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ORIGINAL ARTICLE

Synthetic radiation-inducible promoters mediated HSV-TK/GCV gene therapy in the treatment of oral squamous cell carcinoma

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OBJECTIVE: To investigate the therapeutic effect of herpes simplex virus thymidine kinase (HSV-TK) gene mediated by synthetic radiation-inducible promoters in the treatment of oral squamous cell carcinoma (OSCC) *in vitro* and *in vivo*.

METHODS: The plasmids pcDNA3.1(+)E6-HSV-TK were constructed, in which the HSV-TK genes were mediated by synthetic radiation-inducible promoters. The recombined plasmids were transfected into the Tca8113 cells and golden hamster buccal carcinoma, respectively. Low-dose radiotherapy was used to upregulate the HSV-TK genes expression. HSV-TK mRNA was assayed by RT-PCR. Apoptosis and proliferating cell nuclear antigen were detected respectively by *in situ* end-labeling and immunohistochemical method.

RESULTS: Compared with control group, the comparative survival rate of Tca8113 cells in HSV-TK/GCV/IR group was markedly decreased and the golden hamster buccal carcinoma in HSV-TK/GCV/IR group was obviously suppressed. Up-regulation of HSV-TK gene expression was found in the Tca8113 cells and in the golden hamster buccal carcinoma resulting from exposure to low-dose irradiation. The apoptosis indexes in Tca8113 cells or golden hamster buccal carcinoma with irradiation were markedly higher than those without irradiation. At the same time, the proliferation indexes in Tca8113 cells or golden hamster buccal carcinoma with irradiation were markedly lower than those without irradiation.

CONCLUSION: The results indicate that the synthetic radiation-inducible promoters can serve as a molecular switch to adjust the expression of HSV-TK gene in the treatment of OSCC, and low-dose induction radiation can significantly improve therapeutic efficiency.

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Introduction

The majority of head and neck cancers comprised oral squamous cell carcinoma (OSCC) (Aguiar *et al*, 2007; Scully *et al*, 2008). Conventional treatments are often inadequate for oral cancer patients. Many novel approaches to the treatment of OSCC have been developed, including gene therapy (Scully and Bagan, 2008). Suicide genes typically code for non-mammalian enzymes that convert non-toxic prodrugs into highly toxic metabolites. This therapy has been performed in a variety of tumor models and has a good therapeutic effect *in vitro* and *in vivo*, including OSCC (Ayllón Barbellido *et al*, 2008; Fukui *et al*, 2001; Yu *et al*, 2008).

One problem with suicide gene therapy involves the inappropriate expression of a gene product that may have adverse effects on normal tissues and may negate the therapeutic index. Several strategies have been developed to limit gene expression in tumors, but there has been no satisfactory scheme for clinical therapy yet (Bonini *et al*, 2007; Yu *et al*, 2008). Novel strategies are needed to further improve the treatment outcome for OSCC.

Combining suicide gene therapy and radiotherapy protocol offers a very promising strategy for cancer-targeted treatment (Coulter *et al*, 2008; Natsume and Yoshida, 2008; Liao *et al*, 2009). With the development of intensity modulated radiotherapy, the targetability of radiation therapy provides a unique opportunity to control therapeutic gene expression within tumors via the promoter regions of radiation-inducible sequence. The radiation-inducible promoter from the early growth response-1 (Egr-1) gene has been used successfully for selective transgene expression following radiation treatment (Ahmed, 2004; Chen *et al*, 2008; Park *et al*, 2008).

Recently, synthetic gene promoters based on the consensus sequence CC(A/T)GG, known as CArG boxes, have been proven to produce efficient and specific gene activation following irradiation (IR) in a gene therapy context (Mezhir *et al*, 2006; Coulter *et al*, 2008; Greco *et al*, 2005). The herpes simplex virus thymidine kinase (HSV-TK) gene is a prototype 'suicide gene' because it encodes a viral enzyme foreign to mammalian cells that phosphorylates nucleoside analogs such as acyclovir and ganciclovir (GCV) to their monophosphate metabolites. In some studies, the HSV-TK/GCV system had significant therapeutic effects *in vitro* and *in vivo* on many malignant tumors (Huang *et al*, 2007; Yu *et al*, 2008).

As OSCC is usually superficial and easily accessible, the target of radiation therapy can be limited to local tumor regions. The synthetic radiation-inducible promoters could be used to control the expression of the HSV-TK gene. This strategy would restrict HSV-TK gene activation to irradiated tissues, thereby providing targeted expression. Damage to surrounding normal tissues would be reduced and at the same time the therapeutic efficiency would be improved. The aim of this study is to investigate the therapeutic effect of HSV-TK gene mediated by radiation-inducible promoters in the treatment of OSCC. It must be indicated that our study was performed in the animal models of buccal carcinoma in golden hamsters and the results are not applicable for the treatment of OSCC in humans at the present time.

