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Transplantation of a Novel Recombinant Adeno-Associated Virus (pAAV-HE1B19K-TE1A) Demonstrates Higher Anti-Tumor Effects in Tumor Cells

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Funds Collection G

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Background: Oncolytic viruses (OVs) can specifically infect and kill tumor cells. Adeno-associated virus (AAV) is a widely-studied OV. This study aimed to construct a tumor-targeted recombinant AAV using genetic engineering technology.

Material/Methods: The transgene plasmid pAAV-HE1B19K-TE1A was constructed with 4 genes (hTERT, E1A, HKII, and E1B19K) and co-transfected with pAAV-RC and pHelper to tumor cells (HepG2, A549, BGC-803) and normal cells (HUVEC). rAAV was verified with fluorescence microscopy. Quantitative PCR (qPCR) assay was used to test the titer of rAAV in each cell line. Apoptosis was analyzed using qPCR and Western blot assay. MTT was used to detect the effect of rAAV on cell viability.

Results: The pAAV-HE1B19K-TE1A transgene plasmid was successfully structured. pAAV-HE1B19K-TE1A was highly expressed in all tumor cells. The titers of pAAV-HE1B19K-TE1A in HepG2, A549, and BGC-803 were 7.4×10^7, 1.4×10^8, and 1.1×10^8 gc/μl, respectively. pAAV-HE1B19K-TE1A significantly decreased cell viability of tumor cells compared to that in HUVEC ($p<0.05$). pAAV-HE1B19K-TE1A remarkably triggered cleaved caspase 3 (C-caspase 3) activity in tumor cells compared to that in untransfected tumor cells ($p<0.05$). pAAV-HE1B19K-TE1A significantly induced release of cytochrome C (Cyto C) in tumor cells compared to that in untransfected tumor cells ($p<0.05$). pAAV-HE1B19K-TE1A demonstrated no toxicity to vital tissues of animals.

Conclusions: Tumor-targeted rAAV was successfully produced using the Helper-free system with recombinant plasmid, demonstrating high efficacy in decreasing viability of tumor cells without adverse effects on normal cells.

MeSH Keywords: **Bioengineering • Dependovirus • Tumor Cells, Cultured**

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Background

Cancer is the second leading cause of death in the world after cardiovascular diseases. Oncolytic viruses (OVs) are replication-defective viruses that can specifically recognize and infect tumor cells, eventually causing cell swelling and destroying tumor cells [1]. Adeno-associated virus (AAV) is one of the most widely applied OVs. AAV, as an un-enveloped single-stranded DNA virus, belongs to the *parvoviridae* [2]. The diameter of the virus particles is about 20–26 nm [3]. The genome is involved in the inverted terminal repeat sequence (*ITR*), capsid (*Cap*) gene sequence, and Rep gene sequence [4,5]. The Cap gene encodes the capsid protein of virus, and *ITR* and *Rep* play critical roles in virus replication, packaging, and integration [6]. AAV is regarded as one of the most promising tools for gene therapy because of its good safety, wide range of host cells, and low immunogenicity. AAV relies on helper viruses, which include E1A, E1B, E2A, E4, and VA RNA genes, to complete its life cycle [7]. E1A can upregulate AAV *Rep* and *Cap* genes. The E1B gene can promote the propagation of AAV, especially for E1B19K [8].

Human telomere is a special structure composed of non-coding repeat sequence (TTAGGG) and binding proteins at the end of the chromosome [9,10]. These regions are gradually shortened in cell division, resulting in the loss of basic genetic information and eventually causing cell death [11,12]. Telomerase is a RNA-dependent reverse-transcriptional DNA polymerase that is responsible for telomere elongation [13]. Telomerase is mainly composed of human telomere reverse transcriptase (hTERT), telomere RNA (TR), and telomerase-associated proteins [14,15]. Telomerase can use its own RNA as a template to transcribe and synthesize telomere DNA, which is also added to the end of the chromosome, maintaining the integrity and stability of chromosomes [16]. The hTERT gene is only highly expressed in most tumors and immortalized cell lines, while expression of the hTERT gene is turned off in mature and differentiated normal cells [16]. Therefore, the telomerase gene is specifically transcribed and expressed in most tumor cells [16]. It has been reported that the activation of telomerase can enable tumor cells to gain the ability of unlimited proliferation, which is a key step in tumor occurrence and development [17]. hTERT is the main regulatory subunit of telomerase activity, the high expression of which is caused by complete activation of its promoter in tumor cells. Therefore, hTERT promoter is considered to be tumor-specific [18].

Hexokinase (HK) is the first enzyme in glycolysis, and is also the rate-limiting enzyme in tumor tissues [19]. With the increase of its expression and activity in tumor tissues, the tumor tissues can still obtain enough energy under the condition of hypoxia [19,20]. Many intermediate products of glycolysis can be used by tumor cells to synthesize proteins, nucleic acids, and lipids, thus providing necessary materials for the growth and proliferation of tumor cells [21]. HK II is an extensively studied glycolytic enzyme that plays a dual role in tumors: one is to upregulate the level of glycolysis, and the other is to bind to volt-dependent anion channel (VDAC) in the outer membrane of mitochondria to inhibit apoptosis [22,23]. Therefore, HK II promoter demonstrates tumor specificity.

In this study, we synthesized a tumor-targeted recombined adeno-associated virus (rAAV)-carrying targeting gene using the AAV Helper-free system and evaluated its anti-tumor effects.

