

Human Sulfatase-1 Improves the Effectiveness of Cytosine Deaminase Suicide Gene Therapy with 5-Fluorocytosine Treatment on Hepatocellular Carcinoma Cell Line HepG2 *In Vitro* and *In Vivo*

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Abstract

Background: Human sulfatase-1 (Hsulf-1) is an endosulfatase that selectively removes sulfate groups from heparan sulfate proteoglycans (HSPGs), altering the binding of several growth factors and cytokines to HSPG to regulate cell proliferation, cell motility, and apoptosis. We investigated the role of combined cancer gene therapy with Hsulf-1 and cytosine deaminase/5-fluorocytosine (CD/5-FC) suicide gene on a hepatocellular carcinoma (HCC) cell line, HepG2, *in vitro* and *in vivo*.

Methods: Reverse transcription polymerase chain reaction and immunohistochemistry were used to determine the expression of Hsulf-1 in HCC. Cell apoptosis was observed through flow cytometry instrument and mechanism of Hsulf-1 to enhance the cytotoxicity of 5-FC against HCC was analyzed in HCC by confocal microscopy. We also establish a nude mice model of HCC to address the effect of Hsulf-1 expression on the CD/5-FC suicide gene therapy *in vivo*.

Results: A significant decrease in HepG2 cell proliferation and an increase in HepG2 cell apoptosis were observed when Hsulf-1 expression was combined with the CD/5-FC gene suicide system. A noticeable bystander effect was observed when the *Hsulf-1* and *CD* genes were co-expressed. Intracellular calcium was also increased after HepG2 cells were infected with the *Hsulf-1* gene. *In vivo* studies showed that the suppression of tumor growth was more pronounced in animals treated with the Hsulf-1 plus CD than those treated with either gene therapy alone, and the combined treatment resulted in a significant increase in survival.

Conclusions: Hsulf-1 expression combined with the CD/5-FC gene suicide system could be an effective treatment approach for HCC.

Key words: Cytosine Deaminase/5-Fluorocytosine Suicide System; Hepatocellular Carcinoma; Human Sulfatase-1

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related deaths and the worldwide incidence of this cancer has been increasing in recent years. Most HCCs are not cured by conventional treatments, such as surgical ablation, chemotherapy and liver transplantation. Patients treated conventionally only have a 50% survival rate at 5 years. There is a need for new therapeutic approaches to treat mid- and advanced-stage HCC.^[1,2] Several reports have suggested that chemotherapy in combination with gene therapy may have more advantages than chemotherapy alone in treating cancers.

Previous studies have shown that the transduction of the cytosine deaminase (*CD*) gene into cancer cells followed by treatment with the prodrug 5-fluorocytosine (5-FC), is a successful suicide gene therapy strategy for various cancers. CD deaminates 5-FC into the cytotoxic drug 5-fluorouracil (5-FU), which is one of the standard drugs used in chemotherapy to treat HCC. The *in situ* generation of 5-FU by CD mediated deamination of 5-FC, reduces systemic exposure to the cytotoxic agent and allows for higher concentrations of the drug in the target tissue.^[3,4] Human sulfatase 1 (Hsulf-1) is a recently characterized endosulfatase that selectively removes sulfate groups from heparan sulfate proteoglycans (HSPG), thereby altering the binding sites on HSPG for signaling molecules. Studies have

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demonstrated that re-expression of Hsulf-1 in ovarian cells suppresses fibroblast growth factor-2 and heparin-binding epidermal growth factor signaling, thereby inhibiting cell proliferation and cell invasion *in vitro*. Further studies of the role of Hsulf-1 in tumorigenesis and tumor progression have also found that expression of Hsulf-1 inhibits both hepatocyte growth factor and vascular endothelial growth factor signalling.^[5-8] Due to these properties Hsulf-1 may be a potentially promising candidate for cancer gene therapy. To date, there have been no studies combining the expression of the *Hsulf-1* gene with the CD/5-FC gene suicide system as a therapeutic strategy to treat cancer.

In this study we investigated the combination of *CD* plus *Hsulf-1* gene therapy to enhance the cytotoxicity of 5-FC against HCC in both *in vitro* and *in vivo* systems. In addition, we explored the bystander effect of *CD* plus *Hsulf-1* gene therapy and investigated a possible mechanism of action for the antitumor activity of Hsulf-1.

METHODS

Cell culture and vectors

The HCC cell line HepG2 was purchased from Shanghai Cell Bank (Shanghai, China) and cultured at 37°C in an atmosphere of humidified air with 5% CO₂ in media, according to the manufacturer's recommendations. Expression vectors pcDNA 3.1(+)-Hsulf-1, pcDNA 3.1(+)-CD and pcDNA 3.1(+)-Hsulf-1-IRES-CD were purchased from Wuhan Genesil Biotechnology (Wuhan, China). Transfections were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). HepG2 cells stably expressing Hsulf-1 and/or CD were selected with 600 µg/mL G418 (Invitrogen), and successful transfections were monitored by reverse transcription polymerase chain reaction (RT-PCR).