Materials and methods

Cells and cell culture

Human oral squamous carcinoma cells (Tca8113 cell line, kindly presented by Prof. He Ronggen, Shanghai, China) were grown in RPMI 1640 (GIBCO, Carlsbad, CA, USA) culture medium supplemented with 10% fetal calf serum (GIBCO), 100 units ml^{-1} penicillin G, and 100 $\mu g\ ml^{-1}$ streptomycin at 37°C in 5% CO_2. Subculture occurred weekly (seeded at 5×10^5 cells ml^{-1}), with twice-weekly feeding with complete medium.

Animals

One hundred golden hamsters (4–6 weeks old, about 50 g) were purchased from the Experiment Animal Research Centre (Sun Yat-sen University, China). Animal experiments were performed with the permission of the Animal Ethical Commission of Sun Yat-sen University, China.

Construction of plasmids

All restriction and modifying enzymes were supplied by Promega or Roche and were used according to the manufacturer's instructions. DNA isolation and purification were carried out using the appropriate kit from Qiagen (Crawley, UK). The six-CArG element enhancer (E6) was cloned using the complementary single-stranded oligodeoxyribo-nucleotides pairs: 5′-AGATCTGCTAG C(CCATATAAGG)$_6$TCGCG AA-3′ and 5′-TCGCGA (CCTTATATGG)$_6$GCTAGCAGATCTA-3′, which were synthesized by Songon Company, Shanghai, China. Linkers were produced by mixing 0.05 nmol of each oligonucleotide in 5 μl volumes, heating them to 55°C for 5 min and then cooling to room temperature. pcDNA3.1(+) HSV-TK, presented by Professor Wu Xinrao (Sun Yat-sen University,China), was digested by BglII/NruI. The synthetic radiation-inducible promoter sequence E6 was cloned into the corresponding site in pcDNA(+)3.1 HSV-TK, upstream of the HSV-TK gene. The sequence integrity of the newly constructed plasmid pcDNA3.1(+)E6-HSV-TK was confirmed by a Thermo Sequence Cycle Sequencing Kit (Amersham Pharmacia, UK) and a Gene Readir DNA Analyzer (LI-COR, USA) by Senyou Biotechnology Company (Shanghai, China).

The killing effect of HSV-TK/GCV mediated by radiation-inducible promoter in vitro

Transfection of DNA. Tca8113 cells were transfected with lipofectamine (GIBCO) by modifying the manufacturer's procedure based on our previous study (Yu *et al*, 2005). Tca8113 cells (2×10^5) were seeded into a six-well plate at 2×10^5 cells per well, and conventionally maintained for 24 h. A total of 24 wells of Tca8113 cells were prepared to be transfected. Two micrograms of plasmid DNA (10 μl) was mixed with 90 μl of serum-free medium. This mixture was then incubated with a mixture of 15 μl lipofectamine (2 mg ml^{-1}) and 85 μl serum-free medium for 45 min at room temperature. Subsequently, the DNA-lipofectamine complex (200 μl) was added to each well. GCV (10^{-4}mol l^{-1}) was added 12 h later.

Radiation procedure. After transfection for 24 h, the transfected cells were irradiated by linear accelerator (Primvs, Siemens, Germany) at a dose of 3 Gy. We chose the radiation dose of 3 Gy based on our previous research (Yu *et al*, 2005). The monolayers of the transfected cells in the control group were also mock irradiated and subsequently treated identically as the irradiated monolayers of transfected cells.

Microculture tetrazolium assay. Viability was assessed using the microculture tetrazolium (MTT) assay. All experiments were performed in triplicate. The results were expressed as percentages of the control, which was considered 100%.

RNA preparation and RT-PCR. After IR for 48 h, total mRNA from transfected Tca8113 cells was extracted using an RNeasy mini kit (Qiagen, USA) according to the manufacturer's instructions. RT-PCR was carried out using an AMV reverse transcription kit (Promega, USA) and human β-actin gene was used as the internal control. The TK gene primers were as follows: 5′-TGTCGAATAAACGCTTTACAAAC-3′ (forward) and 5′-AACGTTTGTAATG ATGGCTTCTG-3′ (reverse). The β-actin primers were as follows: 5′-GGTCG GAGTCAACGGATTTGGTCG-3′ (forward) and 5′-CCTCCGACGCCTGCTTCAC CAC-3′ (reverse). The transcript levels were normalized according to the internal control β-actin transcription, and the products were resolved by agarose electrophoresis. The intensity

was quantified by image-analysis computer software (NIH Image; Apple, Cupertino, CA, USA).

