimer or a diploid monomer in AAV viral particle (16, 17). Therefore, McCarty et al developed a novel double-stranded AAV vector, so called self-complementary vector (scAAV) for improved transduction as well as enhanced expression of transgene. They demonstrated that scAAV increased in vitro transduction efficiency by 5 - 140-fold over conventional rAAV vector. Moreover, their in vivo delivery of scAAV/mEpo vectors into mouse liver resulted in greatly faster and higher transgene expression than the full-length single-stranded DNA vector. Since most of cancer gene therapies require faster and stronger expression of therapeutic gene, scAAV vectors will be extremely useful to be exploited to develop an AAV vectors for rapid and high transgene expression in clinical applications (18, 19).

Table 2. Gene therapy Clinical Applications for Treatment of Various Diseases (1)

Indications	Gene Therapy Clinical Trials	
	Number	%
Cancer diseases	797	66.9
Gene marking	50	4.2
Healthy volunteers	19	1.6
Infectious diseases	78	6.5
Monogenic diseases	102	8.6
Others	40	3.4
Vascular diseases	106	8.9
Total	1192	

6. AAV VECTORS IN CANCER GENE THERAPY

Previously, AAV2 was suggestive of having tumor-suppressive and anti-proliferative properties. Several groups have shown that AAV2 induces apoptosis by Rep78-mediated cell death and disruption of the cell cycle. On the other hand, Raj *et al* demonstrated that AAV2 selectively induces apoptosis particularly in the cells that lack active p53, and explained that the hairpin structures at both ends of single-stranded AAV genome elicit a DNA damage response that leads to cell death in the absence of active p53. They also showed that AAV inhibited tumor growth in mice (20-22). Recently, preliminary data from clinical trials for treatment of hemophilia and CF verified the safety of rAAV gene delivery vectors, which can be suggestive of rAAV as an alternative to more frequently used adenoviruses and retrovirus-based vector for human cancer gene therapy. Previous studies have demonstrated that rAAV can mediate *in vitro* and *in vivo* gene transfer to various human cancer cell lines as well as to solid tumors in a hepatoma animal model with great transduction efficiency. Moreover, recent study reported that rAAV showed higher *in vivo* transduction efficiency in gliomas than adenoviral vectors (23–28). Park *et al*, Hacker *et al* and Lee *et al* have also suggested that AAV2 with the highest transduction efficiency in various human cancer cell lines is the most promising gene delivery vector for cancer gene therapy (4, 5, 19). In this review, antiangiogenesis therapy, immunotherapy and suicide gene therapy will be mainly summarized.

6.1. Anti-angiogenesis therapy

The establishment of an angiogenic requirement for tumor growth and metastasis led to develop anti-angiogenic therapies, ranging from suppressed expression of angiogenic molecules, through overexpression of antiangiogenic factor. Therefore, in vivo targeting the vasculature in solid tumors using anti-angiogenesis strategy has been proven to be successful in treatment of various cancers (29-37). Davidoff et al demonstrated the prominent antitumor efficacy using an AAV/a soluble, truncated form of the VEGF receptor-2 (Flk-1) vector in murine models of pediatric kidney tumor (29). Ma *et al* used an AAV/angiostatin vector for intratumoral or intramuscular gene therapy of malignant brain tumor in a rat model and showed the increased survival rate (30, 31). An AAV/endostatin vector was also examined to treat pancreatic cancer and liver metastasis by intraportal injection, which resulted in some success of anti-cancer effectiveness (32). Recently, Ponnazhagan delivered both of endostatin and angiostatin in a single AAV vector and demonstrated synergic protective efficacy in a mouse tumor xenograft model (33). Zacchigna et al demonstrated that AAV/Timp1 transduction significantly retarded endothelial cell migration, reduced the invasion of a Matrigel barrier and strongly inhibited angiogenesis in Kaposi's sarcoma engrafted nude mice (34). An AAV/a mutant endostatin (P124A), a soluble VEGFR1/R2, or antisense VEGF-A also showed the remarkable efficacy of anti-angiogenesis, and indicated a potential gene therapy for treating cancers (35-38).

