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ORIGINAL PAPER

Anti-tumor effect of ^{125}I-UdR in combination with Egr-1 promoter-based IFNγ gene therapy in vivo

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Abstract Although ^{125}I-UdR treatment of malignant tumors in animal models and patients has achieved a certain effect, the short half-life of ^{125}I-UdR in vivo and its cellular uptake only in S phase of the cell cycle are limiting factors with regard to tumor eradication, and therefore its combination with other applications is a promising strategy in cancer therapy. In this study, we show that ^{125}I-UdR radionuclide therapy in combination with Egr-1 promoter-based IFNγ gene therapy is more effective than ^{125}I-UdR therapy alone in suppressing tumor growth and extending survival duration in mice bearing H22 hepatomas. Combined therapy could significantly inhibit cell proliferation and tumor angiogenesis, induce apoptosis and enhance cytotoxic activities of splenic CTL of the mice. Our results suggest that ^{125}I-UdR in combination with Egr-1 promoter-based IFNγ gene therapy may provide novel approaches for cancer treatment.

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Introduction

^{125}I-UdR is a thymidine (TdR) analog, which can be integrated into DNA molecules of proliferating tumor cells. ^{125}I releases Auger electrons in proximity to nuclear DNA, which generate double-strand breaks that may lead to cell death (Kassis et al. 1987; Makrigiorgos et al. 1989). Although ^{125}I-UdR treatment of malignant tumors in animal models and patients has achieved a certain effect, the short half-life of ^{125}I-UdR in vivo and its cellular uptake only in S phase of the cell cycle are limiting factors in tumor eradication, and therefore its combination with other applications is considered as a promising strategy in cancer therapy (Baranowska-Kortylewicz et al. 1991; Kassis et al. 2000, 1987). The discovery of the early growth response-1 (Egr-1) gene with its radiation-sensitive promoter provides a new perspective and establishes the theoretical foundation for effective combination of radiotherapy or radionuclide therapy with gene therapy (Hallahan et al. 1995). Weichselbaum and colleagues successfully constructed a recombinant plasmid containing both the radiation-inducible CArG elements of Egr-1 promoter and TNFα cDNA. Combined treatment with pEgr-TNFα using the cationic liposome delivery system and radiation resulted in increased intratumoral TNFα production, which was not associated with the limiting toxicities encountered when this cytokine was administered systemically (Weichselbaum et al. 1994). Importantly, gene expression could be controlled by radiation in space and in time (Weichselbaum et al. 2002).

Furthermore, Egr-1 promoters can be activated by internal radiation using radioisotopes as well as external radiation (Kawashita et al. 2005). Takahashi et al. (1997) reported that administration of gallium-67 to tumor cells transfected with plasmids containing the Egr-1 promoter and the herpes

simplex virus thymidine kinase (HSV-tk) gene resulted in activation of the Egr-1 promoter and induction of the HSV-tk gene. Manome et al. (1998) demonstrated that irradiation with both external beam radiotherapy and Auger electrons emitted by ^{125}I-UdR could activate adenoviral vectors containing the Egr-1 promoter and the beta-galactosidase reporter gene in malignant glioma. In preliminary studies, we have successfully constructed recombinant plasmid pcDNAEgr-IFNγ containing the Egr-1 promoter and the mIFNγ gene and found that ^{125}I-UdR can effectively activate radiation-inducible Egr-1 promoter to express its downstream IFNγ gene in a dose-dependent manner in tumor cells transfected by pcDNAEgr-IFNγ in vitro (Zhao et al. 2007). In the present study, we investigated the efficacy of ^{125}I-UdR radionuclide therapy combined with Egr-1 promoter-based IFNγ gene therapy in mice bearing H22 hepatomas and its mechanism on the premise that increased expression of IFNγ in tumor induced by Auger electrons emitted by ^{125}I-UdR would up-regulate host anticancer immunity and inhibit cell proliferation and angiogenesis in H22 hepatoma.

Materials and methods

All experiments comply with the current laws of China, according to which they were performed.

Cell line and cell culture

The mouse H22 hepatoma cell line was grown in RPMI 1640 (Gibco BRL, USA) supplemented with 10% fetal bovine serum, 100 μg/ml streptomycin and 100 U/ml penicillin. The cell line was incubated at 37°C in 5% CO_2.

Construction of recombinant plasmid

Recombinant plasmid pcDNAEgr-IFNγ was constructed by inserting the IFN-γ gene into the multiple cloning site of plasmid PcDNAEgr-1, and the transgene expression is driven by the Egr-1 promoter (Yang and Li 2005).

Preparation of ^{125}I-UdR

Preparation of ^{125}I-UdR was done according to the method described by Keough WG (1978): 10 mg UdR (Sigma) was dissolved in 0.5 ml deionized water. After adding 740 MBq Na^{125}I (China Institute of Atomic Energy) and 0.1 ml 2 mol/l HNO_3, the mixture was sealed and placed in a water bath at 80°C for 30 min. The reaction was terminated by adding strong ammonia solution. The reaction mixture was purified by Sephadex G-10 column chromatography. Radiochemical purity was 98.5%.