Detection of apoptosis by flow cytometry. After transfection for 48 h, both adherent and floating cells were harvested and centrifuged. The cell pellets were washed with phosphate-buffered saline (PBS) and then fixed with 70% ethanol overnight. After incubation in phosphate-citric acid, the cells were re-suspended with PBS containing propidium iodide (10 μg ml^{-1}) and RNase (100 μg ml^{-1}). The cells were quantified with a FACScan flow cytometer (Becton Dickinson). The Salmon sperm DNA (ssDNA) was used as the DNA toxicity control.

Tumor inhibition effect in vivo

Animal models of buccal carcinoma. The animal models of buccal carcinoma in golden hamsters were established by painting 0.5% DMBA (Sigma, USA) according to the method reported by Yu (Yu *et al*, 2008). One hundred golden hamsters were used to establish animal models of buccal carcinoma with 93 cases of animal models of buccal carcinoma obtained. Seventy cases were selected for the subsequent experiment, in which the diameters of tumors were about 8 mm and there were no serious complications such as ulcers, bleeding or hypoalimentation. Seventy golden hamsters with buccal carcinoma were divided randomly into five groups:

Control group ($n = 10$): no intervention.
GCV group ($n = 10$): GCV injection only.
IR group ($n = 10$): IR only.
HSV-TK/GCV group ($n = 20$): transfection and GCV injection.
HSV-TK/GCV/IR group ($n = 20$): transfection and GCV injection and IR.

Transfection of DNA. Fifty micrograms of plasmid DNA (100 μl) was mixed with 900 μl of serum-free medium. This mixture was incubated for 45 min at room temperature with a mixture of 200 μl lipofectamine (2 mg ml^{-1}) and 80 μl serum-free medium. Subsequently, the DNA-lipofectmine complex (100 μl) was injected into the tumors using a microliter syringe fitted with a 27-gauge needle (25 mm) at an injection position in different directions when the diameter of the neoplasm was about 8 mm. Afterwards, GCV (30 mg kg^{-1}) was injected abdominally into the golden hamster once a day for 1 week.

Radiation procedure. After transfection for 24 h, the golden hamsters were irradiated (3 Gy) by linear accelerator (Primvs, Siemens, German) at a dose rate of 1.6 Gy per minute.

Evaluation of tumor growth. The tumors sizes were measured (length and width) with a caliper every 4 days. The size of the tumors was measured three times by different observers. Tumor parameters were calculated on day 20 by the following formulas: tumor volume = ($1/2 \times$ length $\times$ width^2); tumor doubling time = ($\ln 2/K$, where K is growth rate); and inhibition rate = (1 – volume change of experimental group/volume change of control group) $\times$ 100%.

RNA preparation and RT-PCR. After transfection for 96 h, 10 golden hamsters from the HSV-TK/GCV group and the HSV-TK/GCV/IR group were killed and the tumor tissues were subsequently collected. The RT-PCR methods employed were the same as those in the experiment *in vitro* described above.

Apoptosis and proliferating cell nuclear antigen detection. The golden hamsters were killed 20 days after transfection, and the tissues from the tumors were collected. Apoptosis was detected by *in situ* end-labeling (ISEL) with an apoptosis labeling kit (Boster, China) according to the manufacturer's protocol: Block endogenous peroxidase by immersing slides in 3% H_2O_2 for 30 min at RT; Incubate slides in ISEL solution in 18°C ice bath for 2 h; Apply ABC solution and incubate at RT for 30 min; Apply DAB for 3–10 min at RT; Immerse in hematoxylin for 90 s for counterstain; Rehydrate and coverslip. The proliferating cell nuclear antigen (PCNA) was detected with immunohistochemical kit (Boster) according to the manufacturer's protocol: incubate for 10 min at RT in 3% H_2O_2 diluted in methanol; add diluted primary antibody and incubate overnight at 4°C; wash section three times with buffer solution; add secondary antibody (rabbit anti-rat monoclone antibody, dilution rate 1/50) and incubate 30 min at RT; add ABC reagent and incubate for 30 min; immerse in hematoxylin for 90 s for counterstain.