Material and Methods

Materials

Plasmid pAAV-IRES-ZsGreen was purchased from Addgene (Cambridge, MA, USA). DNA and RNA extraction kits, DNA fragment gel extraction kit, restriction enzymes, and *E. coli* DH5α were purchased from TaKaRa (Dalian, China). Trypsin, fetal bovine serum (FBS), and Dulbecco's modified Eagle's medium (DMEM) were obtained from Gibco BRL Co. (Grand Island, NY, USA). All primers were synthesized by Thermo Fisher (Hudson, NH, USA). Cell lines, including HepG2, A549, BGC-803, and human umbilical vein endothelial cells (HUVEC), were purchased from ATCC (Manassas, VA, USA). C57b1/6 mice, age 6–8 weeks, were purchased from Ensiweier Biotech (Chongqing, China). Anti-Adenovirus Type 5 E1A and Anti-BNIP3L/NIX antibodies were purchased from Abcam Biotech (Cambridge, MA, USA).

AAV transgene plasmid construction

The core promoter of HKII (294 bp) and E1B19K (528 bp) and the core promoter of hTERT (370 bp) and E1A (984 bp) were reported on NCBI. The elements were synthesized in tandem and cloned into *Mlu1* and *BamH1* by GenScript, and then were inserted into pUC57 for delivery. The transgene plasmid pAAV-HE1B19K-TE1A was constructed from pAAV-IRES-ZsGreen by inserting a fragment of HE1B19K-TE1A between *Mlu1* and *BamH1* sites. pAAV-IRES-ZsGreen was digested with *Mlu1* and *BamH1*. The large fragment (4723 bp) containing the plasmid backbone was purified. This fragment was ligated to the HE1B19K-TE1A (2353 bp) fragment isolated from pUC57-HE1B19K-TE1A. The new transgene plasmid was named pAAV-HE1B19K-TE1A.

Helper-free production of rAAV

HepG2 and HUVEC cells were cultured in DMEM supplemented with 10% FBS and 10% penicillin-streptomycin. A549 was cultured with F12K medium containing 10% FBS and 10% penicillin-streptomycin. BGC-803 was cultured in RPMI-1640 medium supplemented with 10% FBS and 10% penicillin-streptomycin. All of the above cells were seeded in 60-mm dishes and

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Table 1. Primers for the PCR assay.

Gene		Sequences
E1B19K	Forward	5'-GAT AAA TGG AGC GAA GAA ACC C-3'
	Reverse	5'-CGG ACG GAA GAC AAC AGT AGC-3'
Caspase 3	Forward	5'-AGC ACT GGA ATG ACA TCT CGG T-3'
	Reverse	5'-ATG GCT CAG AAG CAC ACA AAC A-3'
Cytochrome C	Forward	5'-AAA GGG AGG CAA GCA CAA GA-3'
	Reverse	5'-GAT CAT TTT TGT TCC AGG GAT GTA-3'
HKII	Forward	5'-GCC TTT CCG TCC CAG CCT TTA GCC-3'
	Reverse	5'-GGA CTC CTG CGC CGG AGT TTC ATG-3'
GAPDH	Forward	5'-AGA TCC CTC CAA AAT CAA GTG G-3'
	Reverse	5'-GGC AGA GAT GAT GAC CCT TTT-3'

cultured at 37°C and in a 5% CO_2 atmosphere to a confluence of 60%–70%. At 2 h before transfection, all cells were cultured in medium containing FBS without antibody. Then, these cells were transiently co-transfected with rAAV plasmids pAAV-RC, pHelper, and pAAV-HE1B19K-TE1A at an equimolar ratio using Lip2000 as a DNA carrier agent. After 72 h, ZsGreen expression was visualized using direct fluorescence microscopy and all cells were harvested and lysed using the freeze-thaw method to obtain recombined pAAV-HE1B19K-TE1A (rAAV). Then, rAAV was used to infect cells again until the third viral generation. The isolation and purification procedure was conducted as described by Crosson et al. [24].

rAAV quantitative analysis

Titers of rAAV were determined by quantitative real-time PCR (qRT-PCR) using SYBR Green PCR master mix. In brief, the purified rAAV was treated with protease K at 50°C for 1 h, then 95°C for 29 min for inactivation. The number of genome copies of rAAV was detected using primers targeting the *ITR* gene (forward primer 5'-GGA ACCCCTAGTGATGGAGTT-3' and reverse primer 5'-CGGCCT CAGTGAGCGA-3'). qPCR was carried out under the following conditions: 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 10 s, and 60°C for 30 s. The qPCR curve of each sample was compared to the standard curve prepared from the dilution series of pAAV-IRES-ZsGreen.

Gene expression determined by PCR assay

qPCR was carried out to assess the expression of E1B19K, caspase-3 and cytochrome C (Cyto C). Total RNAs were isolated and complementary DNA (cDNA) was synthesized using the reverse transcription method. The conditions for PCR assay were: initial denaturation at 95°C for 5 min, 30 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 1 min, followed by final extension at 72°C for 10 min. The transcript level of the housekeeping gene GAPDH was detected to normalize expression of target genes. The expression levels of these 3 genes were analyzed using $2^{-\Delta\Delta CT}$ method [25]. The gene-specific primers for the reference gene and target genes, including E1B19K, caspase 3, cytochrome C, HKII, and GAPDH, are illustrated in Table 1. The primers were designed using the Primer 5 primer designing tool and synthesized by Western Biotech (Chongqing, China),

Protein expression determined by Western blot assay

Western blot analysis for cleaved caspase-3 (C-caspase 3) and cytochrome C were performed to analyze apoptosis levels of all cell lines. Total proteins were extracted from lysed cells and the concentration was determined using the BCA Protein Assay Kit (Cat. No. P0010, Beyotime Biotech, Shanghai, China) according to the protocol of the manufacturer. Samples were separated on 10% SDS-PAGE gel and electro-transferred onto PVDF membranes before blocking with 5% non-fat dry milk for 1 h at room temperature. Then, the PVDF membrane was incubated with rabbit anti-cleaved caspase 3 antibody (Cat. No. AC033, Beyotime Biotech, Shanghai, China), mouse anti-cytochrome C (Cat. No. sc-13156, Santa Cruz Biotech, Santa Cruz, CA, USA), and mouse anti-β-actin antibody (Cat. No. sc-8432, Santa Cruz Biotech) at room temperature for 30 min. After washing with PBS 3 times, PVDF membranes were incubated with secondary antibodies at room temperature for 2 h. After washing with BPS 3 times, the specific protein bands were visualized using an ECL kit. β-actin was used as the internal control to normalize the C-caspase 3 and cytochrome C expression.