Tumor-bearing mice model

Female Kunming mice weighing 18 ± 2 g were purchased from the Animal Center of Jilin University. H22 hepatoma cells (5×10^5 cells per mouse in 100 μl saline) were implanted subcutaneously in the right hind leg of the mice. When the diameter of tumor reached about 6 mm, the mice were divided randomly into control, ^{125}I-UdR therapy, pcDNAEgr-IFNγ gene therapy and pcDNAEgr-IFNγ + ^{125}I-UdR group. There were each 18 mice in the control and the pcDNAEgr-IFNγ gene therapy group and each 48 mice in the other two groups. Tumors of the mice were injected with recombinant plasmid pcDNAEgr-IFNγ solution (20 μg plasmid + 40 μg lipofectamin (Gibco BRL, USA)/100 μl). Forty-eight hours later, 370 kBq ^{125}I-UdR was administered intratumorally by injection. All mice drank 0.1% solution of potassium iodide instead of water for 3 days before the trial to block the thyroid. All the animal experiments were conducted in accordance with the Guidelines for the Welfare of Animals in Experimental Neoplasia.

Measurement of radioactive content of tumors after ^{125}I-UdR injection

Tumors of the mice in ^{125}I-UdR therapy and pcDNAEgr-IFNγ + ^{125}I-UdR group were dissected, and samples were removed for the determination of the incorporated radioactivity in a γ-radio-immuno counter (Hefu Inc. Shanghai, China) at various times after ^{125}I-UdR injection. The Microcal Origin 6.0 software was used to fit non-linear regression equation. The MIRDOSE 3.0 software was applied to calculate the absorbed radiation dose (Stabin 1996; Koppe et al. 2004).

Measurement of tumor volume

The tumor growth was monitored by measuring the tumor diameters in two dimensions with a caliper every second day. The tumor volumes were calculated as follows: L (long diameter) $\times$ S^2 (short diameter)/2. The formula for tumor inhibition rate is as follows: TIR (%) = (1 − [experimental volume/control volume]) × 100.

RNA preparation and reverse transcription PCR analysis of IFN-γmRNA expression

Tumors were excised and quick-frozen in liquid nitrogen at various times after gene-radionuclide therapy. Total cellular RNA was isolated using Trizol reagent (Sangon Inc. Shanghai, China) according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA that was used for PCR amplification. Mouse ß-actin was used as an

internal reference. The following primers were used: ß-actin forward primer 5′-GTGGGCCGCTCTAGGCACCA-3′ and reverse primer 5′-CGGTTGGCCTTAGGGTTCAGGGGGG-3′; IFNγ forward primer 5′-GATCCTTTGGACCCTCTGACTT-3′ and reverse primer 5′-AGACAGTGATAAACTATAAATGAGCG-3′. RT-PCR was performed as following: denaturation at 95°C for 3 min, 30 cycles at 95°C for 45 s, at 56°C for 45 s, at 72°C for 40 s and extension at 72°C for 10 min.

Measurement of IFNγ content in H22 tumors

Tumors were homogenized in 500 μl sodium chloride–Tris buffer (pH 7.5) containing EDTA and protease inhibitors on ice for 30 s, followed by 4 cycles of freezing/thawing. Samples were sedimented at 10,000 rpm for 5 min, and the supernatant was assayed for IFNγ using a mIFNγ ELISA kit (JingMei BIOTECH, China) according to the manufacturer's instructions. IFNγ levels were corrected for total protein using the Bio-Rad Protein Micro-Assay kit.

Cytotoxic activity assay of cytotoxic T lymphocytes (CTL)

^{3}H-TdR releasing assay was used to measure the cytotoxicity. Single-cell suspensions of splenocytes from mice were prepared, washed and resuspended in RPMI 1640 medium at 1×10^7 ml^{-1}. A total of 2×10^6 H22 hepatoma cells were cultured with ^{3}H-TdR (740 kBq/20 μl) (China Institute of Atomic Energy) at 37°C in 5% CO_2 for 4 h, shaken once every 30 min and washed three times with Hank's solution. CTL cytotoxicity assay was performed using ^{3}H-labeled H22 hepatoma as target cells, with a splenocyte to ^{3}H-labeled H22 hepatoma ratio of 100:1. Triplicates were set for each sample, and cpm were recorded 18 h after incubation with a scintillation counter (Sweden). The formula for calculating cytotoxicity is as follows: cytotoxicity (%) = (1 − [experimental cpm/self-releasing cpm]) × 100.

Immunohistochemical studies for proliferating cell nuclear antigen, CD34 and cleaved caspase-3

Tumor tissues were fixed and embedded in paraffin. Tumor sections of 5 μm were cut from the embedded tissue and incubated with specific primary antibodies, including rabbit monoclonal antibody to mouse proliferating cell nuclear antigen (PCNA) (KeyGen Biotech. Nanjing, China), rabbit monoclonal antibody to mouse CD34 (eBioscience, Inc.) and rabbit polyclonal antibody to mouse cleaved caspase-3 (KeyGen Biotech. Nanjing, China) for 1 h at 37°C followed by incubation overnight at 4°C in a humidity chamber. Negative controls were incubated only with universal negative control antibodies under identical conditions. The sections were then incubated with appropriate biotinylated secondary antibody for 60 min at room temperature. Thereafter, sections were incubated with conjugated horseradish peroxidase streptavidin (KeyGen Biotech. Nanjing, China) for 60 min, followed with 3,3′-diaminobenzidine (Sigma Chemical Co.) working solution and counterstained with hematoxylin. The proliferation index was determined as number of PCNA-positive (brown) cells/total number of cells × 100, and tumor microvessel density was quantified by counting the CD34-positive cells and the total number of cells in 9 randomly selected fields (400×). The level of cleaved caspase-3 expression was also quantified as number of positive (brown) cells/total number of cells × 100 in 9 randomly selected fields (400×).