Apoptosis index (AI) and proliferation index (PI) were calculated by observing the proportion of positive-staining cells. Staining was considered positive when the cell nuclei had been stained brown. The number of positive cells and total observed cells was counted under light microscopy (×400 magnification) with a 10×10 grid. The observers selected distinct fields randomly during three separate evaluations in each slide. Ten sections were used to evaluate every group. AI or PI was calculated by the following formula: AI or PI = (positive cells/total observed cells) $\times$ 100%.

Statistical analysis

The experimental data were presented as mean $\pm$ s.d. The data were processed by the statistical analysis software SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). The significance of the differences in mean values was evaluated by the one-way analysis of variance (ANOVA). Significance was defined at the $P < 0.05$ level.

Results

Inhibition effect on Tca8113 cells in vitro

The variability of Tca8113 cells was detected by MTT assay. Figure 1 shows that Tca8113 cells grew rapidly in the blank control group, GCV group and HSV-TK group, and there was no marked difference between the three groups. Tca8113 cells either in HSV-TK/GCV

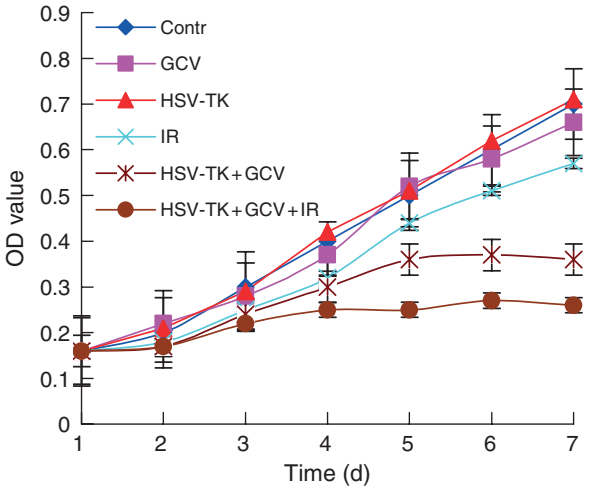

Figure 1 Evaluation of Tca8113 cell growth *in vitro*. The growth of Tca8113 cells in the HSV-TK/GCV/IR group was significantly inhibited compared with any other group. There was significant difference between the HSV-TK/GCV/IR group and the HSV-TK/GCV group

group or IR group were significantly inhibited compared with the control group. Furthermore, the growth of Tca8113 cells in the HSV-TK/GCV/IR group was significantly more inhibited than in any other group.

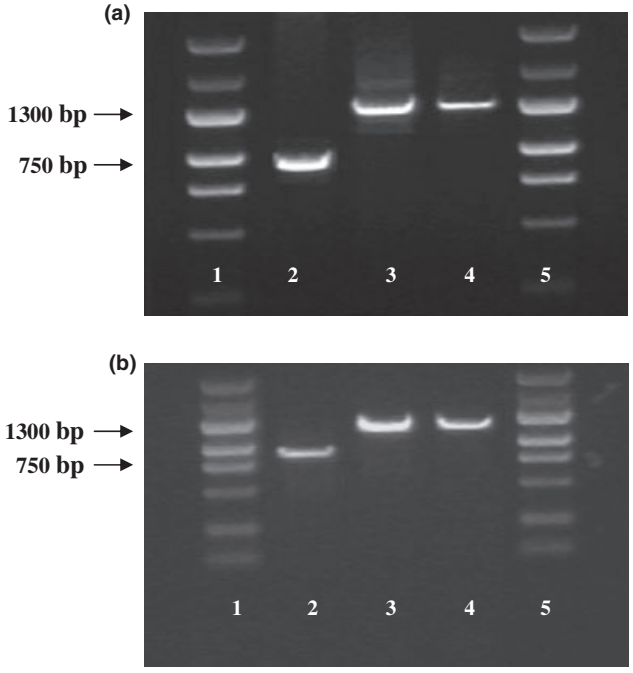

Figure 2 TK transcription after transfection into tumor cells. (**a**) RT-PCR was performed 96 h after HSV-TK gene transfection in Tca8113 cells. Lines 1 and 5, DNA maker; Line 2, product of β-action (750 bp); Line 3, product of HSV-TK gene in the HSV-TK/GCV/IR group; Line 4, product of HSV-TK gene in the HSV-TK/GCV group. (**b**) RT-PCR was performed 96 h after HSV-TK gene transfection in golden hamster buccal carcinoma. Line 1 and 5, DNA maker; Line 2, product of β-action (750 bp); Line 3, product of HSV-TK gene in the HSV-TK/GCV/IR group; Line 4, product of HSV-TK gene in the HSV-TK/GCV group

Expression of HSV-TK mRNA in transfected Tca8113 cells

After IR for 48 h, the HSV-TK expression was detected by RT-PCR. Figure 2(a) shows that the mRNA levels in the HSV-TK/GCV/IR group (0.856 ± 0.023) were significantly higher compared with those in the HSV-TK/GCV group (0.531 ± 0.018) ($P < 0.01$). This result suggested that the synthetic radiation-inducible promoters combined with the IR upregulated the HSV-TK gene expression in transfected Tca8113 cells.