Cell viability evaluation with MTT assay

MTT assay was carried out to detect cell survival and growth (cell viability). Cells were seeded onto a 96-well plate and we

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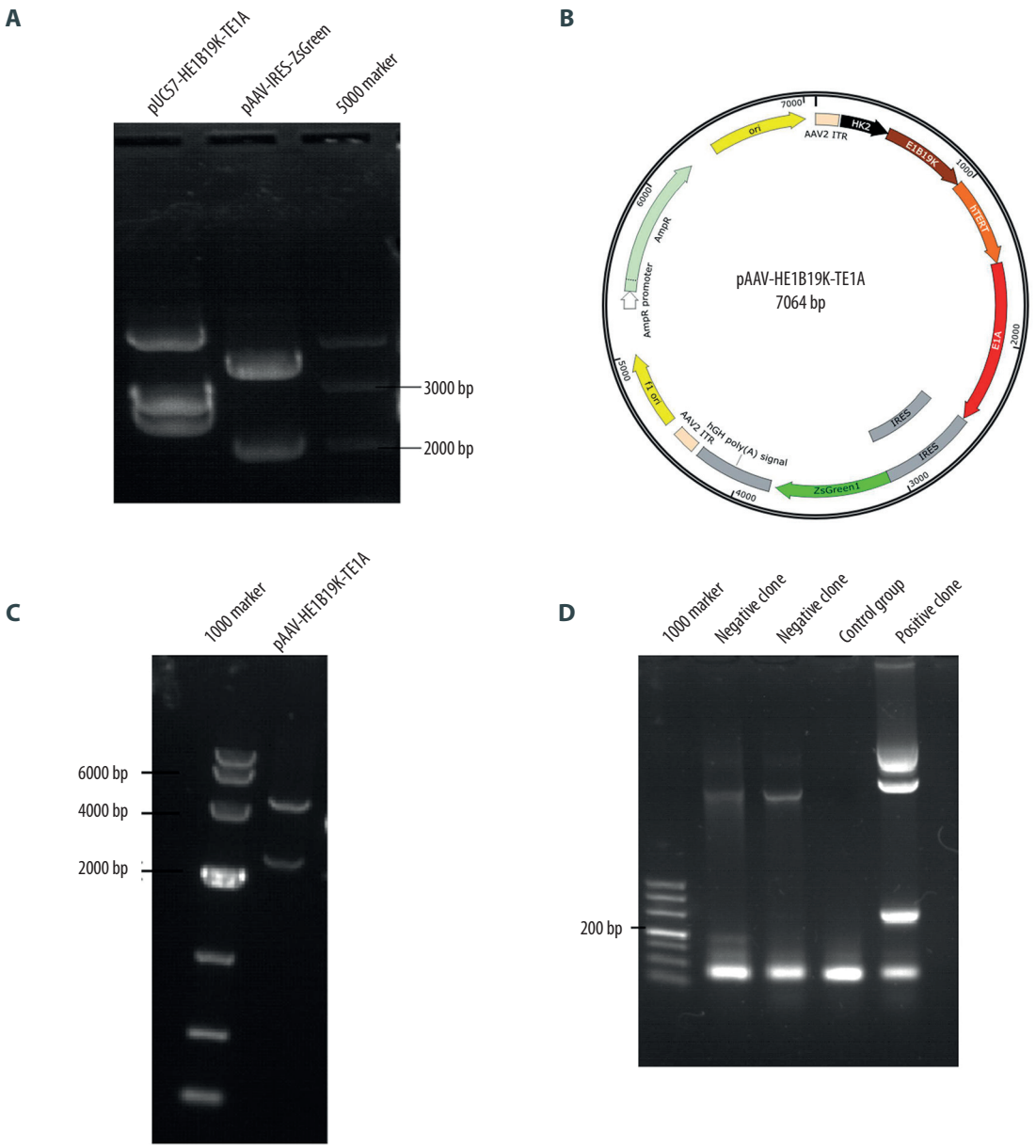

Figure 1. Synthesis of the new transgene plasmid pAAV-HE1B19K-TE1A. (**A**) Restriction digestion of pUC57-HE1B19K-TE1A and pAAV-IRES-ZsGreen with *Mlu1* and *BamH1*. (**B**) The structure of the pAAV-HE1B19K-TE1A. (**C**) Positive clones were verified by restriction digestion. (**D**) Positive clones were verified by PCR assay.

adjusted the density to 2×10^4 cells per well. After infecting with rAAV for 24 h and 72 h, each cell line was incubated with MTT solution (at final concentration of 5 mg/ml) for 4 h, and then the absorbance of cells in plates was detected using an ELISA reader at a wavelength of 595 nm.

Acute toxicity test

Acute toxicity testing was conducted to assess the general toxicity of rAAV. Total of 10 ml rAAV (10^7 infectious units) was injected into C57b1/6 male mice through the tail vein. Fourteen days after injection, the heart, liver, spleen, lung, and kidney tissues were collected to assess the safety of rAAV using hematoxylin-eosin (HE) staining.

