Adenovirus-mediated herpes simplex virus thymidine kinase gene therapy combined with ganciclovir induces hepatoma cell apoptosis

HAITAO ZHANG¹, LING QIN², CHAOLU LI³, JIANYI JIANG¹, LIBO SUN¹, XIAOFEI ZHAO¹ and NING LI¹

Departments of ¹General Surgery and ²Biomedical Information Center, Beijing Youan Hospital, Capital Medical University, Beijing 100069; ³Department of Surgery, Shijingshan Hospital of Beijing, Beijing 100040, P.R. China

Received September 5, 2017; Accepted May 2, 2018

DOI: 10.3892/etm.2019.7147

Abstract. The present study aimed to examine the apoptotic effects of adenovirus (ADV)-mediated herpes simplex virus thymidine kinase (ADV-TK) combined with ganciclovir (GCV) in tissues obtained from patients with hepatocellular carcinoma in order to provide a theoretical basis for the development of this gene therapy program. Apoptosis detection was conducted using the terminal deoxynucleotidyl-transferase-mediated dUTP nick end labelling assay and the apoptosis index was compared between the experimental; and control groups. Furthermore, the protein expression levels of caspase-3, B-cell lymphoma-2 (Bcl-2), Bcl-2-assoicated protein X (Bax) and nuclear factor (NF)-κB were examined in pathological specimens using immunohistochemical staining. The Bax/Bcl-2 ratio and the release of cytochrome c were examined using western blot analysis. Results indicated that combined ADV-TK and GCV treatment significantly increased the number of apoptotic cells compared with the control group (P<0.05). Immunohistological analysis revealed that ADV-TK and GCV treatment significantly increased the number of caspase-3-positive cells, reduced the Bax/Bcl-2 ratio and NF-κB expression levels and promoted the release of cytochrome c compared with the control group (P<0.01). In conclusion, the present results suggest that combined ADV-TK and GCV treatment exerts its effect through the apoptotic signaling pathway.

Introduction

Hepatocellular carcinoma (HCC) is a complicated type of malignant tumor with a high global incidence rate compared with other malignant tumor types (1). The percentage of cases of nonalcoholic steatohepatitis HCC has increased in the past; the overall survival has not. Solely 31% of patients with HCC identified via screening/surveillance received any curative treatment (2,3). Despite the reasonable progression in the understanding of the disease mechanism and its therapeutic possibilities in the past three decades, poor therapeutic outcomes have been reported in response to conventional treatments, including liver transplantation and surgical resection, and the recurrence rate remains considerably high (4,5). In addition, the presence of multifocal tumors in the liver is considered to be a notable risk factor for HCC incidence and due to tumor cell invasion and intrahepatic metastasis, alternative treatments have been described that may improve clinical outcomes, including intratumorally injected gene therapy (6).

Gene-mediated cytotoxic immunotherapy (GMCI) is a clinical intervention that forms a tumor-specific vaccine effect via intratumoral delivery of adenovirus (ADV)-mediated herpes simplex virus thymidine kinase (ADV-TK) followed by a systemic anti-herpetic prodrug, such as valacyclovir or ganciclovir (GCV), in combination with standard tumor resection surgery or radiation (7). Necrotic and apoptotic cell death, and acute inflammation form surgery activate and entice antigens, which induce immune cells and T-cell expansion (7). Stimulation of T cell proliferation and the production of inflammatory cytokines may be induced by herpes simplex virus type 1 thymidine kinase (HSV-TK) protein, which has been described to resemble a super-antigen molecule (7). This immunostimulatory environment forms a systemic anti-tumor immune response that leads to the release of autologous tumor-associated antigens (TAAs) (7). Notably, GMCI treatment towards local tumors led to protection against metastases in mouse syngeneic models (8-10). In addition, tumor growth inhibition has been presented in splenocytes from tumors treated with ADV-TK combined with GCV but not controls treated with ADV-TK plus saline (11). These findings suggest that TAA release and tumor cell death are required to induce a tumor-specific response. Therefore, GMCI may induce immune protection against recurrence from minimal residual disease after tumor debulking.