6.2. Immunotherapy

Since the immunotherapy has offered great promises for treatment of cancers, many approaches have been exploited to use AAV vectors to deliver genes to stimulate the immune response against tumors (39-47). Mohr *et al* demonstrated that the tumor growth in the mice transduced with an AAV2/TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) vector was greatly suppressed (41). Liu *et al* explored to develop a potential vaccine using a rAAV vector and showed that tumor cells or dendritic cells (DC) infected with an AAV vector induced very strong cytotoxic T-lymphocyte (CTL) response to tumor cells (42). An AAV/a soluble TRAIL vector used for transduction of tumor also resulted in a significant inhibition of tumor growth and increased survival rate (43, 44). Among many tested cytokines interferon (IFN) has been shown to inhibit tumor cell proliferation or modulate immune responses with great success. Streck *et al* demonstrated that liver-targeted delivery of AAV containing human IFN-beta in murine tumor models resulted in both of tumoricidal effect as well as antitumor efficacy through anti-angiogenesis (45, 46).

6.3. Suicide gene therapy

Several groups demonstrated that AAV-mediated delivery of the herpes simplex virus thymidine kinase (HSV-tk) selectively killed cancer cells in a mouse model with alpha-fetoprotein-positive hepatocellular carcinoma cells, a glioma model or a human oral squamous cell carcinoma. A bystander effect was also observed with the following administration of ganciclovir (GCV) (47-50).

7. CONCLUSION AND FUTURE PROSPECTS

Until recently, among 1,192 clinical trials about 67% is for treating various cancer patients as shown in Table 2 (1). It is very likely that an anti-cancer drug can be the first approved gene therapy medicine that be released worldwide. Although AAV-mediated gene delivery vectors are currently used only about 4% of total clinical trials (See Table 3), AAV has many prominent features as an ideal gene delivery vector, particularly safety, and it has also been greatly improved to be suitable for caner gene therapy as reviewed in this article.

Table 3. Gene delivery vectors currently used in clinical applications (1)

Vector	Gene Therapy Clinical Trials	
	Number	%
Adeno-associated virus	40	3.4
Adenovirus	301	25.3
Adenovirus + Retrovirus	3	0.3
Flavivirus	5	0.4
Gene gun	5	0.4
Herpes simplex virus	40	3.4
Lentivirus	6	0.5
Lipofection	99	8.3
Listeria monocytogenes	1	0.1
Measles virus	3	0.3
Naked/Plasmid DNA	205	17.2
Naked/Plasmid DNA + Adenovirus	1	0.1
Newcastle disease virus	1	0.1
Poliovirus	1	0.1
Poxvirus	59	4.9
Poxvirus + Vaccinia virus	23	1.9
Recombinant Poxvirus	1	0.1
Retrovirus	285	23.9
RNA transfer	15	1.3
Saccharomyces cerevisiae	2	0.2
Salmonella typhimurium	2	0.2
Semliki forest virus	1	0.1
Simian virus 40	1	0.1
Vaccinia virus	55	4.6
Unknown	37	3.1
Total	1192	

8. ACKNOWLEDGEMENTS

This work was supported by a grant from the Ministry of Science and Technology (M10534040005-05N3404-00511), and partly supported by the Chungbuk Pioneering Bio-industry R&D Grant, Chungbuk, Korea.