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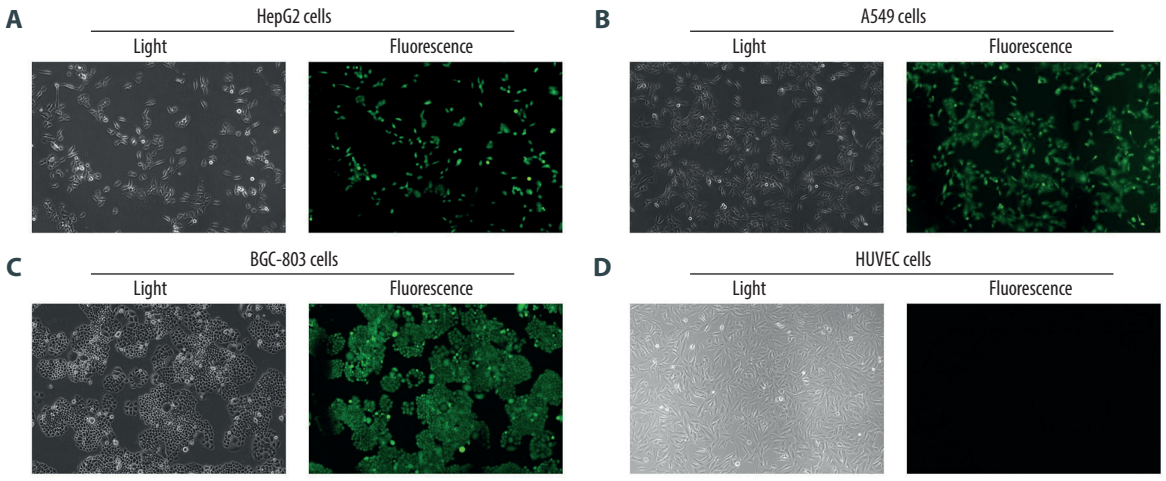

Figure 2. Determination for pAAV-HE1B19K-TE1A transfection in tumor cells. ZsGreen expression was observed in HepG2 (**A**), A549 (**B**), and BGC-803 (**C**), but not in HUVEC cells (**D**).

Statistical analysis

Data were analyzed with SPSS software 20.0 (SPSS, Inc., Chicago, IL, USA). Data were expressed as mean±standard deviation (SD) and were analyzed with Tukey's post hoc test validated by ANOVA to compare differences among groups. At least 6 replicates were carried out for every experiment or test. A p vale less than 0.05 was defined as a significant difference.

Results

pAAV-HE1B19K-TE1A transgene plasmid was successfully structured

The pAAV-IRES-ZsGreen and pUC57-HE1B19K-TE1A plasmids were digested with restriction endonuclease *Mlu1* and *BamH1* (Figure 1A). Through digestion, the fragment HE1B19K-TE1A and open-circular pAAV-IRES-ZsGreen plasmid were obtained (Figure 1A). The large fragment of digested pAAV-IRES-Green and small fragment of digested pUC57-HE1B19K-TE1A (HE1B19K-TE1A) were purified and then ligated to construct pAAV-HE1B19K-TE1A (Figure 1B). The positive clones were verified with restriction digestion (Figure 1C) and PCR assay (Figure 1D). The results showed that both restriction (Figure 1C) and PCR (Figure 1D) images demonstrated an obvious fragment (294 bp) in the positive clone, which was the HKII gene.

pAAV-HE1B19K-TE1A was highly expressed in tumor cells

At 72 h after transfection, the ZsGreen signal was obviously visible in A549 (Figure 2A), HepG2 (Figure 2B), and BGC-803 (Figure 2C), but without signals in HUVEC (Figure 2D). These results suggest that hTERT promoter is only highly expressed in tumor cells. After transfecting with pAAV-HE1B19K-TE1A, expression of E1B19K in tumor cells was significantly higher compared to that in the pAAV-IRES-Zs Green group (Figure 3, $p<0.05$). However, there were no obvious effects of pAAV-HE1B19K-TE1A transfection on E1B19K expression in normal HUVECs (Figure 3). These results suggest that both hTERT and HKII promoters are highly activated in tumor cells; therefore, E1B19K and E1A protein may play important roles in rAAV packaging.

Titers of pAAV-HE1B19K-TE1A

Due to the appropriate amplification plot of ITR for pAAV-IRES-ZsGreen (Figure 4A) and pAAV-HE1B19K-TE1A (Figure 4B) plasmid, the titers of pAAV-HE1B19K-TE1A were detected using qPCR with ITR primers. After collecting pAAV-HE1B19K-TE1A from lysed cells, HepG2, A549, and BGC-803 cells demonstrated 7.4×10^7, 1.4×10^8, and 1.1×10^8 gc/μl virus genome copies, respectively (Figure 4C).

pAAV-HE1B19K-TE1A reduced tumor cell viability

The inhibitory effects of pAAV-HE1B19K-TE1A on viability of tumor cells were determined with MTT assay. The pAAV-HE1B19K-TE1A infection demonstrated an obvious inhibitory effect on the cell viability of tumor cells (HepG2, A549, and BGC-803 cells) at 24 h (Figure 5A) and 48 h (Figure 5B) after transfection, in a time-dependent manner. However, no obvious inhibitory effect of pAAV-HE1B19K-TE1A on cell viability of the normal HUVEC cells was discovered (Figure 5).

pAAV-HE1B19K-TE1A triggered caspase 3 activity in tumor cells

To clarify the mechanism by which pAAV-HE1B19K-TE1A causes a decrease of cell viability in tumor cells, caspase 3 activity was