FACScan analysis of cells apoptosis

After IR for 48 h, the percentage of cell apoptosis in the HSV-TK/GCV/IR group was about (27.12 ± 1.34)%, which was significantly higher than (15.23 ± 0.92)% in the HSV-TK/GCV group ($P < 0.05$) and (8.87 ± 0.52)% in the IR group ($P < 0.01$) (Figure 3). These results indicated that IR markedly enhanced the anti-cancer ability of HSV-TK/GCV gene therapy system *in vitro*.

Tumor inhibition effect on buccal carcinoma in golden hamster

Buccal carcinoma was induced in 93% of the golden hamsters, which was proven by histopathology (Figure 4). A total of 70 animals were subjected to further experiment. As demonstrated by the tumor growth curve (Figure 5), tumor growth was fast in the blank control or GCV group, but significantly repressed in the IR group, HSV-TK/GCV group and HSV-TK/GCV/IR group. As shown in Table 1, tumor-doubling time was prolonged in the IR group, HSV-TK/GCV group and HSV-TK/GCV/IR group. Further more, there was a significant difference between HSV-TK/GCV group and HSV-TK/GCV/IR group. The results indicated

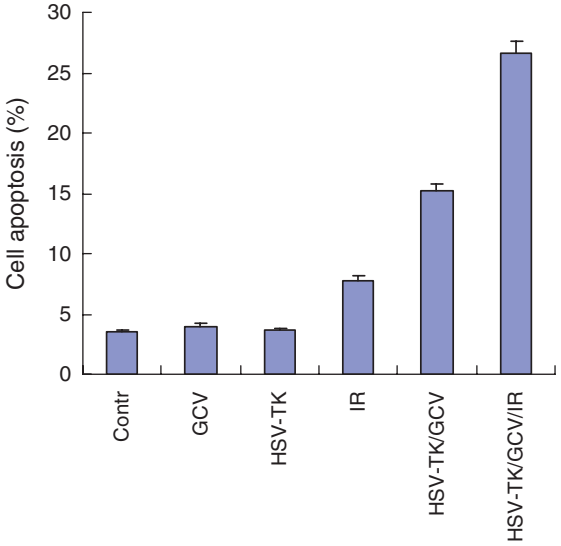

Figure 3 Cells apoptosis in different group were analyzed by flow cytometry 96 h after HSV-TK gene transfection. The apoptosis index was highest (27.12 ± 1.34%) in the HSV-TK/GCV/IR group, which was significantly higher than (15.23 ± 0.92%) in the HSV-TK/GCV group ($P < 0.05$) and (8.87 ± 0.52%) in IR group ($P < 0.01$)