A previous study on hepatic metastasis in lung cancer has demonstrated that treatment with HSV-TK followed by ADV-TK led to significant tumor regression and prolongation of survival (12). In an interleukin (IL)-2 adenoviral vector
expressing murine model, IL-2 treatment alone was ineffect- 
ive, whereas combination therapy with HSV-TK resulted in 
further tumor regression and prolonged animal survival (12). 
In addition, a previous study indicated a similar trend was also 
exhibited in liver metastases in breast cancer, whereby signifi- 
cant tumor regression was presented in response to treatment 
with ADV-TK combined with GCV, which was assessed by 
computerized morphometric analysis towards the residual 
tumor (13). In addition to this result, a significant prolongation 
of survival was also indicated in ADV-TK and GCV-treated 
animals (13). Notably, recombinant ADV-TK and GCV 
expression significantly suppressed the growth of SMMC-7721 
and inhibition of angiogenesis in a HCC model in vitro (16). 
However, to best of our knowledge, no study has reported the 
underlying mechanism of combined ADV-TK and GCV treat- 
ment in clinically collected tissue-based samples. The present 
study aimed to illustrate the therapeutic effect of ADV-TK and 
GCV combined with partial hepatectomy via examining the 
cell death and apoptosis-associated protein expression levels 
in patient’s tissue.

Materials and methods

Specimens. A total of 34 hepatocellular carcinoma tissues 
(1x1x0.5 cm³) were obtained from surgical specimens of 
hospitalized patients between January 2004 and January 2012 
at the Department of General Surgery, Beijing Youan Hospital, 
Capital Medical University (Beijing, China). A partial 
Liver resection was performed in all patients as previously 
described (17). A total of 14 patients received ADV-TK 
(Shenzhen Tiandaxing Gene Engineering Co., Ltd., Shenzhen, 
China) therapy prior to partial hepatectomy. An intratumoral 
dose of 5.0x10¹¹ ADV-TK particles was administered and 
caused an objective response with no significant toxicity. The 
dose was based on a phase I dose escalation trial (15). To ensure 
uniform dosing, a total of 5.0x10¹¹ ADV-TK particles in 60 ml 
of 0.9% saline were injected into peritoneum tissues around 
liver at a doses of 1.25x10¹⁷ viral particles each, including the 
lesser curvature of stomach, abdominal aorta side, head of 
the pancreas surface of the right kidney and the area under 
the right diaphragm. The first dose of GCV (5 mg/kg; Roche 
Diagnostics, Basel, Switzerland) was administered intra-
venously and following 24 h further doses were administered 
twice daily for 10 days until partial hepatectomy. Patients in 
control group received liver resection only and were not treated 
with ADV-TK/GCV. During partial hepatectomy, tumor tissue 
specimens were collected; necrotic and tumor junction areas 
were not included in the analysis. Detailed patient information 
is presented in Table I. Written, informed consent was obtained 
from all patients and the present study was approved by the 
Institutional Review Board of Beijing Youan Hospital, Capital 
Medical University. All tissues were sliced into 5-µm sections 
and were fixed with 10% neutral formaldehyde solution for 
12-18 h at room temperature prior to conventional dehydration 
and paraffin embedding.

Terminal deoxynucleotidyl-transferase-mediated dUTP nick 
end labeling (TUNEL) assay. With xylene and gradient concen-
trated ethanol (100, 95, 90, 80 and 70%), each section was 
was washed twice and subsequently rinsed with phosphate-buffered 
saline (PBS). Sections were fixed with Proteinase K solution 
for 20 min at 37°C and rinsed twice with PBS. Once sections 
were dry, 50 µl of TUNEL reaction mixture from in-situ 
cell death kit (11684809910; Roche Diagnostics) were diluted 1:2 
with PBS, added to each slice and incubated at 37°C for 1 h. 
All slides were washed with PBS three times, treated with 
1 µg/ml 4',6-diamidino-2-phenylindole (DAPI) working solu-
tion and incubated at 37°C for 4 min. Following, the coverslip 
was treated with mounting media. Images were acquired using 
a conventional fluorescent microscope under an excitation 
fluence wavelength of 500 nm and a detection wavelength of 550 nm. 
Six field of view on each slide were observed. Magnifications 
of x20 or x40 were used to confirmed whether the labeling 
was successful or not and to observe the labeled nuclei (intact 
or fragmented). TUNEL-positive puncta were quantified using 
an x10 objective. The following equation was used to calcu-
late the apoptosis index (AI): (number of TUNEL-positive 
cells/total number of cells) x100%.