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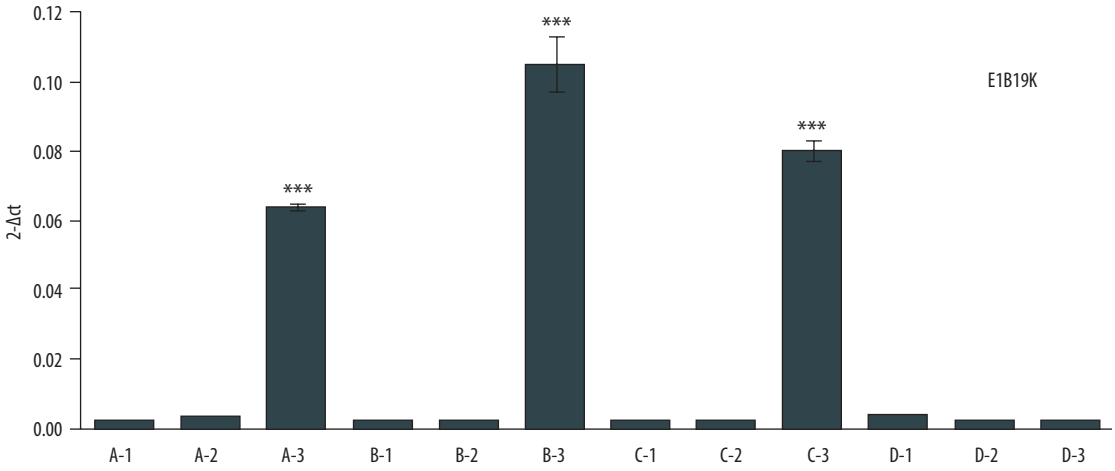

Figure 3. E1B19K expression in cells detected using qPCR assay. (A – HepG2 cells, B – A549 cells, C – BGC-803 cells, D – HUVEC cells. 1 – cells without transfection, 2 – cells transfected with pAAV-IRES-ZsGreen, pHelper, and pAAV-RC, 3 – cells transfected with pAAV-HE1B19K-TE1A, pHelper, and pAAV-RC). *** $p<0.0001$ *vs.* pAAV-IRES-ZsGreen group in HepG2, A549, or BGC-803 cells.

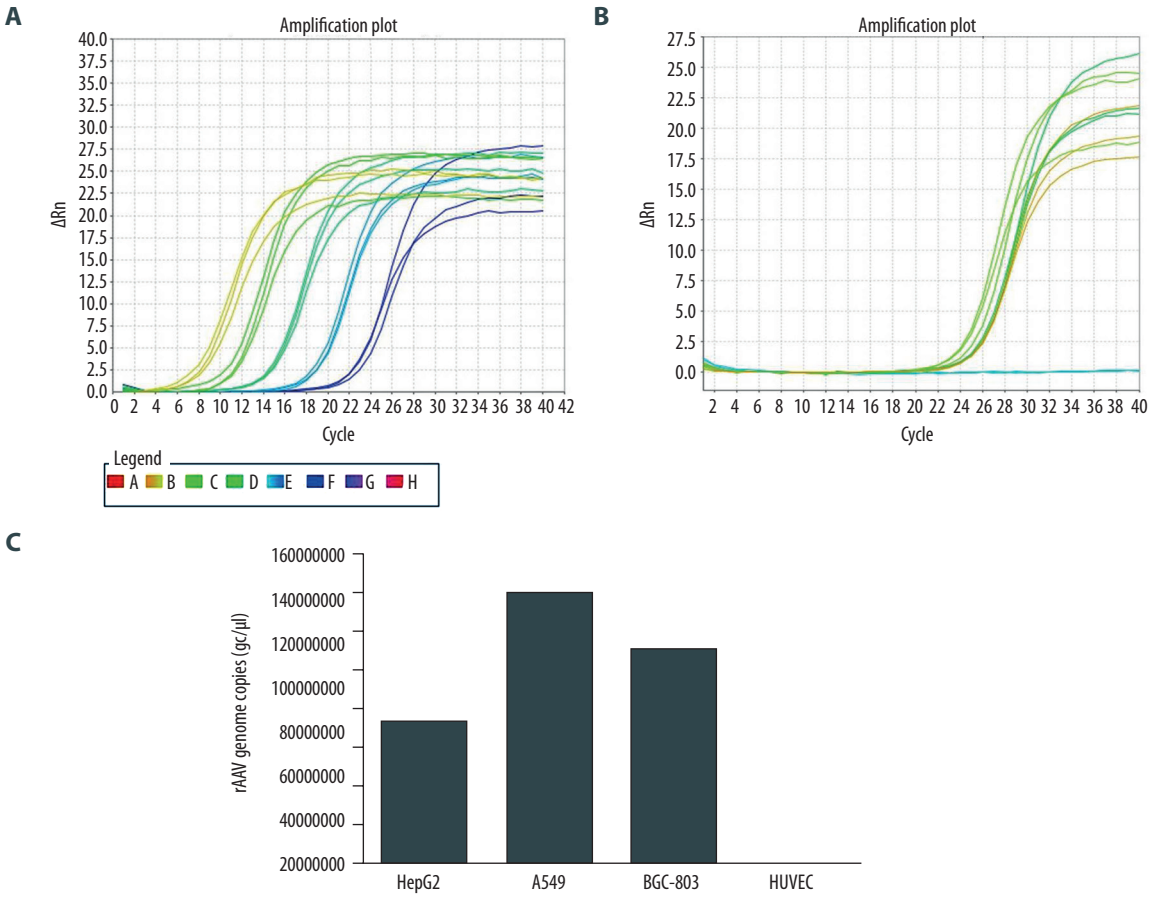

Figure 4. Titers of pAAV-HE1B19K-TE1A were detected using qPCR with ITR primers. (**A**) Standard amplification plot of ITR for plasmid pAAV-IRES-ZsGreen. (**B**) Amplification plot of ITR for pAAV-HE1B19K-TE1A. (**C**) pAAV-HE1B19K-TE1A genome copies in each cell line.