Reverse transcription polymerase chain reaction

Total RNA was isolated from HCC cells using an RNeasy kit (Qiagen, Valencia, CA, USA). Taq enzyme and PCR reagents were purchased from Tiangen Corp (China). Primers were designed according to the gene sequences published in GenBank and purchased from Shanghai Sangon Biological Technology. The forward and reverse primers were as follows: 5'-CTCACAGTCCGGAGCGGAAC-3' (forward) and 5'-CACGGCGTTGCTGCTATCTGCCAGCATCC-3' (reverse) for *Hsulf-1*; and 5'-GCCAATATGCTGCAAGTGCT-3' (forward) and 5'-GTTGTGTGCTGGCAATCACCT-3' (reverse) for *CD*; yielding amplicons of 371 bp and 234 bp, respectively. Semiquantitative RT-PCR products were analyzed on 1% agarose gels stained with ethidium bromide.

Immunohistochemistry

Stably transfected HepG2 cells were plated at a density of 1 × 10⁵ cells/well. After a 24 h incubation, the cells were washed with phosphate-buffered saline (PBS) three times, and fixed with 4% paraformaldehyde for 10 min. An anti-Hsulf-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 0.3% Triton X 100 was incubated at 4°C overnight,

followed by incubation with the secondary antibody for 2 h at room temperature. Horseradish peroxidase-DAB was used as the detection system (R and D Systems China, Shanghai, China) and the cells counter-stained with hematoxylin.

Chemosensitivity *in vitro*

HepG2 cells stably transfected with plasmids pcDNA 3.1(+)-Hsulf-1, pcDNA 3.1(+)-CD, pcDNA 3.1(+)-Hsulf-1-IRES-CD and pcDNA 3.1(+)(blank control) were cultured and seeded in 96-well plates at 4 × 10³ cells/well. After a 24 h incubation, the cells were exposed to 5-FC at various concentrations (0, 0.01, 0.1, 1, 10, 100, 1000 µmol/L) for 96 h and then cell viability was assessed by MTT. Twenty microliters of MTT dye (5 mg/ml) was added to each well and the cells incubated for 4 h followed by the addition of 100 µl DMSO for 10 min to dissolve the crystals. The absorbance was measured with a microtiter plate reader at 490 nm. Results were expressed as cell survival rate compared with the control. Cell survival rate was calculated by: Survival rate (%) = A/B × 100%. (A: OD value of HepG2/Hsulf-1, HepG2/CD, HepG2/Hsulf-1-IRES-CD or HepG2/blank cells; B: OD value of HepG2 parent cells).

Bystander effect

Cocultures of 10% of parent cells and 90% of each type of transfected cells were inoculated onto a 96-well plate at 4 × 10³ cells/well in triplicate. After a 24 h incubation, the mixed cell cultures were exposed to 1 mmol/L 5-FC or 10 mmol/L 5-FC. After a 96 h incubation an MTT assay was performed to assess viability.

Cellular apoptosis assay

To assess apoptosis, stably transfected cells were plated at a density of 4 × 10³ cells/well and incubated for 24 h and then 1 mmol/L 5-FC was added to the culture for 24 h. The cells were harvested 96 h later and diluted to 6 × 10⁵ cells/ml. Apoptotic cells were quantified by annexin-V/propidium iodide (PI) double staining (Jingmei, Shenzhen, China), according to the manufacturer's instructions. Briefly, cells were collected, washed twice in cold PBS, resuspended in 250 µl of binding buffer and stained with 5 µl of annexin-V-FITC and 10 µl of PI for 15 min in the dark at room temperature. The cells were analyzed using flow cytometry.

Laser scanning confocal microscopy

Laser scanning confocal microscopy was used to visualize the intracellular calcium levels. Stably transfected cells were plated at a density of 1 × 10⁵ cells/well on a LabTek 8-well chamber slide and incubated for 24 h. The HepG2 cells were then exposed to 0.4 µmol/L Fluo-3/AM working solution for 20 min, followed by 1% fetal bovine serum in Hank's balanced salt solution for 40 min, and finally washed twice with HEPES buffered saline. Fluorescence was observed by laser scanning confocal microscopy, with excitation at 488 nm and emission at 528 nm.