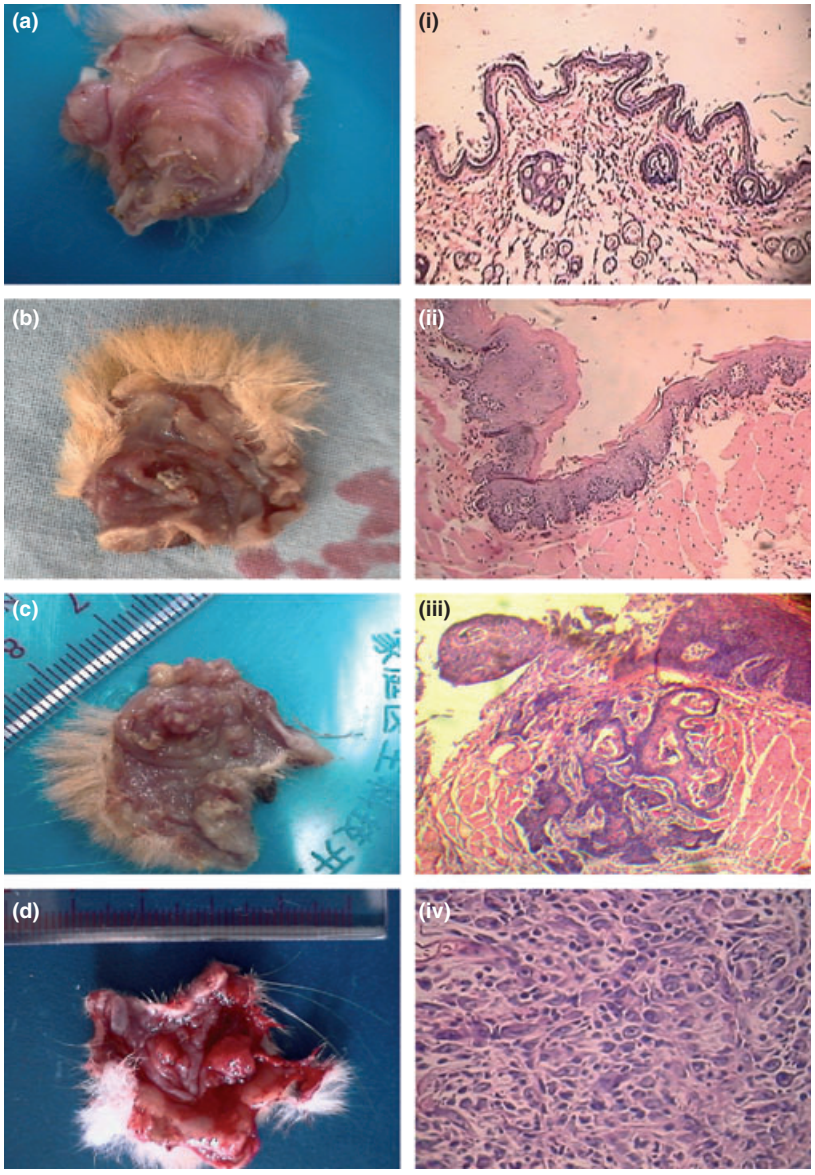

Figure 4 Gross appearance and histology on the procession of golden hamsters buccal carcinoma induced by painting DSAM. (**a**) normal buccal cyst of golden hamster; (**i**) normal buccal mucosa; (**b**) buccal cyst of golden hamster 6 weeks after painting; (**ii**) severe atypical hyperplasia in buccal mucosa 6 weeks after painting; (**c**) neoplasm in buccal cyst of golden hamster 10 weeks after painting; (**iii**) invasive carcinoma in buccal cyst of golden hamster 10 weeks after painting; (**d**) neoplasm in buccal cyst of golden hamster 12 weeks after painting; (**iv**) carcinoma in buccal cyst of golden hamster 12 weeks after painting

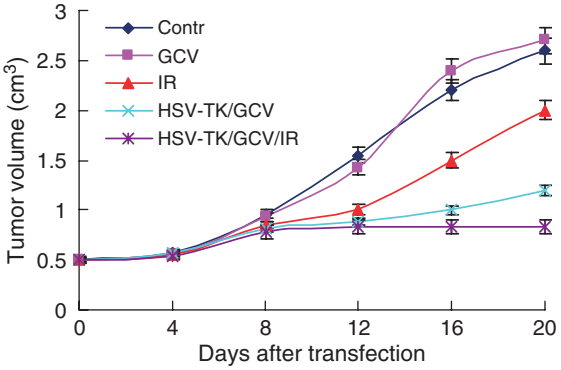

Figure 5 Tumor growth curves of buccal carcinoma of golden hamster. Tumor growth was fast in the blank control and the GCV groups, but significantly depressed in the IR, HSV-TK/GCV or HSV-TK/GCV/IR groups. Furthermore, there were significant differences among the three groups

that the suppression effect of HSV-TK/GCV gene therapy system was markedly improved by IR.

Expression of HSV-TK mRNA in golden hamster buccal carcinoma

Figure 2(b) shows that mRNA levels in the HSV-TK/GCV/IR group (1.537 ± 0.215) were significantly higher compared with those in the HSV-TK/GCV group (1.153 ± 0.139) ($P < 0.01$). This result suggests that synthesis radiation-inducible promoter combined with IR upregulated the HSV-TK gene expression in transfected golden hamster buccal carcinoma.

Proliferation index and apoptosis index

Immunohistiochemical staining showed that PI in the HSV-TK/GCV/IR group was significantly lower than that in any other group ($P < 0.05$) (Figure 6a–e, Table 1). On the other hand, the results of ISEL showed that AI in the HSV-TK/GCV/IR group was signifi-

Table 1 Inhibition effect on the golden hamster buccal carcinoma 20 days after transfection