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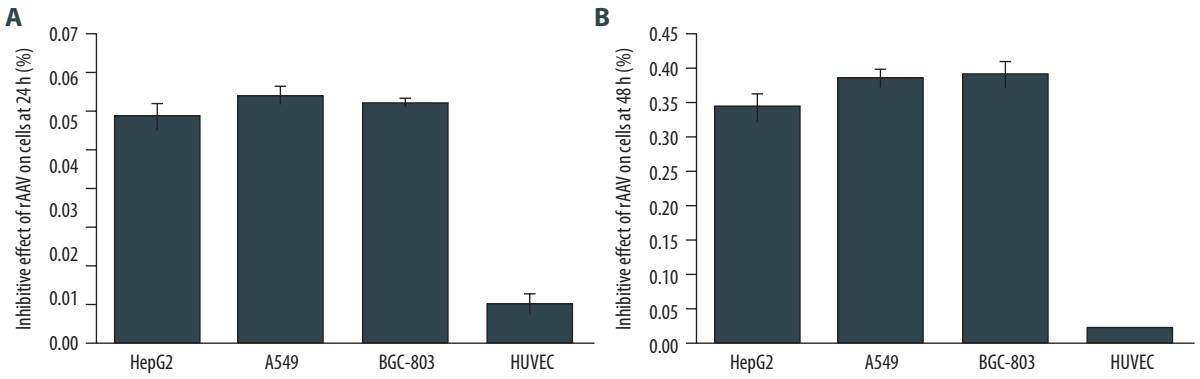


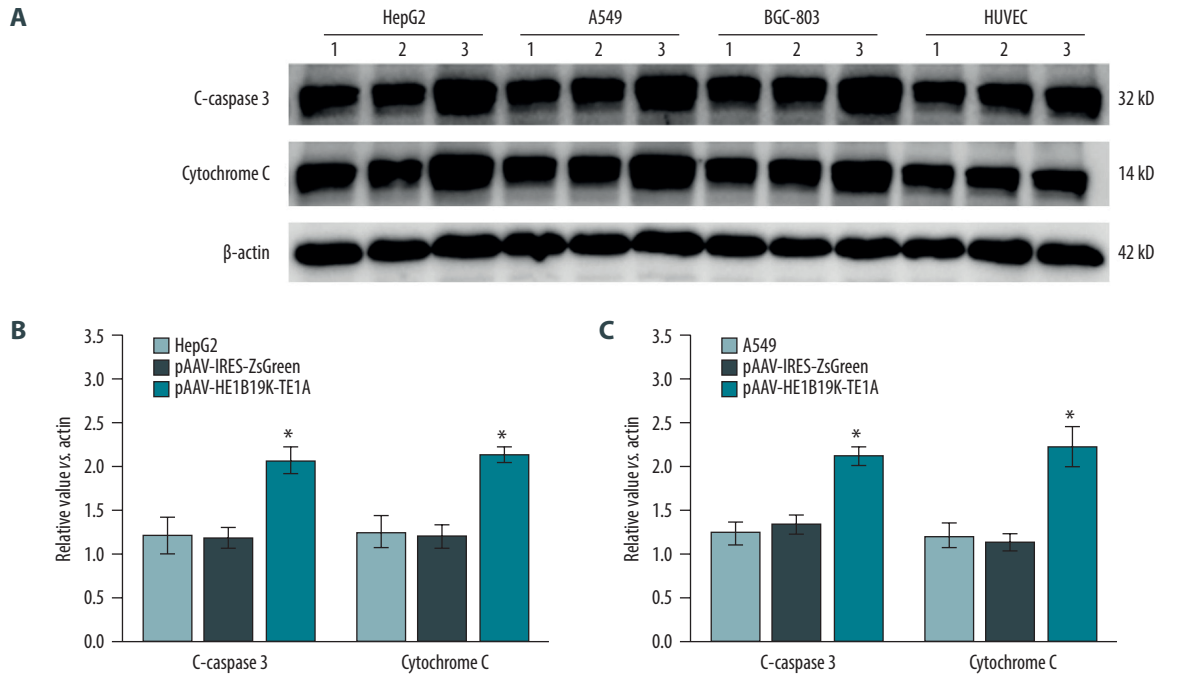
Figure 5. MTT assay for measuring the inhibitory effect of pAAV-HE1B19K-TE1A on tumor cells. (**A**) Inhibitive effects at 24 h after transfection. (**B**) Inhibitive effects at 48 h after transfection.

determined using Western blot assay (Figure 6A) to examine cleaved caspase 3 (C-caspase 3) levels. The findings indicated that pAAV-HE1B19K-TE1A transfection remarkably promoted the C-caspase 3 expression compared to that in un-transfected tumor cells (or pAAV-IRES-Zs Green group) in HepG2 cells (Figure 6B, $p<0.05$), A549 cells (Figure 6C, $p<0.05$), and BGC-803 cells (Figure 6D, $p<0.05$). However, there were no significant effects of pAAV-HE1B19K-TE1A transfection on C-caspase 3 expression in HUVEC cells (Figure 6E, $p>0.05$).