Immunohistochemical staining. For the immunohistochemical 
staining of targeted proteins, HCC tissue sections were depar-
affinized and dehydrated. Once sections were washed with 
distilled water, all sections were soaked in methanol with 3.0% 
hydrogen peroxide for 15 min at room temperature in order to 
block the peroxidase in tissues and further washed with distilled 
water. Antigens were retrieved with citric acid buffer by being 
heating samples at 100°C for 20 min prior to cooling for 
20 min at room temperature. Once sections were washed with 
PBS five times, the slides were incubated with primary rabbit 
polyclonal antibody against nuclear factor (NK) -x (1:1,000; 
ab886299; Abcam, Cambridge, UK) and caspase-3 (1:500; 
ab2302; Abcam); and primary mouse monoclonal antibodies 
against B-cell lymphoma (Bcl-2; 1:1,100; ZA-0611; OriGene 
Technologies, Inc., Beijing, China) and B-cl2-associated 
protein X (Bax; 1:200; ZA-0611; OriGene Technologies, Inc.) 
overtight at 4°C. The slides were subsequently washed five 
times with PBS and then incubated with horseradish peroxidase 
(HRP)-conjugated goat secondary antibody (1:500; TA130005; 
OriGene Technologies, Inc.) for 10 min at room temperature. 
Slides were washed with PBS and treated with diaminoben-
zidine substrate from a kit (OriGene Technologies, Inc.) for 
5 min at room temperature to visualize the reaction between 
antigen and antibody. Sections were counterstained at room 
temperature with Mayer’s hematoxylin (Sigma-Aldrich; Merck 
KGaA, Darmstadt, Germany) for 30 sec, dehydrated, cleared 
and mounted. Class- and species-matched irrelevant antibodies 
and incubations were used as controls. Sections were observed 
under an Olympus BX53 fluorescence microscope (magnification, 
x10; Olympus Corporation, Tokyo, Japan).

Western blot analysis. For assessing cytochrome c release, 
subcellular fractions were separated using the technique
indicated by Ott et al (18). Liver tissues were homogenized in radioimmunoprecipitation assay buffer (Sigma Aldrich; Merck KGaA). Cell lysates were mixed with Laemmli sample buffer and boiled for 3 min. Protein concentrations were determined by bicinchoninic acid assay (Thermo Fisher Scientific, Inc.). Equal amounts of proteins (20 µg) were separated on 10% SDS-PAGE gels and transferred onto nitrocellulose membranes. Membranes were blocked with 5% skimmed milk with TBS for 1 h at room temperature and incubated with anti-Bax antibody (1:500; ab32503; Abcam), anti-Bcl-2 antibody (1:200; ab196495; Abcam) and anti-cytochrome c antibody (1:200; ab13575; Abcam) overnight at 4˚C. Mitochondrial and cytosolic fractions (20 µg each) were resolved in 1-mm thick 12% Novex Tris-glycine polyacrylamide gel and immunoblotted as described above. Subsequently, the membranes were incubated with a HRP-conjugated secondary antibody (1:500; ZB-2306; OriGene Technologies, Inc.) at room temperature for 1 h. Protein bands were visualized with an ECL system (Clinx Scientific Instruments, Shanghai, China). The relative band intensity was determined using a gel image analysis system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Densitometry was performed using Quantity-One image analysis Software (Version 4.6.9; Bio-Rad Laboratories, Inc.). Protein expression levels in each sample were quantified and the ratio of protein to GAPDH (1:200; ab8245; Abcam) was defined as the protein expression.

Statistical analysis. All statistical analyses were performed using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation for normal distribution, whereas the median was representative of skewed distribution. When data were satisfactory for qualification of normal distribution, the independent sample Student's t-test was performed. When data did not meet this qualification, the Kruskal-Wallis test was then used. χ² test was used for enumeration data. One-way analysis of variance was used for multiple group comparison followed by a Bonferroni post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

**Results**

**ADV-TK combined with GCV significantly induced apoptosis.** TUNEL assay examination revealed that few TUNEL-positive apoptotic cells and limited DAPI staining of the same tissue section was indicated in the control group. Conversely, the number of TUNEL-positive cells was markedly increased in the experimental group (Fig. 1A). In addition, DAPI staining in the experimental group revealed multiple condensed and fragmented nuclei, suggesting the cells were going through apoptosis in the identical tissue sections subjected to combined ADV-TK and GCV treatment. In the experimental group, the AI in HCC tissues was significantly elevated compared with the control group (P<0.01; Fig. 1B). These findings suggest that treatment with ADV-TK combined with GCV may lead to significantly increased apoptosis.