Group	*TDT (days)*	*TV (cm^3)*	*PI (%)*	*AI (%)*
Control	2.72	3.138 ± 0.146	38.25 ± 4.32	5.76 ± 0.54
GCV	2.63	3.115 ± 0.232[a]	36.22 ± 3.43[a]	5.72 ± 0.93[a]
IR	3.85	2.212 ± 0.201[b]	25.43 ± 3.03[b]	15.32 ± 1.63[b]
HSV-TK/GCV	4.76	1.863 ± 0.087[b]	22.37 ± 2.52[b]	20.34 ± 3.91[b]
HSV-TK/GCV/IR	7.41	0.634 ± 0.025[bc]	15.86 ± 2.12[bc]	28.27 ± 4.23[bc]

TDT, tumor doubling time; TV, tumor volume at 20 days; PI, proliferation index; AI, Apoptosis index.
[a]Compared with control, $P > 0.05$.
[b]Compared with control, $P < 0.01$.
[c]Compared with HSV-TK/GCV, $P < 0.05$.

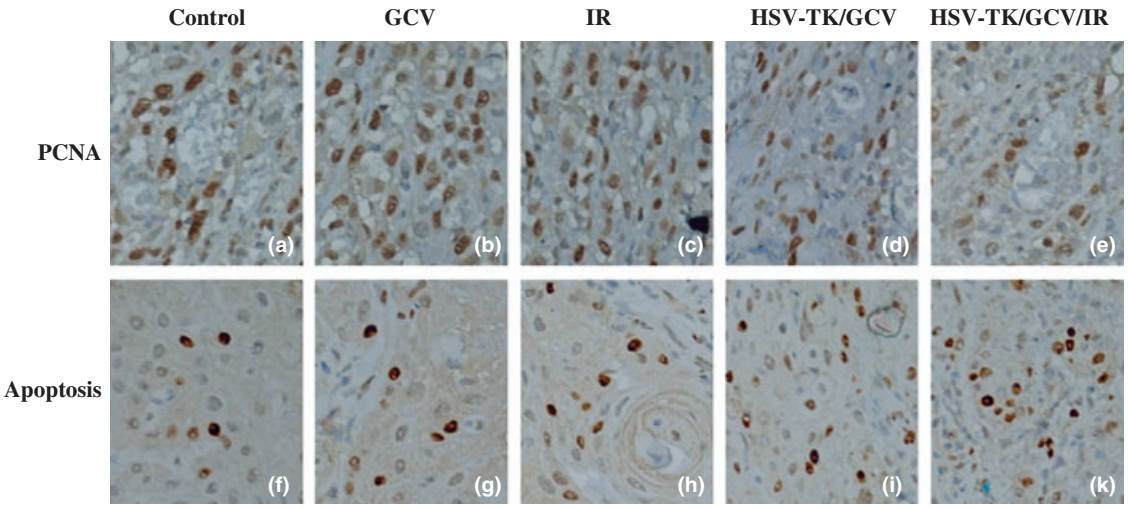

Figure 6 Expression of PCNA and apoptosis cells in the golden hamster buccal carcinoma 20 days after transfection. The proliferating cell nuclear antigen (PCNA) was detected by immunohistochemical method (**a–e**, ×200). Apoptosis were detected by *in situ* end-labeling (**f–k**, ×200)

cantly higher than that in any other group ($P < 0.05$) (Figure 6f–k, Table 1). There were significant differences between PIs (or AIs) in the HSV-TK/GCV/IR group and PIs (or AIs) in the HSV-TK/GCV group (Table 1, $P < 0.05$), which demonstrated that IR improved the tumor inhibition effect of the HSV-TK/GCV gene therapy system by enhancing apoptosis and reducing proliferation.

Discussion

The HSV-TK/GCV gene therapy system has been proven to induce effectively the killing and wounding of many malignant tumors (Huang *et al*, 2007; Yu *et al*, 2008). However, this clinical application is blocked badly because of lack of tumor specific targeting capability (Portsmouth *et al*, 2007). The control of gene expression is an important issue at all times in gene therapy.

Recently, researchers have focused on physical targeting of gene therapy by IR. This is a new strategy that combines suicide gene therapy with radiotherapy. A RecA promoter was used as a radiation-inducible promoter to increase tumor necrosis factor-α (TNF-α) production in *clostridium sp* (Nuyts *et al*, 2001). The survivin promoter has also been used as a radiation-inducible promoter to improve the therapeutic ratio of virotherapy for malignant glioma (Nandi *et al*, 2008). The promoter from the Egr-1 gene has been used successfully for selective transgene expression following radiation treatment (Marples and Collis, 2008). The combination of TNF-α gene and Egr-1 promoters has been demonstrated to be a successful therapeutic strategy for treating limb sarcomas and melanomas (James *et al*, 2002; Bickenbach *et al*, 2008).