Tumor model of human hepatocellular carcinoma in nude mice

Male BALB/c athymic nude mice aged 4–6 weeks (18–22 g)

were purchased from Wuhan University Experimental Animal Center and handled in accordance with the Wuhan University Experimental Animal Center Committee guidelines (Wuhan, China). The nude mice were randomly divided into four groups: HepG2/Hsulf-1 group ($n = 10$); HepG2/CD group ($n = 10$); HepG2/Hsulf-1-IRES-CD group ($n = 10$); and HepG2/blank cells group ($n = 10$). Each of the transfected cell types were subcutaneously inoculated in the right hind flank at a concentration of 1×10^7 cells/ml. Six days after initial tumor inoculation, all the mice received an intraperitoneal injection of 5-FC (500 mg/kg), which was repeated once/day for 2 weeks. Tumor growth was monitored twice a week using vernier calipers and the tumor volume calculated from the square of the longest diameter (B) multiplied by a smaller diameter (A) of tumor ($V = AB^2$). To determine the survival rate of the mice, they were observed until they were close to death when they were humanely euthanized.

Statistical analysis

All data were analyzed using GraphPad Prism 5.02 for Windows. Data are expressed as the mean \pm standard error. The significance of differences between groups was determined by two-sided *t*-tests. Comparison between each two groups was performed by one-way analysis of variance. $P < 0.05$ were considered to be statistical significance.

RESULTS

Reverse transcription polymerase chain reaction verification of cell transfection

In the control HepG2/blank cells there were no detectable mRNAs from either the *Hsulf-1* or *CD* gene. In the HepG2/Hsulf-1-IRES-CD and HepG2/Hsulf-1 transfected cells RT-PCR demonstrated the presence of Hsulf-1 mRNA. In the HepG2/Hsulf-1-IRES-CD or HepG2/CD transfected cells CD mRNA was detected [Figure 1].

The expression of human sulfatase-1 protein

Immunohistochemical methods were used to detect the expression of Hsulf-1 protein in the HepG2 cells infected with

the plasmids. The results showed that Hsulf-1 was expressed in HepG2/Hsulf-1-IRES-CD and HepG2/Hsulf-1 transfected cells. No Hsulf-1 protein was detected in transfected HepG2/CD and control HepG2/blank cells [Figure 1b].

Chemosensitivity on HepG2 cells

Figure 2a shows the effects of the combination gene therapy with 5-FC treatment on HepG2 cells. The HepG2/Hsulf-1-IRES-CD and HepG2/CD transfected cells were sensitive to 5-FC at concentrations above 1 $\mu\text{mol/L}$ and exhibited a dose-dependent response. In the HepG2/Hsulf-1 cells cytotoxicity was reduced in the presence of 5-FC, although there was limited dose-dependency. The control HepG2/blank cells were insensitive to 5-FC, with approximately 10% of the cells exhibiting cytotoxicity to 5-FC at a concentration of 1 mmol/L. In the HepG2/Hsulf-1-IRES-CD cells cytotoxicity was significantly greater than that in HepG2/CD cells exposed to 5-FC. The concentrations of 5-FC that induced 50% cell lethality were 4.3 $\mu\text{mol/L}$ and 13.2 $\mu\text{mol/L}$ for HepG2/Hsulf-1-IRES-CD and HepG2/CD transfected cells respectively. These results demonstrated that cytotoxicity was highest in HepG2/Hsulf-1-IRES-CD cells.

Bystander effect *in vitro*

We investigated the bystander effect of each group in response to 5-FC treatment. Cocultures consisting of 10% of HepG2/Hsulf-1-IRES-CD, HepG2/CD, HepG2/Hsulf-1 and control HepG2/blank cells and 90% bystander HepG2 cells were incubated with 1 mmol/L 5-FC, and cell survival rates were $23.23 \pm 1.52\%$, $35.72 \pm 1.97\%$, $89.23 \pm 3.19\%$ and $95.83 \pm 3.51\%$ respectively. Significant reductions in cell survival were observed when comparing HepG2/Hsulf-1-IRES-CD group or HepG2/CD group to the other groups ($P < 0.05$). There was no significant difference in the percent cell survival between the HepG2/Hsulf-1 and HepG2/blank groups. In the presence of 10 mmol/L 5-FC, the cell survival rates were $13.25 \pm 0.96\%$, $22.03 \pm 1.34\%$, $86.56 \pm 2.89\%$ and $94.23 \pm 3.67\%$ in HepG2/Hsulf-1-IRES-CD, HepG2/CD, HepG2/Hsulf-1 and control HepG2/blank cells respectively. When the concentration of