**ADV-TK combined with GCV induces apoptosis through alternating the protein expression of caspase-3 and NF-kB, the Bax/Bcl-2 expression ratio and cytochrome c release.** As indicated in Fig. 2A, the protein expression levels of Bax, Bcl-2, caspase-3 and NF-kB was detected by immunohistochemistry. Brown particles revealed positive protein expression, which was primarily located in the cell membrane, cell nucleus and cytoplasm. The positive staining was diffuse and focal distribution was exhibited. Notably, the number of caspase-3-positive cells was significantly elevated in the experimental group compared with the control group (P<0.01; Fig. 2B). By contrast, the Bax/Bcl-2-positive cell ratio was significantly increased in the experimental group compared with the normal control group (P<0.01; Fig. 2C). Furthermore, a significantly increased number of NF-kB-positive cells was observed in the control group compared with the experimental group (P<0.01; Fig. 2D). In order to confirm the effect of combined ADV-TK

### Table I. General clinical data of all patients.

| Characteristics                  | Experimental (14 cases) | Control (20 cases) | P-value |
|----------------------------------|-------------------------|-------------------|---------|
| Age (years)                      | 51.14±12.64             | 60.40±9.80        | 0.109*  |
| Sex                              |                         |                   |         |
| Male                             | 10/14                   | 2/20              |         |
| Female                           | 4/14                    | 18/20             | 0.354*  |
| AFP before surgery (ng/ml)       | 2227±5474               | 101±161           | 0.232*  |
| Neoplasm stage                   |                         |                   |         |
| Phase I                          | 10 cases                | 10 cases          |         |
| Phase II                         | 2 cases                 | 6 cases           |         |
| Phase III                        | 2 cases                 | 4 cases           | 0.439*  |
| Postoperative pathological grades|                         |                   |         |
| High differentiation             | 2 case                  | 6 cases           |         |
| Moderate differentiation         | 4 cases                 | 8 cases           |         |
| Poor differentiation             | 8 cases                 | 6 cases           | 0.274*  |

*Independent sample t-test; †rank sum test. AFP, alpha-fetoprotein.
and GCV therapy on the apoptotic signaling pathway, western blot analysis was performed to further assess the Bax/Bcl-2 ratio and cytochrome c release. Representative western blot analysis images were demonstrated (Fig. 2E). Results indicated that the Bax/Bcl-2 protein ratio was significantly increased with combined ADV-TK and GCV treatment compared with the control (P<0.01; Fig. 2F).

**Discussion**

The present study was performed to assess the therapeutic effects of ADV-TK and GCV treatment combined with partial hepatectomy by comparing the biological activities in tissues.
from patients with HCC. The present results demonstrated that increased apoptotic cell death was observed in patients with HCC who underwent ADV-TK and GCV treatment combined with partial hepatectomy compared with those who were only subjected to partial hepatectomy. Furthermore, the expression levels of apoptosis-associated proteins were assessed in the present study. Notably, the protein expression levels of caspase-3, the ratio of Bax/Bcl-2 protein and the release of cytochrome C were significantly increased, whereas the protein expression levels of NF-κB were decreased in HCC tissues obtained from patients treated with combined ADV-TK and GCV therapy compared with the control.

Suicide gene therapy using HSV-TK gene transduction in combination with GCV is a therapeutic strategy that has been used in a wide variety of cancer treatments, and its application has been assessed in over 17 clinical trials in the United States (19). Phosphorylation of GCV results in its conversion into a non-diffusible nucleoside analogue, which terminates DNA synthesis and consequently results in cell death (20). Another benefit of this approach is the ‘bystander effect’, in which the cytotoxic GCV-triphosphate molecule is taken up by untransduced cells through gap junctions, which ultimately enhances tumor cell death (21).