TUNEL detection of apoptotic cells in tumor tissue

One day after gene-radionuclide therapy, apoptotic cells in tumor tissue were detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) staining, using an In Situ Cell Death Detection Kit (KeyGen Biotech. Nanjing, China) following the manufacturer's specifications. In brief, tumor histological sections were permeabilized using a mixture containing 0.1% sodium citrate and 0.1% Triton X-100 and incubated with TUNEL reaction mixture containing terminal deoxynucleotidyltransferase and fluorescein-dUTP at 37°C for 60 min. The apoptotic index was calculated as number of apoptotic cells/total number of cells × 100 in 9 randomly selected fields (400×).

Data analysis

All data were calculated as Mean ± SD. Comparisons between treatment groups and controls were made by *t*-test. A *P*-value less than 0.05 between groups was considered to be significant difference.

Results

Radioactive content of tumor after ^{125}I-UdR injection

The radioactive content of tumors in the ^{125}I-UdR therapy and the pcDNAEgr-IFNγ + ^{125}I-UdR groups at various times after ^{125}I-UdR injection was measured as shown in Fig. 1. The MIRDOSE 3.0 was applied to calculate the absorbed radiation dose. The biological half-life of ^{125}I-UdR in tumors in the ^{125}I-UdR therapy and the pcDNAEgr-IFNγ + ^{125}I-UdR group was 3.46 h and 3.42 h, respectively. The deposited dose in tumors of the

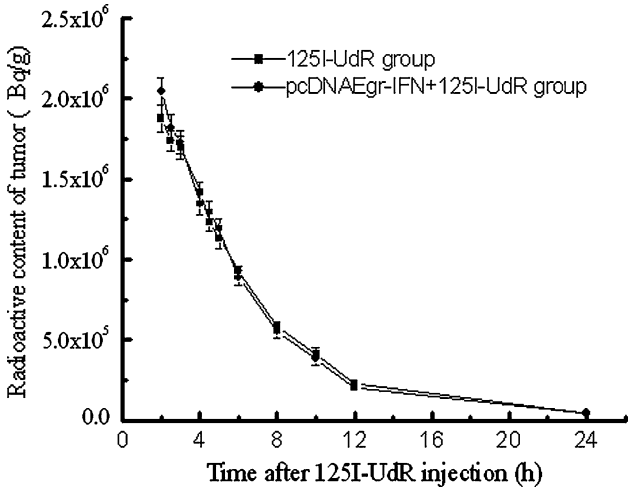

Fig. 1 Radioactive content of tumors at different time point after ^{125}I-UdR injection. $n = 3$

^{125}I-UdR therapy and the pcDNAEgr-IFNγ + ^{125}I-UdR group during 24 h after ^{125}I-UdR injection was 0.0546 Gy and 0.0551 Gy, respectively.

Effect of ^{125}I-UdR radionuclide therapy combined with IFNγ gene therapy on tumor volume of mice

The tumors were injected with recombinant plasmid pcDNAEgr-IFNγ, and 370 kBq ^{125}I-UdR was administered intratumorally by multifocus injection, followed by observing the growth of the tumors as shown in Fig. 2. At 6–15 days after gene-radionuclide therapy, the tumor volumes in the ^{125}I-UdR group and the pcDNAEgr-IFNγ + ^{125}I-UdR group were obviously lower than those of the control group ($P < 0.05$–0.01). There was no significant difference between the tumor volumes in the control group and the pcDNAEgr-IFNγ group ($P > 0.05$). The tumor volumes in the pcDNAEgr-IFNγ + ^{125}I-UdR group was obviously lower than that in the ^{125}I-UdR group 6–15 days after gene-radionuclide therapy ($P < 0.05$–0.01). Fifteen days after gene-radionuclide therapy, the tumor inhibition rates in the ^{125}I-UdR group and the pcDNAEgr-IFNγ + ^{125}I-UdR group were 40.54% and 61.94%, respectively.

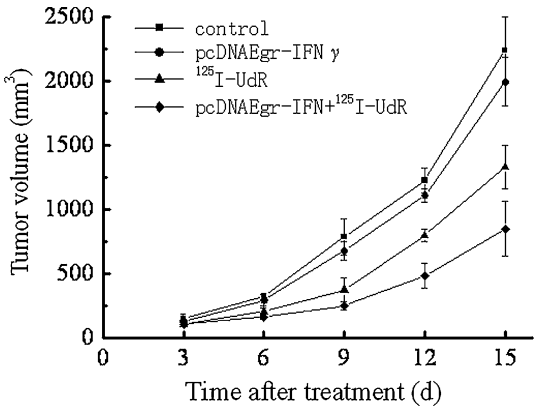

Fig. 2 Effect of ^{125}I-UdR radionuclide therapy combined with IFNγ gene therapy on H22 tumor growth in mice. The tumor volumes of mice 3–15 days after treatment are shown. $n = 6$

Effect of gene-radionuclide therapy on mean survival time of mice

The effects of the different treatments on survival were evaluated using Kaplan–Meier plots as shown in Fig. 3. Mean survival times of the mice in the ^{125}I-UdR group and the pcDNAEgr-IFNγ + ^{125}I-UdR group were longer than that of the mice in the control group ($P < 0.05$–0.01). There was no significant difference between the mean survival times of control group and pcDNAEgr-IFNγ group ($P > 0.05$). The mean survival time of the mice in the pcDNAEgr-IFNγ + ^{125}I-UdR group was the longest in all groups (58.33 ± 4.32 days), which was significantly longer than that of ^{125}I-UdR group ($P < 0.05$).