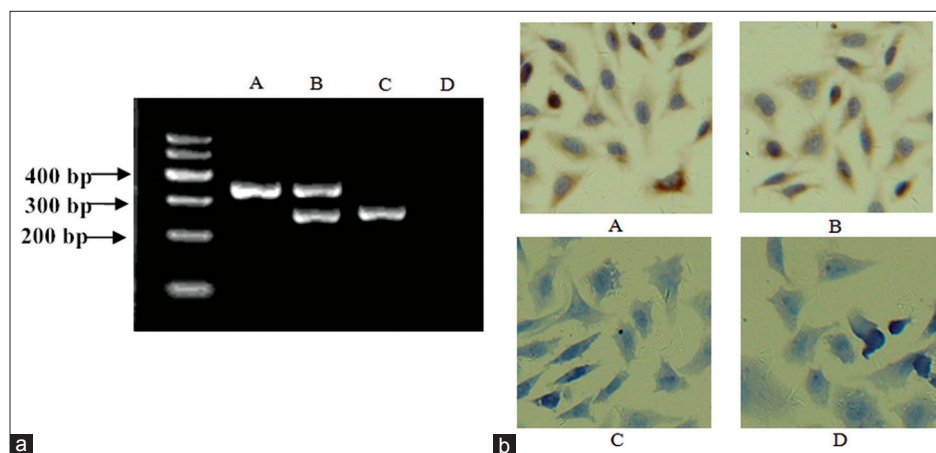


Figure 1: (a) Expression of human sulfatase-1 (Hsulf-1) mRNA by reverse transcription polymerase chain reaction; (b) Hsulf-1 protein by immunohistochemistry A: HepG2/Hsulf-1 cells; B: HepG2/Hsulf-1-IRES-cytosine deaminase (CD); C: HepG2/CD cells; D: HepG2/blank cells.

5-FC was increased there were no changes in the survival of the bystander cells in the HepG2/Hsulf-1 and HepG2/blank groups. In contrast, a dose-dependent bystander effect was observed in both the HepG2/CD and HepG2/Hsulf-1-IRES-CD groups, and this was more pronounced in the HepG2/Hsulf-1-IRES-CD group than that in HepG2/CD group ($P < 0.05$). The largest observed bystander effect was in the HepG2/Hsulf-1-IRES-CD cells when they were treated with 5-FC [Figure 2b].

Induction of cell apoptosis

To address whether the combination of *Hsulf-1* plus *CD* gene therapy enhanced apoptosis in the presence of 5-FC we measured apoptosis in the transfected cells following treatment with 1 mmol/L 5-FC. The percentage of cells exhibiting apoptosis in each group were $62.13 \pm 2.69\%$ in the HepG2/Hsulf-1-IRES-CD group, $43.52 \pm 2.13\%$ in the HepG2/CD group, $22.32 \pm 1.56\%$ in the HepG2/Hsulf-1 group and $3.19 \pm 0.13\%$ in the control HepG2/blank group. Statistically significant differences were observed when comparing each of the two groups ($P < 0.05$). These results suggested that the positive cytotoxic interaction of *CD* plus *Hsulf-1* gene therapy combined with 5-FC treatment may have caused cell death by inducing apoptosis [Figure 3].

Intracellular calcium concentration

To establish a possible mechanism for *Hsulf-1* in combination with *CD* in inducing chemosensitivity to 5-FC we visualized

intracellular calcium distribution. The results demonstrated that there were no changes in intracellular calcium concentrations in the control HepG2/blank and HepG2/CD transfected cells, while intracellular calcium concentrations increased significantly in HepG2/Hsulf-1 and HepG2/Hsulf-1-IRES-CD transfected cells. There was an almost 10-fold increase in calcium levels in *Hsulf-1* transfected cells compared with HepG2 cells not transfected with the *Hsulf-1* gene [Figure 4].

In vivo antitumor activity

We established a nude mice model of human HCC to address the effect of *Hsulf-1* expression on the *CD*/5-FC suicide gene therapy *in vivo*. It took about 4–6 days for a measurable tumor, approximately 0.5 cm in diameter, to be discernible following tumor cell inoculation. Six days after the tumor inoculation chemotherapy with 5-FC was initiated. A time-dependent increase in tumor volume was seen in all groups and we observed that tumors grew fastest in the HepG2/blank group, while tumor growth was slower, though not inhibited, in the HepG2/Hsulf-1, HepG2/CD and HepG2/Hsulf-1-IRES-CD groups. Thirty days after initial tumor inoculation, the smallest volume tumors were in the HepG2/Hsulf-1-IRES-CD group, with larger tumors in HepG2/CD and HepG2/Hsulf-1 groups, while the largest tumors were in the control HepG2/blank group. Significant differences were observed between each group ($P < 0.05$) [Figure 2d].