The role of HSV-TK and GCV in the promotion of cell death has been demonstrated in the treatment of diverse types of cancer in vitro and in vivo (22-24). One of the mechanisms involved in combined ADV-TK and GCV-induced cell-death is apoptosis (25,26). A study on colorectal cancer with combined ADV-TK and GCV treatment suggested that its cytotoxic effect may be dependent on the tumor cell type, as evidenced by early apoptosis in the G1 phase of cell cycle and late apoptotic or necrotic cell death at the sub-G1 phase with DNA fragmentation (27). Cell death in ADV-TK and GCV transduced oral carcinoma has been revealed to be mediated through the apoptotic signaling pathway (28). By contrast, it was proposed that nonapoptotic biological activity may be a central manifestation in cell death induced by combined ADV-TK and GCV therapy (29). The present results demonstrated combined ADV-TK and GCV treatment significantly increased the number of apoptotic cells according to TUNEL analysis, which suggested that combined ADV-TK and GCV treatment induced apoptotic cell death in HCC tissues.

Caspase-3 is one of the key effector caspases in the cell. Inactive procaspase is cleaved into an active molecule with a lower molecular weight (~17KD), which activates other proteins to trigger the apoptotic process (30). In ADV-TK and GCV transduced NT2e cells, cleaved caspase could not be detected (31). Similarly, it has also been reported that no cleaved or activated caspase-3 band was observed following combined HSV-TK and GCV treatment (32). These findings suggest that the biological activity serving a role in the cytotoxic effect of HSV-TK and GCV treatment on NT2e cells other than apoptosis requires further investigation. The results in the present study suggested that combined ADV-TK and GCV treatment increased the caspase-3 protein expression levels and was subsequently responsible for the activation of the early stage apoptosis signaling pathway.

The mitochondrial apoptosis-induced channel is responsible for cytochrome c release in the early stage of apoptosis (33). It has been reported that the combined ADV-TK and GCV system effectively inhibited the proliferation of non-small cell lung cancer cells in vitro and in vivo via the potent induction of apoptosis, in which the release of the apoptosis initiator cytochrome c was increased (34). The therapeutic mechanism of suicide gene therapy was further investigated by Beltinger et al (26). Their study suggested that treatment with ADV-TK and GCV led to mitochondrial perturbations, including loss of the mitochondrial membrane potential and release of cytochrome c from the mitochondria into the cytosol, as well as caspase activation and nuclear fragmentation. As major members of the Bcl-2 family, Bcl-2 and Bax serve a crucial role in the inhibition of the intrinsic apoptotic signaling pathway and tumor progression triggered by mitochondrial dysfunction (35). In a previous study, the expression level of Bcl protein was significantly lower, whereas Bax protein expression level was significantly higher following 70-HSV-TK and GCV administration combined with Mn$_{3}$Zn$_{9}$Fe$_{2}$O$_{4}$ nanoparticles for HCC treatment in vitro and in vivo (36). A study suggested that the balance between Bcl and Bax proteins is important in assessing the sensitivity of tumor cells to GCV (37). Following the induction of glioma cell death, it was suggested that cytosine deaminase/5-fluorocytosine- and ADV-TK/GCV-induced apoptosis does not require cell death receptors or p53, but gathering at a mitochondrial pathway caused by different mechanisms of Bcl-2 modulation (38). A previous study suggested that downregulation of Bcl-2 and increased caspase-3 expression was a possible apoptosis mechanism in BIU87 cells induced by a HSV-TK/GCV system combined with allitride (39). Along with the present analysis of caspase-3 activity and apoptosis suggest that combined ADV-TK and GCV treatment induces apoptosis in host tumor cells by increasing the Bax/Bcl-2 ratio and inducing the release of cytochrome c from mitochondria to the cytosol.

NF-κB-inducing kinase serves an important role in promoting the processing of p100/NF-κB2, which is defined as the non-canonical NF-κB signaling pathway (40). Furthermore, NF-κB was overexpressed in various types of cancer and the inhibition of NF-κB was implied to reduce cell growth and enhance cell apoptosis (41,42). A previous study indicated that apoptosis is the central mechanism in cell death and tumor progression, whereby latent membrane protein 1 regulated the NF-κB-positive cells upon their treatment with the pVLTR-TK and GCV system (43). However, to the best of our knowledge, no study has ever reported the NF-κB-associated mechanism in combined ADV-TK and GCV-induced apoptosis. The present results demonstrated that combined treatment with ADV-TK and GCV could significantly reduce the expression of NF-κB protein, suggesting that NF-κB may be associated with ADV-TK and GCV-induced apoptosis in HCC. However, further investigations are required to fully elucidate this potential mechanism.