IFNγ mRNA expression in H22 tumors in mice after gene-radionuclide therapy

Because the H22 tumors received only a single injection of recombinant plasmid pcDNAEgr-IFNγ, it was important to determine both the amount and the duration of IFNγ expression in the tumor cells. H22 hepatoma tumors growing in the right hind legs of mice were excised, and IFN-γ mRNA levels were semi-quantitatively determined by RT-PCR as shown in Fig. 4. IFNγ mRNA levels in the

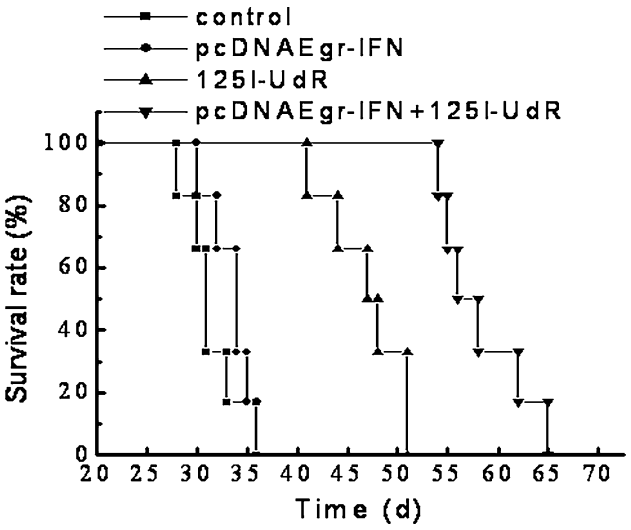

Fig. 3 Effect of ^{125}I-UdR radionuclide therapy combined with IFNγ gene therapy on survival of mice bearing H22 tumors. The survival rate of mice at different time points after treatment is shown. $n = 6$

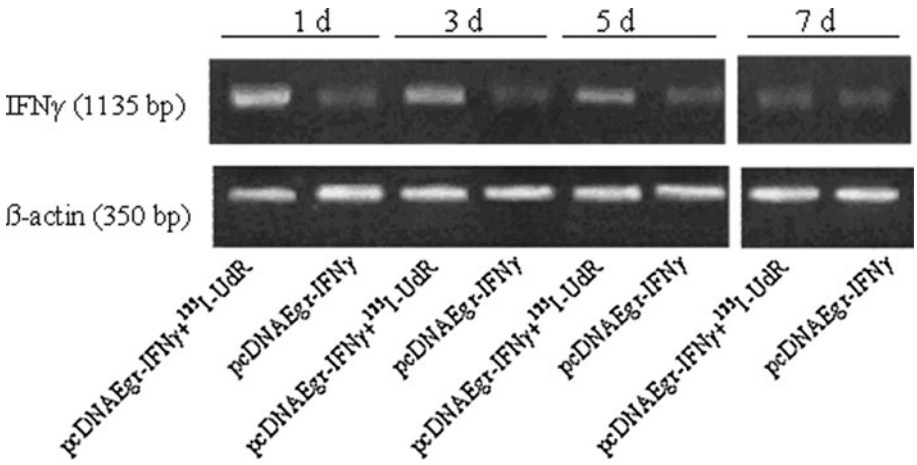

Fig. 4 Detection of IFNγ mRNA expression in H22 tumors in mice after gene-radionuclide therapy by RT-PCR

pcDNAEgr-IFNγ + ^{125}I-UdR group were significantly higher than those of the pcDNAEgr-IFNγ group on day 1, 3 and 5 after treatment ($P < 0.05$). IFNγ mRNA levels showed no significant difference between the pcDNAEgr-IFNγ + ^{125}I-UdR group and the pcDNAEgr-IFNγ group 7 days after treatment. No IFNγ mRNA was detected in H22 tumors in the control group and the ^{125}I-UdR group.

IFNγ production in H22 tumors in mice after gene-radionuclide therapy

IFNγ levels in tumors of the pcDNAEgr-IFNγ + ^{125}I-UdR group peaked at 522 ± 34 pg/mg total protein and were 5.16 times higher than that of the pcDNAEgr-IFNγ group 1 day after treatment. IFNγ levels of the pcDNAEgr-IFNγ + ^{125}I-UdR group were significantly higher than those of the pcDNAEgr-IFNγ group on days 1, 3 and 5 after treatment, as shown in Fig. 5 ($P < 0.05$–0.01). However, there was a progressive time-dependent reduction of IFNγ levels in the pcDNAEgr-IFNγ + ^{125}I-UdR group. Thus, 7 days after treatment, IFNγ levels showed no significant difference between the pcDNAEgr-IFNγ + ^{125}I-UdR group and the pcDNAEgr-IFNγ group. No IFNγ was detected in H22 tumors in the control group and the ^{125}I-UdR group. These data demonstrate that a significant amount of IFNγ protein can be induced after a single injection of 370 kBq ^{125}I-UdR using the cationic liposome delivery system.