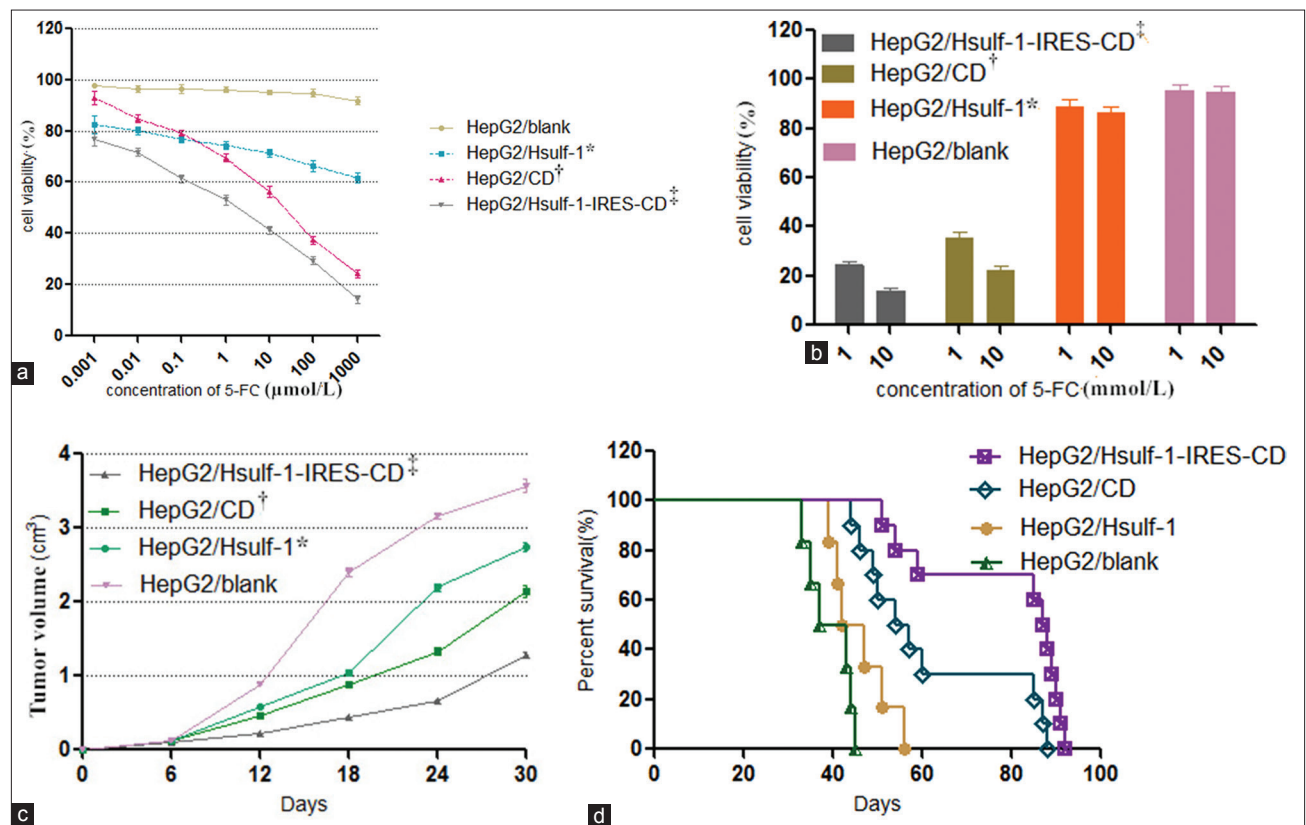


Figure 2: Chemosensitivity and bystander effect *in vitro*. (a and b) Tumor growth curve and survival analysis of nude mice inoculated with cytosine deaminase (*CD*) and/or human sulfatase-1 (*Hsulf-1*) gene therapy with 5-fluorocytosine treatment; (c and d) $*P < 0.05$ compared with HepG2/blank; $†P < 0.05$ compared with HepG2/Hsulf-1 or HepG2/blank; $‡P < 0.05$ compared with HepG2/CD, HepG2/Hsulf-1 or HepG2/blank.