In the present study, a simple approach was applied to understand the cell death modulation in tissues from patients with HCC who had received combined ADV-TK and GCV therapy. The results concluded that combined ADV-TK and GCV treatment promoted cell death in HCC tissues. Furthermore, the present findings suggested that cell death was primarily mediated by the apoptotic signaling pathway.

Acknowledgements
Not applicable.
Funding
The study was supported by National Science and Technology Major Project (grant no. 2017ZX10203205-006-003), Changes of T Cell Immune Mechanism Before and After Hepatectomy for Hepatocellular Carcinoma (grant no. 20150303), Beijing Key Laboratory (grant no. BZ0373), the National Key Research and Development Program of China: The cluster construction of human genetic resource Bio-bank in North China (grant no. 2016YFC1201703).

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors' contributions
NL conceived and designed the study. HTZ and LQ performed experiments, and were major contributors in writing the manuscript. CLL and JYJ made significant contributions to acquisition of resources. HTZ, LQ and LBS were responsible for biological samples collection. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of Beijing Youan Hospital, Capital Medical University. Written informed consent was obtained from each patient.

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Wallace MC, Preen D, Jeffrey GP and Adams LA: The evolving epidemiology of hepatocellular carcinoma: A global perspective. Expert Rev Gastroenterol Hepatol 9: 765-779, 2015.
2. Kim NG, Nguyen PP, Dang H, Kumari R, Garcia G, Esquivel CO and Nguyen MH: Temporal trends in disease presentation and survival of patients with hepatocellular carcinoma: A real-world experience from 1998 to 2015. Cancer 124: 2588-2598, 2018.
3. Libbrecht L, Desmet V and Roskams T: Prenecplastic lesions in human hepatic carcinogenesis. Liver Int 25: 16-27, 2005.
4. Faloppi L, Scartozzi M, Maccaroni E, Di Pietro Paolo M, Berardi R, Del Prete M and Cascinu S: Evolutionary strategies for the treatment of hepatocellular carcinoma: From clinical-guided to molecularly-tailored therapeutic options. Cancer Treat Rev 37: 169-177, 2011.
5. Rahbari NN, Mehrabi A, Mollberg NM, Müller SA, Koch M, Büchler MW and Weitz J: Hepatocellular carcinoma: Current management and perspectives for the future. Ann Surg 253: 453-469, 2011.
6. Lou L, Ye W, Chen Y, Wu S, Jin L, He J, Tao X, Zhu J, Chen X, Deng A and Wang J: Arbidlipin (5845) inhibits survival, invasion and metastasis of human hepatocellular carcinoma cells. Phytomedicine 19: 603-608, 2012.
7. Aguilar LK, Guzik BW and Aguilar-Cordova E: Cytotoxic immunotherapy strategies for cancer: Mechanisms and clinical development. J Cell Biochem 112: 1969-1977, 2011.
8. Hall SJ, Mutchnik SE, Chen SH, Woo SL and Thompson TC: Adenovirus-mediated herpes simplex virus thymidine kinase gene and ganciclovir therapy leads to systemic activity against spontaneous and induced metastasis in an orthotopic mouse model of prostate cancer. Int J Cancer 70: 183-187, 1997.
9. Perez-Cruet MJ, Trask TW, Chen SH, Goodman JC, Woo SL, Grossman RG and Shine HD: Adenovirus-mediated gene therapy of experimental gliomas. J Neurosci Res 39: 506-511, 1994.
10. Vile RG, Nelson JA, Castleden S, Chong H and Hart IR: Systemic gene therapy of human melanoma using tissue-specific expression of the HSVtk gene involves an immune component. Cancer Res 54: 6228-6234, 1994.
11. Agard C, Ligueza C, Dupas B, Izembart A, El Kouri C, Moullier P and Ferry N: Immune-dependent distant bystander effect after adenovirus-mediated suicide gene transfer in a rat model of liver colorectal metastasis. Cancer Gene Ther 8: 128-136, 2001.