9. REFERENCES

1. The Journal of Gene Medicine, John Wiley and Sons, Ltd: http://www.wiley.co.uk/genmed/clinical (2006)
2. Peng Z. The Mechanism, Safety and Efficacy of Gendicine: The 9th American Society Gene Therapy Annual Meeting, Pre-meeting sessions, 001:Gene Therapy From Around The Globe (2006)
3. Warrington KH & RW Herzog: Treatment of human disease by adeno-associated viral gene transfer. *Hum Genet* 119, 571-603 (2006)
4. Hacker UT, L Wingenfeld, DM Kofler, NK Schuhmann, S Lutz, T Herold, SB King, FM Gerner, L Perabo, J Rabinowitz, DM McCarty, RJ Samulski, M Hallek & H Büning: Adeno-associated virus serotypes 1 to 5 mediated tumor cell directed gene transfer and improvement of transduction efficiency. *J Gene Med* 7, 1429-1438 (2005)
5. Park K, YJ Kim, DK Kim, YH Cho, JK Kang, SY Hwang, HJ Kim, JH Lee, SH Cho, SY Yi, SK Kim & EJ Park: Transduction Efficiency Screening for 7 Different AAV Serotypes in 12 Human Cancer Cell Lines and 2 Xenograft Animal Models. *Mol Therapy* 13 (Supplement 1), S368, #955 (2006)
6. Kotin RM, RM Linden & KI Berns: Characterization of a preferred site on human chromosome 19q for integration of adeno-associated virus DNA by non-homologous recombination. *EMBO J* 11, 5071-5078 (1992)
7. Grimm D & MA Kay: From Virus Evolution to Vector Revolution: Use of Naturally Occurring Serotypes of Adeno-associated Virus as Novel Vectors for Human Gene Therapy. *Current Gene Therapy* 3, 281-304 (2003)
8. Xie Q, W Bu, S Bhatia, J Hare, T Somasundaram, A Azzi & MS Chapman: The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proc Natl Acad Sci USA* 99, 10405-10410 (2002)
9. Somia N & IM Verma: Gene therapy: trials and tribulations. *Nature Rev Genet* 1, 91-99 (2000)
10. Ponnazhagan S, G Mahedra, S Kumar, JA Thomson & Jr M Castillas: Conjugate-based targeting of recombinant adeno-associated virus type 2 vectors by using avidin-linked ligands. *J Virol* 76, 12900-12907 (2002)
11. Stachler MD & JS Bartlett: Efficient Targeting of AAV Vectors to Tumor-Associated Angiogenic Vasculature Following Intravascular Delivery. *Mol Therapy* 15 (Supplement 1), S147, #383 (2007)
12. Hauck B & W Xiao: Characterization of tissue tropism determinants of adeno-associated virus type 1. *J Virol* 77, 2768-2774 (2003)
13. Rabinowitz JE, DE Bowles, SM Faust, JG Ledford, SE Cunningham & RJ Samulski: Cross-dressing the virion: the transcapsidation of adeno-associated virus serotypes functionally defines subgroups. *J Virol* 78: 4412-4432 (2004)
14. Grimm D, JS Lee, TA Storm & MA Kay: Molecular evolution of adeno-associated viral (AAV) vectors via DNA family shuffling of primate and non-primate serotypes. *Mol Therapy* 13 (Supplement 1), S287, #742 (2006)
15. Li W & RJ Samulski: Directed evolution of adeno-associated virus (AAV) by DNA shuffling yields enhanced gene delivery vectors. *Mol Therapy* 13 (Supplement 1), S287, #743 (2006)
16. Dong JY, PD Fan & RA Frizzell: Quantitative analysis of the packaging capacity of recombinant adeno-associated virus. *Hum Gene Ther* 7, 2101-2112 (1996)
17. Hirata RK & DW Russell: Design and packaging of adeno-associated virus gene targeting vectors. *J Virol* 74, 4612-4620 (2000)
18. McCarty DM, PE Monahan & RJ Samulski: Self-complementary recombinant adeno-associated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Therapy* 8, 1248-1254 (2001)
19. Lee HS, OK Shin, SJ Kim, WI Lee, S Jeong, K Park, H Choe & H Lee: Efficient gene expression by self-complementary adeno-associated virus serotype 2 and 5 in various human cancer cells. *Oncology Reports* 18, 611-616 (2007)
20. Batchu RB, MA Shammas, JY Wang & NC Munshi: Interaction of Adeno-associated Virus Rep78 with p53: Implications in Growth Inhibition. *Cancer Research* 59, 3592-3595 (1999)
21. Schmidt M, S Afione & RM Kotin: Adeno-Associated Virus Type 2 Rep78 Induces Apoptosis through Caspase Activation Independently of p53. *J Virol* 74, 9441-9450 (2000)
22. Raj K, P Ogston & P Beard: Virus-mediated killing of cells that lack p53 activity. *Nature* 412, 914-917 (2001)
23. High KA & E Theodore: AAV-mediated gene transfer for hemophilia. *Am Clin Climatol Asso* 114, 337-351 (2003)
24. Wagner JA, IB Nepomuceno, AH Messner, ML Moran, EP Batson, S Dimiceli, BW Brown, JK Desch, AM Norbash, CK Conrad, WB Guggino, TR Flotte, JJ Wine, BJ