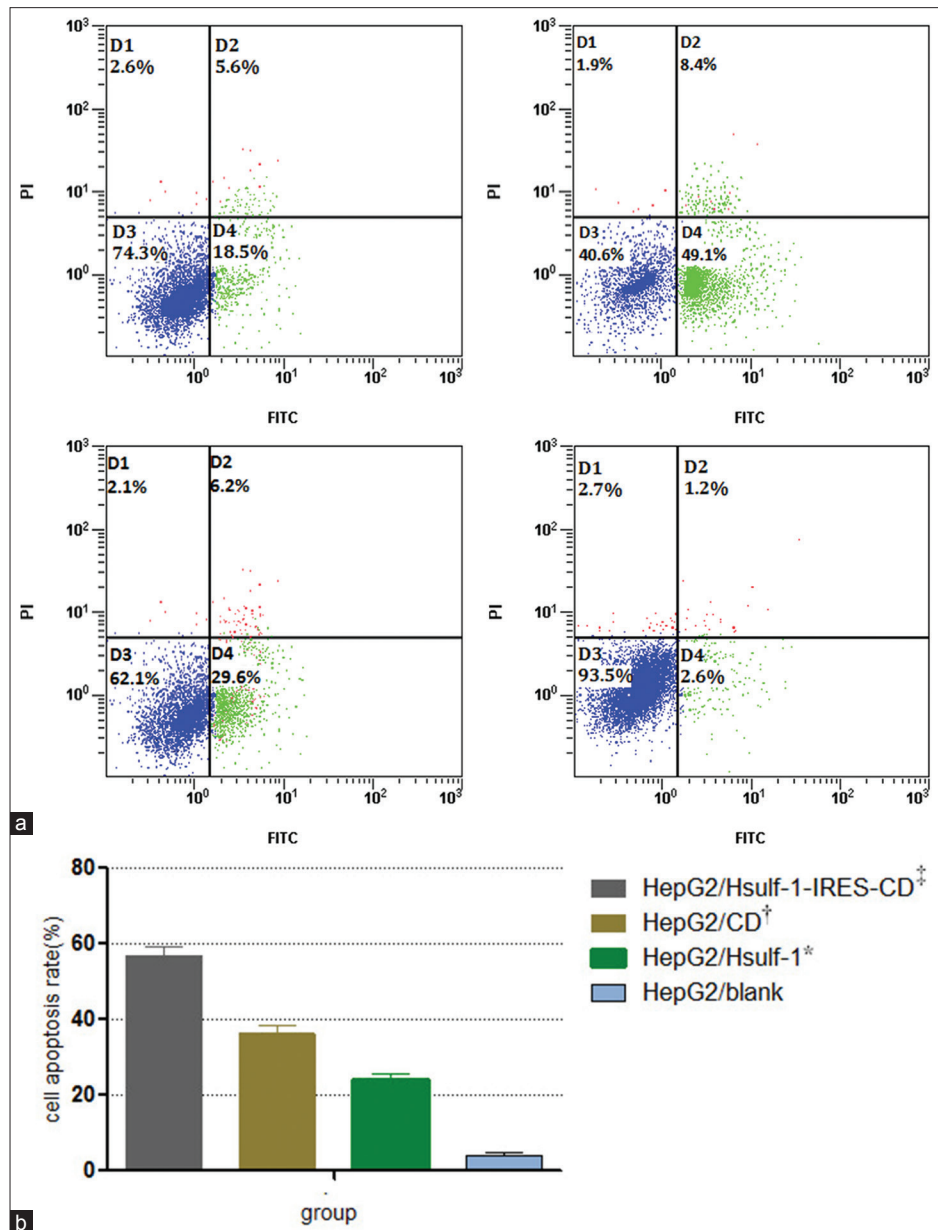


Figure 3: Induction of apoptosis in HepG2 cells with cytosine deaminase (CD) and/or human sulfatase-1 (Hsulf-1) gene therapy. (a) The apoptotic cells were detected by flow cytometry. (b) The cell apoptosis rate was calculated using flow cytometry. * $P < 0.05$ compared with HepG2/blank; [†] $P < 0.05$ compared with HepG2/Hsulf-1 or HepG2/blank; ^{*} $P < 0.05$ compared with HepG2/CD, HepG2/Hsulf-1 or HepG2/blank.

Survival analysis

Survival analyses were conducted on the mice inoculated with the tumor and treated with 5-FU. The median survival times for the HepG2/blank and HepG2/Hsulf-1 groups were 41 and 46 days respectively, and no mice from these two groups survived beyond 56 days. While 30% of the mice in the HepG2/CD group and 70% of the mice in the HepG2/Hsulf-1-IRES-CD group survived for >60 days. The mice in the HepG2/Hsulf-1-IRES-CD group survived significantly longer than mice in the other groups ($P < 0.05$) [Figure 2c].

DISCUSSION

Surgical and chemotherapeutic approaches to treating HCC have limited success in extending survival rates

in humans. In particular the limitations of systemic chemotherapy including the low local drug concentrations at the target site coupled with systemic side-effects limit the effectiveness of chemotherapy. In order to address this problem, researchers have developed prodrug/enzyme activation systems that may improve the local therapeutic effectiveness of the active drug. One such approach is the CD/5-FU gene suicide system, where the transduction of the CD gene into cancer cells is followed by chemotherapy with the prodrug 5-FU, the precursor to 5-FU.^[9,10] The Hsulf-1 protein is an arylsulfatase that has been shown to remove specific sulfate groups from cell surface HSPGs, which results in a down-regulation of receptor tyrosine kinase signaling. Therefore, Hsulf-1 might be considered to act like a tumor-suppressor gene. Hsulf-1 also affects