Cytotoxic activities of splenic CTL of the mice 3 days after therapy

To investigate the immune mechanism of gene-radionuclide therapy, a ^{3}H-TdR releasing assay was used to measure the cytotoxicity of splenic CTL of the mice 3 days after therapy. The cytotoxic activity of splenic CTL of the mice in the pcDNAEgr-IFNγ + ^{125}I-UdR group was significantly higher than that in the control group, pcDNAEgr-IFNγ group and ^{125}I-UdR group, as shown in Fig. 6

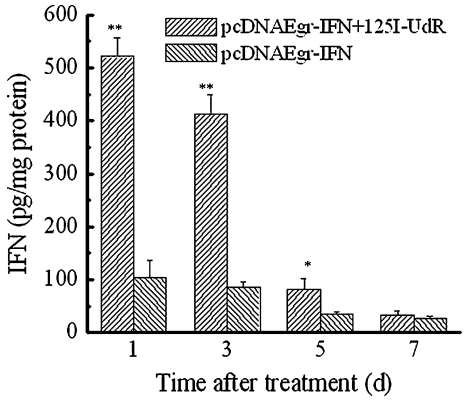

Fig. 5 IFNγ production in H22 hepatoma in mice after gene-radionuclide therapy. $n = 3$, * $P < 0.05$, ** $P < 0.01$ versus pcDNAEgr-IFNγ group

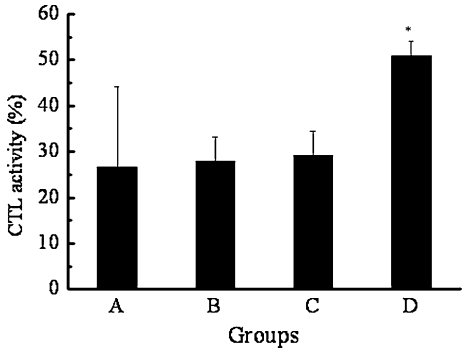

Fig. 6 Cytotoxic activities of splenic CTL of the mice 3 days after therapy. **a** control group; **b** pcDNAEgr-IFNγ group; **c** ^{125}I-UdR group; D: pcDNAEgr-IFNγ + ^{125}I-UdR group. $n = 6$, * $P < 0.01$ versus control/pcDNAEgr-IFNγ/^{125}I-UdR group

($P < 0.01$). There was no significant difference between the cytotoxic activities of splenic CTL of the mice in control group, pcDNAEgr-IFNγ group and ^{125}I-UdR group ($P > 0.05$).

Effect of gene-radionuclide therapy on cell proliferation in H22 tumors

We examined the cell proliferation in H22 tumors 3 days after gene-radionuclide therapy by immunohistochemical analysis of PCNA expression. In microscopic observation of the tumors, lower numbers of PCNA-positive cells were observed in the ^{125}I-UdR group ($P < 0.01$) and the pcDNAEgr-IFNγ + ^{125}I-UdR group ($P < 0.001$) when compared with the control group, as shown in Fig. 7. There was no significant difference between the number of PCNA-positive cells in the control group and the pcDNAEgr-IFNγ group ($P > 0.05$). In addition, PCNA-positive cells were significantly decreased in the pcDNAEgr-IFNγ + ^{125}I-UdR group compared to the ^{125}I-UdR group ($P < 0.001$).

Effect of gene-radionuclide therapy on tumor angiogenesis in H22 tumors

The effect of gene-radionuclide therapy on tumor angiogenesis was also analyzed 7 days after therapy by staining of CD34, which is a specific marker of endothelial cells. The microscopic examination revealed lower numbers of CD34-positive cells in tumors of the pcDNAEgr-IFNγ + ^{125}I-UdR group when compared to the control group ($P < 0.001$), as shown in Fig. 8. There was no significant difference between the number of CD34-positive cells in tumors of the mice in the control group, the pcDNAEgr-IFNγ group and the ^{125}I-UdR group ($P > 0.05$). These data demonstrate that tumor angiogenesis could significantly be inhibited by gene-radionuclide therapy.

Effect of gene-radionuclide therapy on apoptosis in H22 tumors

TUNEL staining was performed to assess the apoptotic effect of gene-radionuclide therapy on tumors 1 day after gene-radionuclide therapy. An increased number of TUNEL-positive cells was observed in the pcDNAEgr-IFNγ + ^{125}I-UdR group ($P < 0.001$) and the ^{125}I-UdR group ($P < 0.001$) when compared to the control group, as shown in Fig. 9. There was no significant difference between the number of TUNEL-positive cells in the pcDNAEgr-IFNγ + ^{125}I-UdR group and the ^{125}I-UdR group ($P > 0.05$).

Because the Auger electrons released from ^{125}I-UdR are known to induce apoptosis through mitochondrial-mediated pathways and subsequent activation of caspase-3 (Urashima et al. 2004), we analyzed the levels of cleaved caspase-3 in tumor sections by immunohistochemistry staining 1 day after gene-radionuclide therapy.