The qPCR assay also illustrated that pAAV-HE1B19K-TE1A transfection remarkably enhanced C-caspase 3 gene expression in HepG2 (Figure 7A, $p<0.05$), A549 (Figure 7B, $p<0.05$), and BGC-803 (Figure 7C, $p<0.05$) compared to that in blank tumor cells. However, pAAV-HE1B19K-TE1A transfection triggered no obvious changes of C-caspase 3 gene expression in HUVEC cells (Figure 7D, $p>0.05$).

pAAV-HE1B19K-TE1A induced release of Cyto C in tumor cells

We assessed cytochrome C (Cyto C) expression in tumor cells using Western blot (Figure 6A) and qPCR assays. The Western blot assay results showed that pAAV-HE1B19K-TE1A transfection obviously induced Cyto C release in HepG2 cells (Figure 6B, $p<0.05$), A549 cells (Figure 6C, $p<0.05$), and BGC-803 cells (Figure 6D, $p<0.05$), but did not trigger Cyto C release in normal HUVEC cells (Figure 6E, $p>0.05$). qPCR assay also showed



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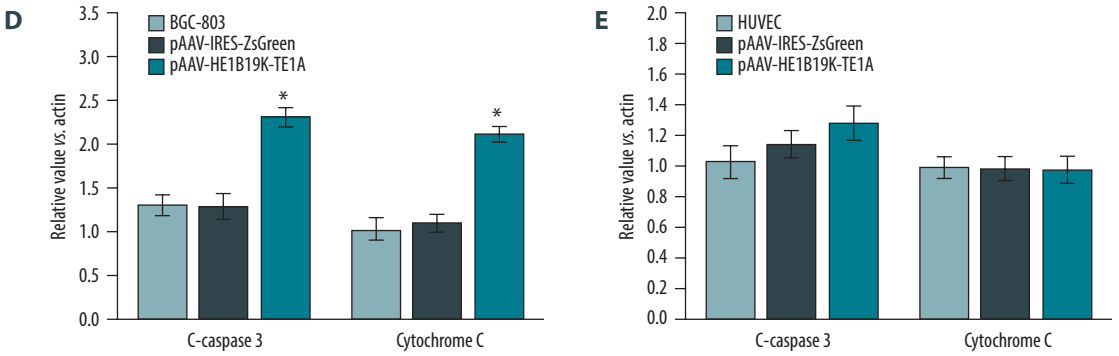

Figure 6. Production of C-caspase 3 and cytochrome C proteins in tumor cells and normal cells. (**A**) Western blot images. Relative C-caspase 3 and cytochrome C expression in HepG2 (**B**), A549 (**C**), BGC-803 (**D**), and HUVEC (**E**) was statistically analyzed. 1 – Tumor cells (HepG2, A549, BGC-803, or HUVEC cells) without transfection, 2 – Tumor cells (HepG2, A549, BGC-803, or HUVEC cells) transfected with pAAV-IRES-ZsGreen, pHelper, and pAAV-RC, 3 – Tumor cells (HepG2, A549, BGC-803, or HUVEC cells) transfected with pAAV-HE1B19K-TE1A, pHelper, and pAAV-RC). * $p<0.05$ *vs.* pAAV-IRES-ZsGreen group in HepG2, A549, or BGC-803 cells.

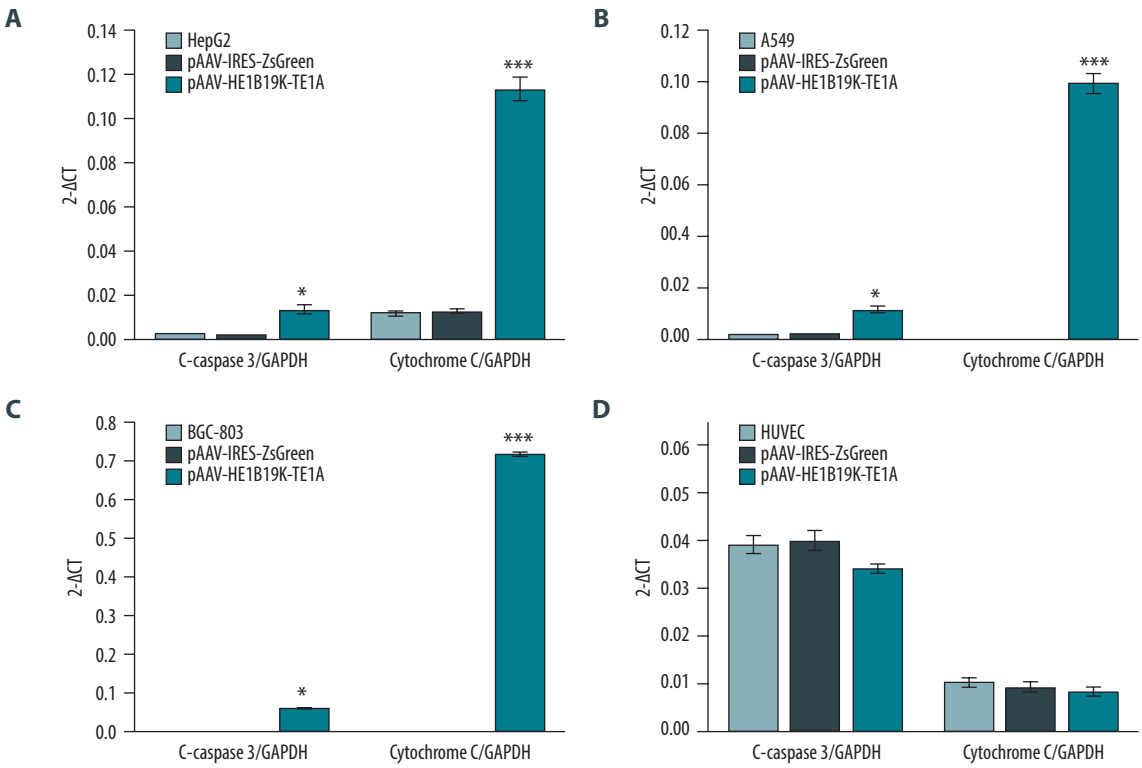

Figure 7. Effects of pAAV-HE1B19K-TE1A transfection on C-caspase 3 and cytochrome C expression in tumor cells and HUVEC cells using PCR assay. (**A**) Relative C-caspase 3 gene expression (C-caspase 3/GAPDH) and relative cytochrome C gene expression (cytochrome C/GAPDH) in HepG2 cells. (**B**) Relative C-caspase 3/GAPDH and relative cytochrome C/GAPDH in A549 cells. (**C**) Relative C-caspase 3/GAPDH and relative cytochrome C/GAPDH in BGC-803 cells. (**D**) Relative C-caspase 3/GAPDH and relative cytochrome C/GAPDH in HUVEC cells. * $p<0.05$ and *** $p<0.0001$ *vs.* pAAV-IRES-ZsGreen group in HepG2, A549, or BGC-803 cells.