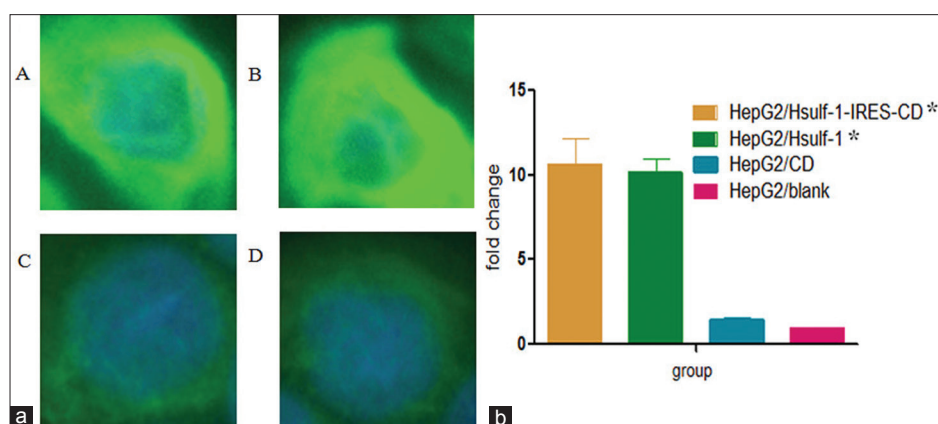


Figure 4: Intracellular Ca^{2+} concentration detected by laser scanning confocal microscopy. (a) Green fluorescence was observed by laser scanning confocal microscopy on behalf of Intracellular calcium concentration. (b) Quantitative analysis of intracellular calcium levels by laser scanning confocal microscopy. * $P < 0.05$ compared with HepG2/human sulfatase-1 or HepG2/blank.

the binding of heparin-binding factors to their receptors in several signaling pathways, as well as suppressing the phosphorylation and activation of receptor tyrosine kinases.^[5-7,11] Although the exact mechanisms by which Hsulf-1 may exert an effect are not fully known, it may be a potential candidate for use in experimental gene therapy approaches to treat cancer.

Our studies, both *in vitro* and *in vivo*, demonstrated a synergistic anti-tumor effect when *Hsulf-1* gene was expressed in combination with CD/5-FC gene suicide therapy. The *in vitro* studies showed that there were significantly positive cytotoxic interactions and bystander effects when Hsulf-1 expression and CD/5-FU gene suicide therapy were combined in HepG2 cells. Flow cytometry data supported these findings and suggested that the combination therapy induced apoptosis in the HepG2 cells. The *in vivo* experiments showed both slower tumor growth and a significantly longer survival time for mice treated with the combined 5-FC/CD and Hsulf-1 therapy compared with either treatment alone. We also demonstrated that the concentration of intracellular calcium increased significantly when HepG2 cells were transfected with the *Hsulf-1* gene. Based on this observation it appears that Hsulf-1 may activate intracellular calcium signaling systems, thereby potentially regulating calcium binding protein enzyme, calnexin and pro-apoptosis proteins in both mitochondria and endoplasmic reticulum. Changes in calcium concentration may play a crucial role in the upstream signal transduction pathways for apoptosis. For example, calcium activated proteases are required to activate the caspases involved in the apoptosis cascade, while Bax gene expression that is required for apoptosis is also calcium dependent. Alterations in calcium signaling in the mitochondria affects both the Bcl-2 family and Bad expression, which may in turn regulate the opening of mitochondria permeability transition pores.^[12-14] These processes may not only directly increase cell apoptosis, but they may also increase the sensitivity of the cells to the chemotherapeutic drug to induce apoptosis.

The combination of CD/5-FC suicide gene therapy and Hsulf-1 expression may exert an effect in HCC via multiple synergistic

pathways. Increased calcium concentrations can directly cause shrinkage of the intracellular skeleton due to dissociation of troponin-actin and myosin-actin complexes, which may result in an increased clearance between cells. It has been shown that both the intracellular distribution and function of actin are associated with changes in cell shape, vascular permeability and other signaling pathways.^[15-18] There may also be an effect on tumor-associated vascular function by alterations in signaling pathways dependent on calcium. An increase in the vascular permeability may increase the local concentration of 5-FU, which is the active chemotherapeutic agent converted from 5-FC by CD. Alternatively, the down-regulation of receptor tyrosine kinase signaling mediated by removal of the sulfate groups from HSPG by Hsulf-1 may also produce an antitumor response, thereby enhancing the effect of CD/5-FC suicide gene therapy.^[5-7] However, it is clearly evident that the Hsulf-1 and CD/5-FC combination therapy induced cell apoptosis and increased the sensitivity of the tumor cells to the chemotherapeutic drug, making this strategy a potential therapeutic approach to HCC.