Carter, TC Reynolds, RB Moss & P Gardner: A Phase II, double-blind, randomized, placebo-controlled clinical trial of tgAAVCF using maxillary sinus delivery in patients with cystic fibrosis with antrostomies. *Hum Gene Ther* 13, 1349-1359 (2002)
25. Hoerer M, C Bogedain, U Scheer, C Heberger, S Steyrer, A Burger & G Maass: The use of recombinant adeno-associated viral vectors for the transduction of epithelial tumor cells. *Int J Immunopharmacol* 19, 473-479 (1997)
26. Maass G, C Bogedain, U Scheer, D Michl, M Hörer, M Braun-Falco, M Volkenandt, D Schadendorf, CM Wendtner, EL Winnacker, RM Kotin & M Hallek: Recombinant adeno-associated virus for the generation of autologous, gene-modified tumor vaccines: evidence for a high transduction efficiency into primary epithelial cancer cells. *Hum Gene Therapy* 9, 1049-1059 (1998)
27. Peng D, C Qian, Y Sun, MA Barajas & J Prieto: Transduction of hepatocellular carcinoma (HCC) using recombinant adeno-associated virus (rAAV): *in vitro* and *in vivo* effects of genotoxic agents. *J Hepatol* 32, 975-985 (2000)
28. Enger PO, F Thorsen, PE Lønning, R Bjerkvig & F Hoover: Adeno-associated viral vectors penetrate human solid tumor tissue *in vivo* more effectively than adenoviral vectors. *Hum Gene Therapy* 13, 1115-1125 (2002)
29. Davidoff AM, AC Nathwani, WW Spurbeck, CYC Ng, J Zhou & EF Vanin: rAAV-mediated Long-term Liver-generated Expression of an Angiogenesis Inhibitor Can Restrict Renal Tumor Growth in Mice[1]. *Cancer Res* 62, 3077-3083 (2002)
30. Ma HI, SZ Lin, YH Chiang, J Li, SL Chen, YP Tsao & X Xiao: Intratumoral gene therapy of malignant brain tumor in a rat model with angiostatin delivered by adeno-associated viral (AAV) vector. *Gene Therapy* 9, 2-11 (2002)
31. Ma HI, P Guo, J Li, SZ Lin, YH Chiang, X Xiao & SY Cheng: Suppression of intracranial human glioma growth after intramuscular administration of an adeno-associated viral vector expressing angiostatin. *Cancer Res* 62, 756-763 (2002)
32. Noro T, K Miyake, N Suzuki-Miyake, T Igarashi, E Uchida, T Misawa, Y Yamazaki & T Shimada: Adeno-Associated Viral Vector-Mediated Expression of Endostatin Inhibits Tumor Growth and Metastasis in an Orthotropic Pancreatic Cancer Model in Hamsters. *Cancer Res* 64, 7486-7490 (2004)
33. Ponnazhagan S, G Mahendra, S Kumar, DR Shaw, CR Stockard, WE Grizzle & S Meleth: Adeno-Associated Virus 2-Mediated Antiangiogenesis Cancer Gene Therapy: Long-Term Efficacy of a Vector Encoding Angiostatin and Endostatin over Vectors Encoding a Single Factor. *Cancer Res* 64, 1781-1787 (2004)
34. Zacchigna S, L Zentilin, M Morini, R Dell'Eva, DM Noonan, A Albini & M Giacca: AAV-mediated gene transfer of tissue inhibitor of metalloproteinases-1 inhibits vascular tumor growth and angiogenesis *in vivo*. *Cancer Gene Therapy* 11, 73-80 (2004)
35. Davidoff AM, CYC Ng, Y Zhang, CJ Streck, SJ Mabry, SH Barton, T Baudino, J Zhou, RS Kerbel, EF Vanin & AC Nathwani: Careful Decoy Receptor Titering is Required to Inhibit Tumor Angiogenesis While Avoiding Adversely Altering VEGF Bioavailability. *Mol Therapy* 11(2), 300-310 (2005)
36. Subramanian IV, R Ghebre & S Ramakrishnan: Adeno-associated virus-mediated delivery of a mutant endostatin suppresses ovarian carcinoma growth in mice. *Gene Therapy* 12, 30-38 (2005)
37. Park K, MY Ahn, SY Hwang, YH Cho, BS Kang, YJ Kim, SH Cho, YM Kim, SY Yi & YI Lee: Cancer Gene Therapeutic Approaches Using an AAV-Mediated Gene Delivery System Containing Antisense VEGF-A cDNA . *Mol Therapy* 11, S278, #717 (2005)
38. Harding TC, AS Lalani, BN Robert, S Yendluri, B Luan, KE Koprivnikar, M Gonzalez-Edick, G Huan-Tu, R Musterer, MJ VanRoey, T Ozawa, RA LeCouter, D Deen, PJ Dickinson & K Jooss: AAV Serotype 8-Mediated Gene Delivery of a Soluble VEGF Receptor to the CNS for the Treatment of Glioblastoma. *Mol Therapy* 13, 956-966 (2006)
39. Paul D, MH Qazilbash, K Song, H Xu, BK Sinha, J Liu & KH Cowan: Construction of a recombinant adeno-associated virus(rAAV) vector expressing murine imterleukin-12 (IL-12). *Cancer Gene Therapy* 7, 308-315 (2000)
40. Veldwijk MR, S Fruehauf, B Schiedlmeier, JA Kleinschmidt & WJ Zeller: Differential expression of a recombinant adeno-associated virus 2 vector in human $CD34^+$ cells and breast cancer cells. *Cancer Gene Therapy* 7, 597-604 (2000)
41. Mohr A, G Henderson, L Dudus, I Herr, T Kuerschner, KM Debatin, H Weiher, KJ Fisher & RM Zwacka: AAV-encoded expression of TRAIL in experimental human colorectal cancer leads to tumor regression. *Gene Therapy* 11, 534-543 (2004)
42. Liu Y, M Chiriva-Internati, C You, R Luo, H You, CK Prasad, F Grizzi, E Cobos, VS Klimberg, H Kay, JL Mehta & PL Hermonat: Use and specificity of breast cancer antigen/milk protein BA46 for generating anti-self-cytotoxic T lymphocytes by recombinant adeno-associated virus-based gene loading of dendritic cells. *Cancer Gene Therapy* 12, 304-312 (2005)
43. Ma H, Y Liu, S Liu, R Xu & D Zheng: Oral Adeno-Associated Virus-sTRAIL Gene Therapy Suppresses Human Hepatocellular Carcinoma Growth in Mice. *Hepatology* 42, 1355-1363 (2005)
44. Shi J, D Zheng, Y Liu, MH Sham, P Tam, F Farzaneh & R Xu: Overexpression of soluble TRAIL Induces Apoptosis in Human Lung Adenocarcinoma and Inhibits Growth of Tumor Xenografts in Nude Mice. *Cancer Res* 65, 1687-1692 (2005)
45. Streck CJ, PV Dickson, CYC Ng, J Zhou, JT Gray, AC Nathwani & AM Davidoff: Adeno-Associated Virus Vector-Mediated Systemic Delivery of IFN-beta Combined with Low-Dose Cyclophosphamide Affects Tumor Regression in Murine Neuroblastoma Models. *Clin Cancer Res* 11, 6020-6029 (2005)
46. Streck CJ, PV Dickson, CYC Ng, J Zhou, MM Hall, JT Gray, AC Nathwani & AM Davidoff: Antitumor efficacy of AAV-mediated systemic delivery of interferon- beta. *Cancer Gene Therapy* 13, 99-106 (2006)
47. Su H, JC Chang, SM Xu & YW Kan: Selective killing of AFP-positive hepatocellular carcinoma cells by adeno-

