

Treatment of hepatitis C virus core-positive hepatocytes with the transfer of recombinant caspase-3 using the 2',5'-oligoadenylate synthetase gene promoter

Ying Wang¹, Shanshan Mao², Bo Li¹, Pingping Tan¹, Deyun Feng^{1*}, and Jifang Wen^{1*}

¹Department of Pathology, Basic Medical College, Central South University, Changsha 410078, China

²Department of Pathology, Shanghai Medical School, Fudan University, Shanghai 200032, China

*Correspondence address. Tel: +86-731-2650410; Fax: +86-731-2650408; E-mail: dyfeng743@yahoo.com.cn (D.F.); Tel: +86-731-2650400; Fax: +86-731-2650408; E-mail: jifangwen@hotmail.com (J.W.)

Hepatitis C virus (HCV) infection is a leading cause of liver-related morbidity and mortality throughout the world. There is no vaccine available and current therapy is only partially effective. Since HCV infects only a minority of hepatocytes, we hypothesized that induction of apoptosis might be a promising approach for the treatment of hepatitis C. In the present study, recombinant caspase-3 gene (re-caspase-3) was used because it has the ability to induce apoptosis that is independent of the initiator caspases. An HCV-specific promoter is required to regulate the cytotoxic caspase-3 expression in HCV-infected cells. It has been reported that HCV core protein can specifically activate the 2',5'-oligoadenylate synthetase (OAS) gene promoter in human hepatocytes. Therefore, we constructed an expression vector consisting of the re-caspase-3 under the OAS gene promoter (pGL3-OAS-re-caspase-3) and then investigated its effect on HCV core-positive liver cells. It was found that the pGL3-OAS-re-caspase-3 construct induced apoptosis in HCV core-positive liver cells, but not in normal liver cells. These results strongly suggested that the transfer of the re-caspase-3 gene under the OAS promoter was a novel targeting approach for the treatment of HCV infection.

Keywords recombinant caspase-3; 2',5'-oligoadenylate synthetase; apoptosis; gene therapy

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Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma worldwide. The current standard therapy for

chronic hepatitis C consists of a combination of pegylated IFN alpha (pegIFN α) and ribavirin. It achieves a sustained viral clearance in only 50–60% of patients [1]. Moreover, this treatment is associated with substantial side effects, precluding its use by many individuals [2]. Thus, current therapies are inadequate for the majority of patients, and there is an urgent need for the novel hepatitis C therapeutic strategies.

Accumulating evidence indicated that HCV-specific immune responses play an important role in spontaneous viral clearance [3,4]. Cytotoxic T lymphocytes use two major mechanisms to eliminate the infected cells: the Fas-mediated cytotoxicity and the perforin-mediated cytolysis [5]. Both pathways ultimately converge to trigger apoptotic death through caspases activation. Apoptosis is the process of programmed cell death that is believed to permit removal of cells from the organ without provoking inflammation. However, HCV utilizes multifaceted arms to subvert various immune effectors, contributing to the development of HCV persistency [6]. It is generally believed that HCV infects only a small fraction of hepatocytes, ~1–20% as judged by the detection of HCV proteins or HCV RNAs in liver biopsy samples [7,8]. Therefore, induction of hepatic apoptosis is a potential approach for treatment of chronic hepatitis C.

Activation of effector caspases is a central and ultimate step in many apoptosis pathways. Caspase-3 is the key executioner caspase, and it exists as an inactive zymogen that is activated by upstream signals [9]. Several research groups have tried to use the human caspase-3 gene as a novel anticancer gene [10–13]. However, overexpression of the wild-type caspase-3 gene in mammalian cells does not induce apoptosis, which is due to their inability to undergo autocatalytic processing without upstream caspase for activation [14]. Constitutively, an active

recombinant caspase-3 (re-caspase-3) has been generated by making its small subunit preceding its large subunit [14]. Unlike its wild-type counterpart, in which the large subunit precedes the small subunit, the re-caspase-3 is capable of autocatalytic processing and inducing apoptosis independent of the upstream initiator caspase molecules. In addition, it could resist the effect of some apoptosis restraining genes. As caspase-3 is the most effective downstream executioner of apoptosis, the re-caspase-3 could be used at a very low concentration to induce apoptosis in targeted cells.

However, if caspases-3 is transferred to normal tissues, it could also induce apoptosis, resulting in undesirable damage. To restrict induction of apoptosis to HCV-infected cells and to increase the safety of this approach, it is needed to establish an HCV-specific caspase expression system. The HCV core protein is currently considered to be a multifunctional protein that plays an important role in persistent infection and hepatocellular carcinogenesis. Naganuma *et al.* [15] found that HCV core protein specifically activated the interferon (IFN)-inducible 2',5'-oligoadenylate synthetase (OAS) gene promoter in human hepatocyte cells, whereas the E1, E2, and NS5A proteins did not activate the OAS gene promoter. Moreover, the activation by the core protein is a general phenomenon, regardless of HCV genotype and strain. Therefore, using the OAS gene promoter that is predominantly active in HCV core-positive hepatocytes would be an ideal strategy to restrict the cytotoxic caspase expression.

In the present study, we constructed the re-caspase-3 expression vector under the OAS promoter (pGL3-OAS-caspase-3) and investigated its antiviral effect on HCV core-positive liver cells *in vitro*.

Materials and Methods

Cell lines and culture conditions

QSG7701, a normal human immortal liver cell line, was obtained from Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences (Shanghai, China). QSG7701/core, a stable cell line expressing the HCV core protein, was prepared as previously described [16]. These cells were routinely grown in DMEM medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. In all the experiments, 70–80% confluent cells were used.