12. Kwong YL, Chen SH, Kosai K, Finegold M and Woo SL: Combination therapy with suicide and cytokine genes for hepatic metastases of lung cancer. Chest 112: 1332-1337, 1997.
13. Kwong YL, Chen SH, Kosai K, Finegold M and Woo SL: Adenoviral-mediated suicide gene therapy for hepatic metastases of breast cancer. Cancer Gene Ther 3: 339-344, 1996.
14. Chen GZ, Hu H, Xu MY and Deng XL: Construction of recombinant adenovirus containing TK gene and its effect against human liver cancer cells. Nan Fang Yi Ke Da Xue Xue Bao 30: 1007-1009, 2010 (In Chinese).
15. Li N, Zhou J, Weng D, Zhang C, Li L, Wang B, Song Y, He Q, Lin D, Chen D, et al: Adjuvant adenovirus-mediated delivery of herpes simplex virus thymidine kinase administration improves outcome of liver transplantation in patients with advanced hepatocellular carcinoma. Clin Cancer Res 13: 5847-5854, 2007.
16. Zhu R, Chen D, Lin D, Lu F, Yin J and Li N: Adenovirus vector-mediated herpes simplex virus-thymidine kinase gene/ganciclovir system exhibits anti-tumor effects in an orthotopic hepatocellular carcinoma model. Pharmazie 69: 547-552, 2014.
17. Chan ACY, Chok K, Dai JWC and Lo CM: Impact of split completeness on future liver remnant hypertrophy in associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) in hepatocellular carcinoma: Complete-ALPPS versus partial-ALPPS. Surgery 161: 357-364, 2017.
18. Ott M, Robertson JD, Gogvadze V, Zhivotovsky B and Orrenius S: Sytochrome c release from mitochondria proceeds by a two-step process. Proc Natl Acad Sci USA 99: 1259-1263, 2002.
19. Rosenberg SA, Blaese RM, Brenzer MK, Deisseroth AB, Ledley FD, Lotze MT, Wilson JM, Nabel GJ, Cornetta K, Economou JS, et al: Human gene marker/therapy clinical protocols. Human Gene Ther 8: 2301-2318, 1997.
20. Moolten FL: Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: Paradigm for a prospective cancer control strategy. Cancer Res 46: 5276-5281, 1986.
21. Kothari V, Joshi G, Nama S, Somasundaram K and Mulherkar R: HDAC inhibitor valproic acid enhances tumor cell kill in adenovirus-HSVtk mediated suicide gene therapy in HNSCC xenograft mouse model. Int J Cancer 126: 733-742, 2010.
22. Ketola A, Määttä AM, Pasanen T, Tulimäki K and Wahlffors J: Osteosarcoma and chondrosarcoma as targets for virus vectors and herpes simplex virus thymidine kinase/ganciclovir gene therapy. Int J Mol Med 13: 705-710, 2004.
23. Määttä AM, Tenhunen A, Pasanen T, Meriläinen O, Pellinen R, Mäkinen K, Alhava E and Wahlffors J: Non-small cell lung cancer as a target disease for herpes simplex type I thymidine kinase-ganciclovir gene therapy. Int J Oncol 24: 943-949, 2004.
24. Kwong KY, Zou Y, Day CP and Hung MC: The suppression of colon cancer cell growth in nude mice by targeting beta-catenin/TCF pathway. Oncogene 21: 8340-8346, 2002.
25. Chu QD, Sun L, Li J, Byrnes K, Chervenak D, DeBenedetti A, Mathis JM and Li BD: Rat adenocarcinoma cell line infected with an adenovirus carrying a novel herpes-simplex virus-thymidine kinase suicide gene construct dies by apoptosis upon treatment with ganciclovir. J Surg Res 143: 189-194, 2007.
26. Beltinger C, Fulda S, Kammertons T, Uckert W and Debatin KM: Mitochondrial amplification of death signals determines thymidine kinase/ganciclovir-triggered activation of apoptosis. Cancer Res 60: 3212-3217, 2000.
27. Taxy SB, Melcher A, Bottlely G, Gough M and Vile R: Cell death associated with genetic prodrug activation therapy of colorectal cancer. Cancer Lett 174: 25-33, 2001.
28. Nishikawa M, Hayashi Y, Yamamoto N, Fukui T, Fukuhara H, Mitsudo K, Tohnaiz I, Ueda M, Mizuno M and Yoshida J: Cell death of human oral squamous cell carcinoma cell line induced by herpes simplex virus thymidine kinase gene and ganciclovir. Nagoya J Med Sci 66: 129-137, 2003.