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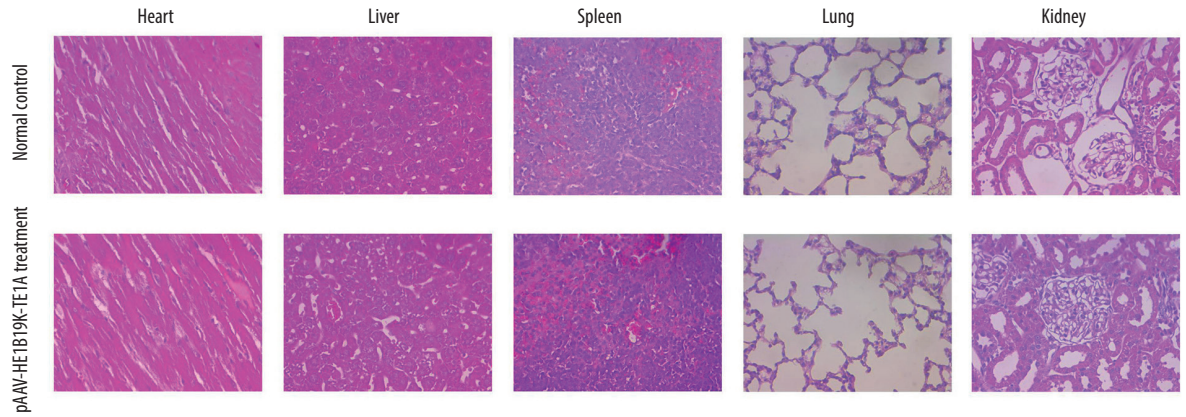

Figure 8. HE staining for analyzing pathological changes of vital organs of mice after pAAV-HE1B19K-TE1A injection and in the vital organs of normal mice. For the pAAV-HE1B19K-TE1A-injected mice, at 2 weeks after injection, the tissues (heart, liver, spleen, lung, and kidney) were collected for toxicity evaluation. Magnification, 400×.

that pAAV-HE1B19K-TE1A transfection triggered the release of Cyto C in HepG2 (Figure 7A, $p<0.001$), A549 (Figure 7B, $p<0.001$), and BGC-803 (Figure 7C, $p<0.001$), but not in HUVEC cells (Figure 7D, $p<0.001$).

pAAV-HE1B19K-TE1A demonstrated no toxicity to tissues of animals

HE staining showed that pAAV-HE1B19K-TE1A caused no obvious pathological changes in the heart, liver, spleen, lung, and kidney of the treated mice, equal to the status of the normal control mice (Figure 8). This result suggests that treatment with pAAV-HE1B19K-TE1A caused no acute toxicity to the heart, liver, spleen, lung, and kidney. Therefore, pAAV-HE1B19K-TE1A appears to be safe for tail vein injection.

Discussion

In contrast to the non-replicating virus vector used in traditional gene therapy, OVs are have high reproduction and are assembled by special plasmids and without propagation in normal cells. In theory, they have higher anti-tumor efficiency and fewer adverse effects. OVs can propagate in tumor cells and occupy the materials, energy, and places of the host cells, eventually destroying the cells and releasing offspring virus to infect neighboring tumor cells [1]. Moreover, OVs can express toxic proteins, induce inflammatory cytokines such as TNF, and trigger the immune response [26]. Gene therapy using conditional replicated AAV has become an important focus of tumor gene therapy [27]. There are 2 main approaches to constructing targeted rAAV. One approach is to remove genes that are necessary for the virus to replicate in normal cells but are not needed in tumor cells, and the other is that gene expression is regulated by a tumor-specific promoter. rAAV vector, which is the best-known gene vector system, overcomes the shortcomings that other gene expression vectors cannot overcome [28,29]. rAAV vector has a wide range of transfection, with high transfection efficiency, to drive gene expression stably *in vivo* for a long time and without adverse (immunogenic) effects [30].

In this study, recombinant AAV was successfully produced using the Helper-free system in tumor cells and obtained replication ability with E1A and E1B19K genes. Tumor-specific promoters, hTERT and HKII, were inserted upstream of E1A and E1B19K gene frames, respectively, to regulate the expression of these 2 genes. This process causes the rAAV to only replicate in tumor cells. The construction of this vector gives the gene therapy vectors dual targeted effects on tumor cells. These results showed that all of the tumor cells (HepG2, A549, and BGC-803) demonstrated higher ZsGreen signals, but HUVEC did not. These results suggest that hTERT promoter is only highly expressed in tumor cells. However, due to the different cell lines with different growth characteristics, the ZsGreen signals were different in various tumor cells lines. Moreover, HKII and hTERT promoters could effectively mediate the tumor-specific expression of E1B19K and E1A genes, but not in normal cells. In addition, *in vitro* tests showed that the rAAV could reduce cell viability of tumor cells through inducing apoptosis, and the effect was enhanced with the increased time of virus action. We plan to verify this effect by *in vivo* tests.

Conclusions

The result of this study indicated that the tumor-targeted rAAV was successfully produced using the Helper-free system with the recombinant plasmid, demonstrating high ability to decrease the cell viability of tumor cells without adverse effects

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on normal cells. In the future, it may be possible that the plasmid pAAV-HE1B19K-TE1A can be inserted with special genes targeted to the tumor markers in rAAV to enhance the ability of specific infection.

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Conflict of interest

None.

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