associated virus transfer if the herpes simplex virus thymidine kinase gene. *Hum Gene Ther* 7, 463-470 (1996)
48. Surosky M Urabe, SG Godwin, SA McQuiston, GJ Kurtzman, K Ozawa & G Natsoulis: Adeno-associated virus Rep proteins target DNA sequences to a unique locus in the human genome. *J Virol* 71, 7951-7959 (1997)
49. Mizuno M, J Yoshida, P Colosi & G Kurtzman: Adeno-associated virus vector containing the herpes simplex virus thymidine kinase gene causes complete regression of intracerebrally implanted human glioma in mice, in conjunction with ganciclovir administration. *Jpn J Cancer* 89, 76-80 (1998)
50. Fukui T, Y Hayashi, H Kagami, N Yamamoto, H Fukuhara, I Tohnai, M Ueda, M Mizuno & J Yoshida: Suicide gene therapy for human oral squamous cell carcinoma cell lines with adeno-associated virus vector. *Oral Oncology* 37, 211-215 (2001)

Abbreviations: AAV: adeno-associated virus; scAAV: self-complementary vector

Key Words: AAV, Cancer gene therapy, Ideal vector, Review

Send Correspondence to: Keerang Park, Ph.D., Department of Biotechnology, Juseong Gene Therapy R&D Center, Juseong College, Chungbuk 363-794, Korea, Tel: 82-43-210-8462; Fax: 82-43-210-8465, E-mail: krpark@jsc.ac.kr

http://www.bioscience.org/current/vol13.htm