In this strategy, the radiation-inducible promoters are inserted upstream of the therapeutic gene to regulate gene expression. The synthetic gene promoters based on the consensus sequence motif $CC(A/T)_6GG$ in native Egr-1 promoter, known as CArG element, have been proven to produce efficient and specific gene activation following IR in the gene therapy context (Scott *et al*, 2002; Coulter *et al*, 2008). As to the advantage of the synthetic promoters, the inducibility can be adjusted by regulating the numbers of CArG elements according to needs. Furthermore, Marples and Scott found that the synthetic promoters were more effective at driving IR-responsive gene expression than native Egr-1 promoters with the same number of CArG motifs in MCF-7 cells (Marples *et al*, 2000; Scott and Greco,

2004). Our previous experiment suggested that six CArG elements and 3 Gy of IR was advisable for Tca8113 cells (Yu *et al*, 2005). We investigated GFP inducibility using a promoter containing six CArG elements in Tca8113 cells under different IR doses. The results indicated that the level of GFP expression was dependent on the radiation dose, with maximum expression seen following 3-Gy IR >161% compared with the control group.

The combination of radiation and HSV-TK gene has been investigated in some tumors but rarely in OSCC (Weichselbaum *et al*, 2001; Kufe and Weichselbaum, 2003; Scully and Bagan, 2007). In present study, the synthetic promoters show radiation-inducible activity to upregulate the HSV-TK gene expression in OSCC following 3Gy IR. The HSV-TK mRNA activity increased significantly. The tumor doubling time in HSV-TK/GCV/IR group (7.32 days) was prolonged compared with HSV-TK/GCV group (4.96 days). A similar result has been reported. In a nude mouse U87-MG glioblastoma xenograft model context, the synthetic promoter-driven HSV-TK construction resulted in a significant tumor regrowth delay (44.3 days) in irradiated mice, compared with unirradiated controls (33.4 days) (Marignol *et al*, 2007). Growth delay experiments in MCF-7 human adenocarcinoma cells transfected with the HSV-TK gene placed under the control of the synthetic radiation-inducible promoter showed a dose-dependent increase in sensitivity to GCV. Reduced surviving fraction was achieved when the promoters were used following a single radiation dose of 3 Gy (Scott *et al*, 2002).

In the new era of improved imaging system, the evaluation of tumor features is facilitated especially for superficial and easily accessible tumors such as OSCC. Using magnetic resonance and optical imaging, we are able to gain a functional MR map of the tumor. With intensity-modulated radiotherapy, it is becoming increasingly possible for IR to be controlled exactly to cast tumor location. Targeted IR will allow therapeutic gene targeted expression to be mediated by radiation-inducible promoters (Greco *et al*, 2002; Scott and Greco, 2004; Marignol *et al*, 2007).

Non-specific suicide gene therapy will implicate the normal tissue and result in serious adverse effects, which is a limitation of this study. One potential promising solution for the specific control of therapeutic gene expression is the transcriptional targeting strategy. Tumor- or tissue-selective promoters were used to activate the transcription of transgenes (Konishi *et al*, 1999; Kurihara *et al*, 2000; Wuzhong *et al*, 2007). For example, adenocarcinoma, which overexpresses MUC-1, preferentially expresses therapeutic genes that are transcriptionally activated by the MUC-1 promoters (Marples and Collis, 2008). Similar strategies have been used for carcinoembryonic antigen (CEA)-expressing tumors with the therapeutic gene containing CEA promoter (Tomasek *et al*, 2005).

To educe synergistic effects by integrating various therapy strategies together is a tendency in current gene therapy. In this study, we demonstrated that synthetic radiation-inducible promoter following 3-Gy IR upregulated the expression of HSV-TK gene in transcriptional level significantly in OSCC. We also proved that the combination of HSV-TK/GCV gene therapy system with 3 Gy of IR could efficiently inhibit the growth of tumor cells by enhancing apoptosis and reducing proliferation *in vivo* or *in vitro*. Although those results are not applicable for the treatment in humans at the present time, it implied that the radiation-inducible promoter can serve as a molecular switch to regulate the expression of HSV-TK gene and that low dose induction radiation can significantly improve the targeting therapeutic efficiency in the future treatment of OSCC.

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Author contributions

D.-S. Yu and H.-Z. Huang contributed to the research design. D.-S. Yu and X.-W. Hu contributed to the plasmids construction and transfection. W. Zhao and X.-Q. Liu contributed to cell culture and animal experiment. H.-K. Tang contributed to immunohistochemistry and flow cytometry. D.-S. Yu and W. Zhao contributed to the statistical analysis and critical revision of the study.

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