In summary, our data demonstrated that combination therapy using Hsulf-1 and CD induced a dose-dependent antitumor effect. The combination therapy was more effective than either of the two gene vectors alone when transfected into HepG2 cells and the HCC nude mouse model. The data indicated that increased intracellular calcium may play a role, although the underlying mechanism of Hsulf-1 on the effect of intracellular calcium increase is not clear and needs to be further investigated.

REFERENCES

1. el-Serag HB. Epidemiology of hepatocellular carcinoma. *Clin Liver Dis* 2001;5:87-107, vi.
2. He J, Gu D, Wu X, Reynolds K, Duan X, Yao C, *et al.* Major causes of death among men and women in China. *N Engl J Med* 2005;353:1124-34.
3. Torimura T, Ueno T, Taniguchi E, Masuda H, Iwamoto H, Nakamura T, *et al.* Interaction of endothelial progenitor cells expressing cytosine deaminase in tumor tissues and 5-fluorouracil administration suppresses growth of 5-fluorouracil-sensitive liver cancer in mice. *Cancer Sci* 2012;103:542-8.

4. Kuriyama S, Mitoro A, Yamazaki M, Tsujinoue H, Nakatani T, Akahane T, *et al.* Comparison of gene therapy with the herpes simplex virus thymidine kinase gene and the bacterial cytosine deaminase gene for the treatment of hepatocellular carcinoma. *Scand J Gastroenterol* 1999;34:1033-41.
5. Forsten-Williams K, Chu CL, Fannon M, Buczek-Thomas JA, Nugent MA. Control of growth factor networks by heparan sulfate proteoglycans. *Ann Biomed Eng* 2008;36:2134-48.
6. Ji W, Yang J, Wang D, Cao L, Tan W, Qian H, *et al.* hSulf-1 gene exhibits anticancer efficacy through negatively regulating VEGFR-2 signaling in human cancers. *PLoS One* 2011;6:e23274.
7. Lai JP, Sandhu DS, Shire AM, Roberts LR. The tumor suppressor function of human sulfatase 1 (SULF1) in carcinogenesis. *J Gastrointest Cancer* 2008;39:149-58.
8. Narita K, Staub J, Chien J, Meyer K, Bauer M, Friedl A, *et al.* HSulf-1 inhibits angiogenesis and tumorigenesis *in vivo*. *Cancer Res* 2006;66:6025-32.
9. Hanna NN, Mauceri HJ, Wayne JD, Hallahan DE, Kufe DW, Weichselbaum RR. Virally directed cytosine deaminase/5-fluorocytosine gene therapy enhances radiation response in human cancer xenografts. *Cancer Res* 1997;57:4205-9.
10. Ichikawa T, Tamiya T, Adachi Y, Ono Y, Matsumoto K, Furuta T, *et al.* *In vivo* efficacy and toxicity of 5-fluorocytosine/cytosine deaminase gene therapy for malignant gliomas mediated by adenovirus. *Cancer Gene Ther* 2000;7:74-82.
11. Lin X. Functions of heparan sulfate proteoglycans in cell signaling during development. *Development* 2004;131:6009-21.
12. Eckenrode EF, Yang J, Velmurugan GV, Foskett JK, White C. Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca²⁺ signaling. *J Biol Chem* 2010;285:13678-84.
13. Ferrari D, Pinton P, Szabadkai G, Chami M, Campanella M, Pozzan T, *et al.* Endoplasmic reticulum, Bcl-2 and Ca²⁺ handling in apoptosis. *Cell Calcium* 2002;32:413-20.
14. Tagliarino C, Pink JJ, Dubyak GR, Nieminen AL, Boothman DA. Calcium is a key signaling molecule in beta-lapachone-mediated cell death. *J Biol Chem* 2001;276:19150-9.
15. Park JH, Okayama N, Gute D, Krsmanovic A, Battarbee H, Alexander JS. Hypoxia/aglycemia increases endothelial permeability: Role of second messengers and cytoskeleton. *Am J Physiol* 1999;277:C1066-74.
16. Rivero F, Köppel B, Peracino B, Bozzaro S, Siegert F, Weijer CJ, *et al.* The role of the cortical cytoskeleton: F-actin crosslinking proteins protect against osmotic stress, ensure cell size, cell shape and motility, and contribute to phagocytosis and development. *J Cell Sci* 1996;109 (Pt 11):2679-91.
17. Dudek SM, Garcia JG. Cytoskeletal regulation of pulmonary vascular permeability. *J Appl Physiol* (1985) 2001;91:1487-500.
18. Yuan SY. Signal transduction pathways in enhanced microvascular permeability. *Microcirculation* 2000;7:395-403.

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