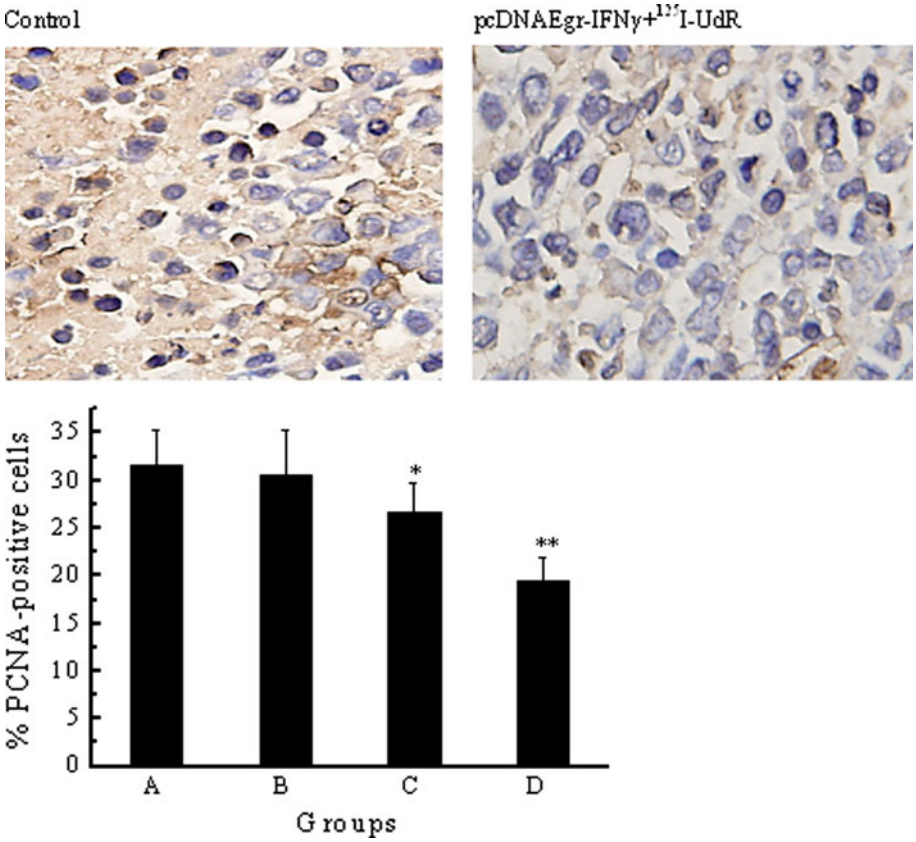

Fig. 7 Effect of gene-radionuclide therapy on cell proliferation in H22 tumors. PCNA expression in tumors was examined by immunohistochemical analysis. The percentage of PCNA-positive cells in tumor are shown. **a** control group; **b** pcDNAEgr-IFNγ group; **c** ^{125}I-UdR group; **d** pcDNAEgr-IFNγ + ^{125}I-UdR group. $n = 9$, * $P < 0.01$, ** $P < 0.001$ versus control group

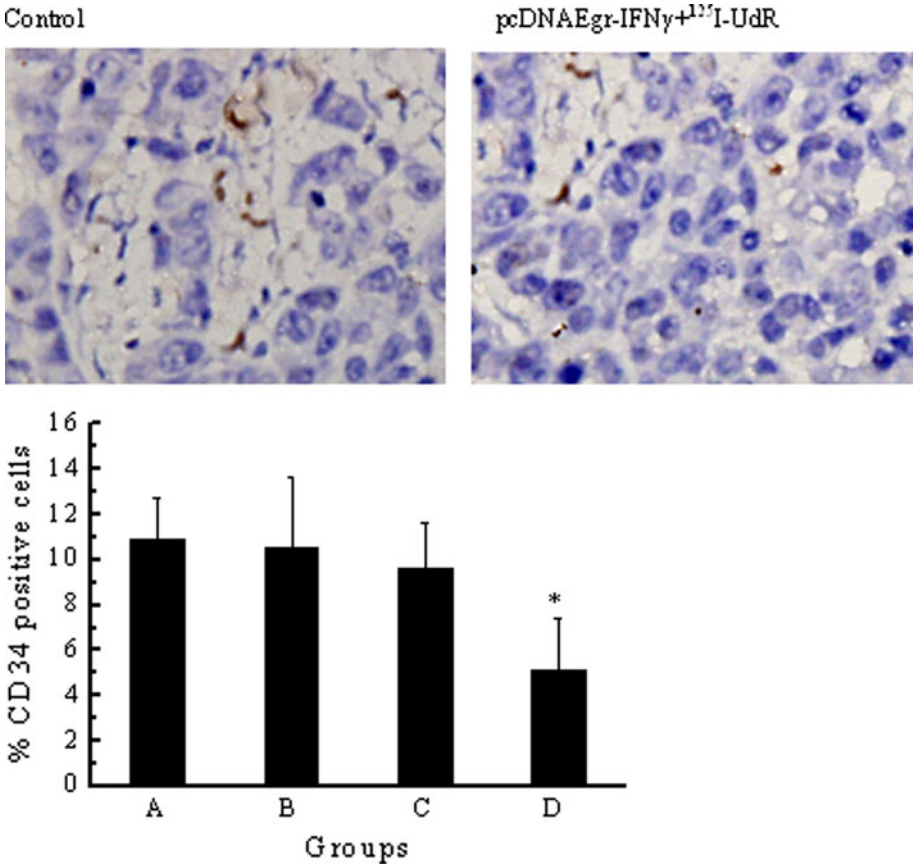

Fig. 8 Effect of gene-radionuclide therapy on tumor angiogenesis in H22 tumors. CD34 expression in tumors was examined by immunohistochemical analysis. The percentages of CD34-positive cells in tumor are shown. **a** control group; **b** pcDNAEgr-IFNγ group; **c** ^{125}I-UdR group; **d** pcDNAEgr-IFNγ + ^{125}I-UdR group. $n = 9$, * $P < 0.001$ versus control group