29. Katabi M, Yuan S, Chan H, Galipeau J and Batist G: The nonapoptotic pathway mediating thymidine kinase/ganciclovir toxicity is reduced by signal from adenovirus type 5 early region 4. Mol Ther 5: 170-176, 2002.

30. Sergeeva TF, Shirmanova MV, Zlobovskaya OA, Gavrina AI, Dudenkova VV, Lukina MM, Lukyanov KA and Zagaynova EV: Relationship between intracellular pH, metabolic co-factors and caspase-3 activation in cancer cells during apoptosis. Biochim Biophys Acta 1864: 604-611, 2017.

31. Srivastava D, Joshi G, Somasundaram K and Mulherkar R: Mode of cell death associated with adenovirus-mediated suicide gene therapy in HNSCC tumor model. Anticancer Res 31: 3851-3857, 2011.

32. Kuratate I: Cell death induction of thymidine kinase gene transfer followed by ganciclovir treatment in oral squamous cell carcinoma cell lines. Yonago Acta Med 48: 63-74, 2005.

33. Martinez-Caballero S, Dejean LM, Jonas EA and Kinnally KW: The role of the mitochondrial apoptosis induced channel MAC in cytochrome c release. J Bioenerg Biomembr 37: 155-164, 2005.

34. Chiu CC, Kang YL, Yang TH, Huang CH and Fang K: Ectopic expression of herpes simplex virus thymidine kinase gene in human non-small cell lung cancer cells conferred caspase-activated apoptosis sensitized by ganciclovir. Int J Cancer 102: 328-333, 2002.

35. Thangarajan S, Ramachandran S and Krishnamurthy P: Chrysir exerts neuroprotective effects against 3-Nitropropionic acid induced behavioral despair-Mitochondrial dysfunction and striatal apoptosis via upregulating Bcl-2 gene and downregulating Bax-Bad genes in male wistar rats. Biomed Pharmacother 84: 514-525, 2016.

36. Tang Q, Lu M, Chen D and Liu P: Combination of PEI-Mn0.5Zn0.5Fe2O4 nanoparticles and pHsp 70-HSV-TK/GCV with magnet-induced heating for treatment of hepatoma. Int J Nanomedicine 10: 7129-7143, 2015.

37. Craperi D, Vicat JM, Nissou MF, Mathieu J, Baudier J, Benabid AL and Verna JM: Increased bax expression is associated with cell death induced by ganciclovir in a herpes thymidine kinase gene-expressing glioma cell line. Hum Gene Ther 10: 679-688, 1999.

38. Fischer U, Steffens S, Frank S, Rainow NG, Schulze-Osthoff K and Kramm CM: Mechanisms of thymidine kinase/ganciclovir and cytosine deaminase/5-fluorocytosine suicide gene therapy-induced cell death in glioma cells. Oncogene 24: 1231-1243, 2005.

39. Qiu SP, Mao XP, Cao KY, Chen XI, Yuan GQ, Xu L and Huang XR: The effect of cell killing and apoptosis by human herpes simplex virus-thymidine kinase/ganciclovir system combined with allitridite in BIU87 cells. Zhonghua Wai Ke Za Zhi 43: 382-386, 2005 (In Chinese).

40. Battaglia L, Bertrand MJ, Remouchamps C, Seleznik G, Reisinger F, Janas M, Bénézech C, Fernandes MT, Marchetti S, Mair F, et al: NIK promotes tissue destruction independently of the alternative NF-kB pathway through TNFR1/RIPI-induced apoptosis. Cell Death Differ 22: 2020-2033, 2015.

41. Seubwai W, Wongkham C, Puapairoj A, Khuntikeo N, Pugkhem A, Hahnvajanawong C, Chaiyagool J, Umezawa K, Okada S and Wongkham S: Aberrant expression of NF-kB in liver fluke associated cholangiocarcinoma: Implications for targeted therapy. PLoS One 9: e106065, 2014.

42. Shinoda K, Kuboki S, Shimizu H, Ohitsu M, Kato A, Yoshitomi H, Furukawa K and Miyazaki M: Pin1 facilitates NF-kB activation and promotes tumour progression in human hepatocellular carcinoma. Br J Cancer 113: 1323-1331, 2015.

43. Lifang Y, Min T, Midan A and Ya C: HSV-tk/GCV gene therapy mediated by EBV-LMP1 for EBV-associated cancer. J Exp Clin Cancer Res 27: 42, 2008.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.