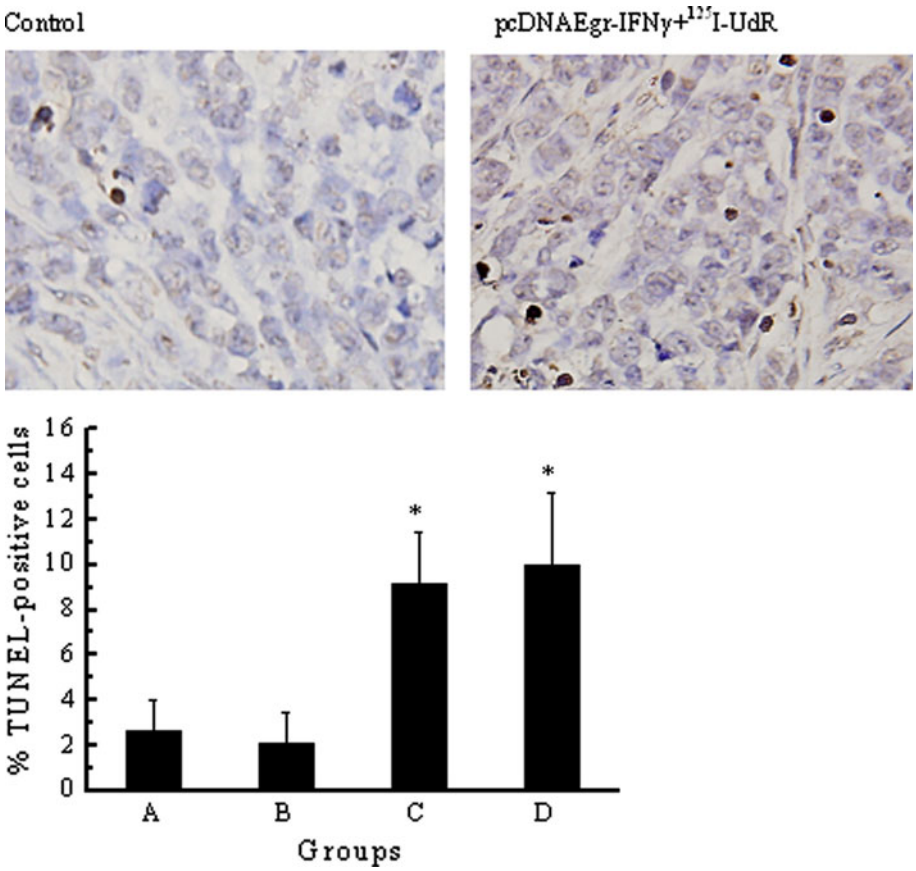

Fig. 9 Effect of gene-radionuclide therapy on apoptosis in H22 hepatoma. The percentages of TUNEL-positive cells in tumor are shown. **a** control group; **b** pcDNAEgr-IFNγ group; **c** ^{125}I-UdR group; **d** pcDNAEgr-IFNγ + ^{125}I-UdR group. $n = 9$, * $P < 0.001$ versus control group

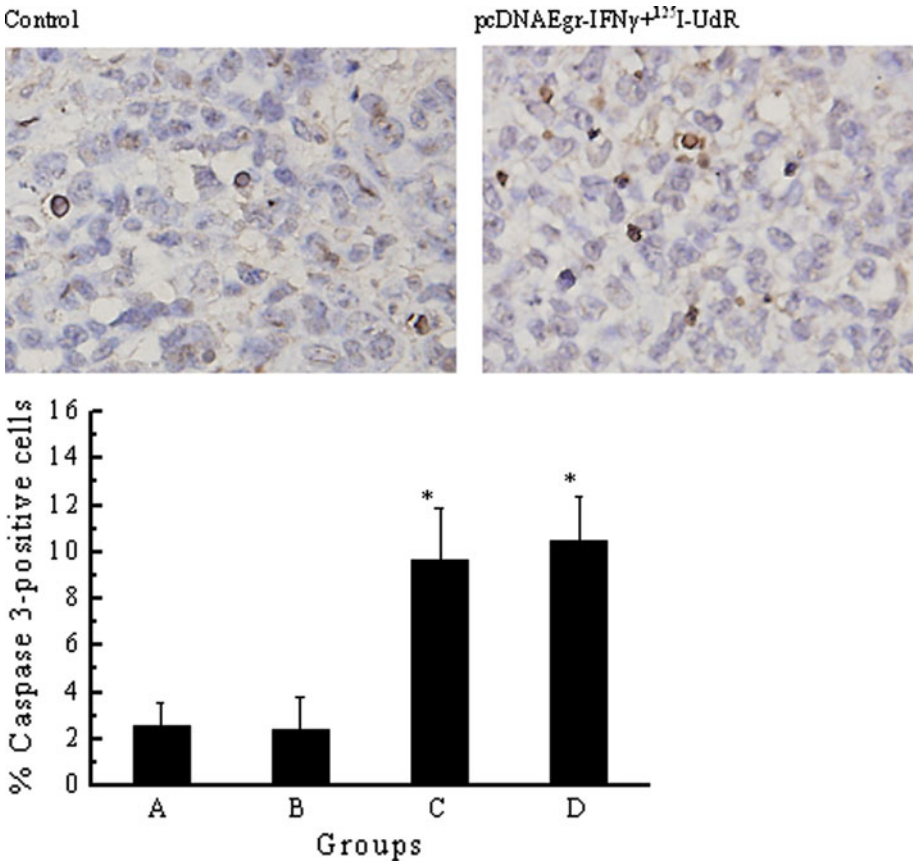

Fig. 10 Effect of gene-radionuclide therapy on cleaved caspase-3 expression in H22 hepatoma. We analyzed the levels of cleaved caspase-3 in tumor sections by immunohistochemistry staining. The percentages of cleaved caspase-3-positive cells in tumor are shown. **a** control group; **b** pcDNAEgr-IFNγ group; **c** ^{125}I-UdR group; **d** pcDNAEgr-IFNγ + ^{125}I-UdR group. $n = 9$, * $P < 0.001$ versus control group

Microscopic examination of cleaved caspase-3 staining showed an increased number of positive cells in the pcDNAEgr-IFNγ + ^{125}I-UdR group ($P < 0.001$) and the ^{125}I-UdR group ($P < 0.001$) compared to the control group, as shown in Fig. 10. There was no significant difference between the number of cleaved caspase-3-positive cells in the pcDNAEgr-IFNγ + ^{125}I-UdR group and the ^{125}I-UdR group ($P > 0.05$).

Discussion

5-iodo-2′deoxyuridine (IUdR) behaves remarkably like TdR. Within the cell, IUdR and TdR are phosphorylated by thymidine kinase to IUdR monophosphate (IdUMP) and TdR monophosphate (dTMP), respectively. dTMP is then further phosphorylated in a stepwise reaction and incorporated into DNA. IdUMP, on the other hand, may either follow a similar fate of phosphorylation and DNA incorporation or be dehalogenated by thymidylate synthetase (TS) to dUMP, which is further converted to dTMP (Semnani et al. 2005). IUdR is stable in vitro but quite unstable in vivo, with an overall half-life in the circulation of 5 min in humans and 7 min in mice. IUdR is rapidly degraded and dehalogenated mainly in the liver and excreted via the kidneys (Kassis 2003). The absolute uptake by tumors and the tumor-to-non-tumor ratios of IUdR radiolabeled with ^{125}I, ^{125}I-UdR are low when this radiopharmaceutical is administered systemically. Intratumoral administration is a good approach to bypass the rapid breakdown, to ensure the availability of radiolabeled IUdR molecules to dividing tumor cells, and to achieve excellent tumor therapy (Buchegger et al. 2004).

IFNγ has been investigated as a potential therapy for various types of cancer. The antitumor activity of IFNγ is attributed to direct actions on tumor cells and indirect mechanisms, such as immunomodulation and antiangiogenesis (Zhao et al. 2007). IFNγ stimulates the expression of Th1 cytokines (e.g., IL-2, TNF-α,GM-CSF) and mediates cellular immunity. IFNγ enhances susceptibility of tumor cells to CTL by up-regulating MHC-I expression on tumor cell surfaces (Yang and Li 2005; Wu et al. 2006). Furthermore, IFNγ is the mediator of the IL-12-induced anti-angiogenic effect, and interferon-inducible protein-10 (IP-10) and monokine induced by IFNγ (MIG) are IFNγ-induced anti-angiogenic factors (Angiolillo et al. 1996; Horton et al. 1998). However, it is difficult to attain a local therapeutic concentration by administration of recombinant IFNγ protein because of its short half-life and significant side effects (Zhao et al. 2007; Wu et al. 2006). Egr-1

promoter-based IFNγ gene-radionuclide therapy may overcome the pitfalls of recombinant IFNγ therapy. The early growth response 1 (Egr-1) gene encodes a 533-amino acid phosphoprotein transcription factor (Sukhatme 1990). Previous studies have demonstrated that irradiation increases transcription of the Egr-1 gene (Datta et al. 1992). The Egr-1 promoter sequence responsible for this effect has been localized to six $CC(A/T)_6GG$ elements. Reactive oxygen intermediates target $CC(A/T)_6GG$ sequences to mediate activation of the early growth response 1 transcription factor gene by ionizing radiation (Datta et al. 1993). The use of such a radiation-inducible promoter allows the expression of a therapeutic gene to be confined within a specific irradiated volume for a chosen period of time. The spatial and temporal control of therapeutic gene expression potentially increases the efficiency and safety of gene therapy (Hallahan et al. 1995; Weichselbaum et al. 1994, 2002; Wu et al. 2008). Cationic liposome was used to transfect plasmids into tumor cells because it has been proven a safe and effective approach in tumor gene therapy (Meidan et al. 2006). It is not necessary to have all the tumor cells transfected with the therapeutic gene. Only a few cells incorporating the gene would be enough to enable the product of gene expression to exert an effect through autocrine and paracrine pathways and circulation (Chien et al. 2005). Moreover, one injection may have a relatively long-standing effect, and injections could be repeated if necessary (Morille et al. 2008).

The experimental results showed that ^{125}I-UdR combined with Egr-1 promoter-based IFNγ gene therapy was more effective than ^{125}I-UdR therapy alone in suppressing tumor growth and extending survival duration in mice bearing H22 tumors. IFNγ mRNA and protein levels in combined therapy group were significantly higher than those of the Egr-1 promoter-based IFNγ gene therapy alone group at various times up to 5 days after injection of ^{125}I-UdR. This suggests that the Egr-1 promoter was activated to induce IFNγ expression strongly and transiently in the tumors after a single intratumoral injection of 370 kBq ^{125}I-UdR. Egr-1 promoter-based IFNγ gene therapy alone showed no significant anti-tumor effects because the Egr-1 promoter was not activated to induce enough IFNγ expression. Combined therapy significantly inhibited cell proliferation and tumor angiogenesis and enhanced cytotoxic activities of splenic CTL compared with ^{125}I-UdR therapy alone. These changes resulted from the increased expression of IFNγ induced by Auger electrons emitted by ^{125}I-UdR.

Tumor gene-radionuclide therapy provides a new approach for cancer treatment. However, as a relatively new subject, some problems remain to be solved, such as selection of proper genes and doses of ^{125}I-UdR administration. It is possible that more encouraging results may occur in the near future with further optimization of the strategy.

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