Promoter cloning and luciferase assay

Template genomic DNA was extracted from QSG7701 cells using DNAzol reagent (Invitrogen, Carlsbad, CA,

USA). Human OAS promoter sequence (–157 to +82) was amplified by PCR from the genomic DNA. The primers incorporating *SacI* and *HindIII* restriction sites (underlined), respectively, were: 5'-CCGAGCTCGGGATCAGGGGAGTGT-3' (forward) and 5'-CCCAAGCTTGCATGCGGAAACACG-3' (reverse). The PCR fragment was digested and cloned into the *SacI/HindIII* sites of promoter-less pGL3-Basic vector (Promega, Madison, WI, USA) to generate pGL3-OAS-Luci. Negative and positive control constructs were pGL3-Basic, without any promoter sequences, and pGL3-control, containing the SV40 promoter and enhancer sequences, respectively.

Transient transfection of luciferase reporter plasmids was performed by using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. To evaluate the effects of IFN on the transcriptional activity of OAS promoter, some cells were treated with human recombinant IFN-α2b (Anhui Anke Biotech, Hefei, China; final concentration: 5–500 U/ml) at 36 h post-transfection. The cells were harvested 48 h post-transfection. Luciferase assays were performed according to the manufacturer's protocols (Promega). Briefly, cells were lysed with reporter lysis buffer, and the luciferase activity was determined using a luminometer. The β-galactosidase expression plasmid (pSV-β-galactosidase; Promega) was co-transfected to normalize transfection efficiency.

Construction of the OAS promoter plasmid carrying re-caspase-3 gene

Total RNA from QSG7701 cells was extracted by using Trizol reagent (Invitrogen), and the entire coding region of human caspase-3 was amplified by RT-PCR using specific primers containing *BamHI* and *XbaI* restriction sites (underlined), respectively, as follows: 5'-CGGGATCCATGGAGAACACTGAAAACCTC-3' (forward) and 5'-GCTCTAGACAACCAACCATTCTTTAGTG-3' (reverse). The PCR product was digested and cloned into the *BamHI/XbaI* sites of the pcDNA3.1 to generate pcDNA3.1-caspase-3.

The large and small subunits of caspase-3 were amplified by PCR using the pcDNA3.1-caspase-3 construct as a template. The primers of large subunit were: 5'-GGAATTCATGGAGAACACTGAAAACCTCAG-3' (forward) and 5'-GCTCTAGATTAGTCTGTCTCAATGCCACAGT-3' (reverse), containing *EcoRI* and *XbaI* sites (underlined), respectively; the primers of small subunit were: 5'-CGGGATCCATGATTGAGACAGACAGT-3' (forward) and 5'-GGAATTCGTGATAAAA

ATAGAGTTC-3' (reverse), containing *Bam*HI and *Eco*RI sites (underlined), respectively. The PCR products of large and small subunits of caspase-3 were digested with *Eco*RI, purified and ligated with T4 DNA ligase (Promega), which placed the small subunit preceding the large subunit. The ligation product was double digested with *Bam*HI/*Xba*I and then subcloned to the pcDNA3.1 to generate pcDNA3.1-re-caspase-3.

To construct the re-caspase-3 expression vector with the OAS promoter, the 830 bp fragment of re-caspase-3 was generated by PCR using pcDNA3.1-re-caspase-3 as a template. The primers were: 5'-CATGCCATGGA TGATTGAGACAGACAGT-3' (forward) and 5'-GC TCTAGA TTAGTCTGTCTCAATGCCACAGT-3' (reverse), containing *Nco*I and *Xba*I sites, respectively (underlined). The PCR product was then inserted into pGL3-OAS-Luci plasmid instead of luciferase digested with *Nco*I/*Xba*I. This is the construct that has the re-caspase3 with the OAS promoter (pGL3-OAS-re-caspase-3).

Caspase-3 activity assay

Caspase-3 activity was measured using CaspACE assay system (Promega) according to the manufacture's instructions. Briefly, pGL3-OAS-re-caspase-3 and pcDNA3.1-re-caspase-3 were transiently transfected into cells and incubated for 48 h, then cells were harvested and resuspended in cell lysis buffer at a concentration of 10^8 cells/ml. The cells were lysed by freeze-thaw, then lysates were centrifuged, and the supernatant fractions were collected. Assays were performed in 96-well plates by incubating cell extract in reaction buffer containing the caspase-3 colorimetric substrate, acetyl-DEVD-*p*-nitroaniline (Ac-DEVD-*p*NA). Upon cleavage by caspase-3, *p*NA produces a yellow color that can be monitored by a spectrophotometer at 405 nm. The pGL3-OAS-Luci plasmid was used as a negative control. The efficiency of transfection was measured by co-transfection of β -galactosidase reporter plasmid.

Apoptosis analysis by Annexin V/propidium iodide staining

Cells were grown on glass coverslips in 6-well plates. The pcDNA3.1-re-caspase-3 and pGL3-OAS-re-caspase-3 constructs were transiently transfected into cells and incubated for 48 h, then cells were washed with phosphate-buffered saline (PBS), stained with Annexin V-FITC and propidium iodide (PI) (BD, Franklin Lakes, NJ, USA). After 15 min in the dark at room temperature, cells were examined under a fluorescence microscope.

The pGL3-OAS-Luci was used as a negative control. The pEBFP (Clontech, Mountain View, CA, USA) expressing the enhanced blue fluorescent protein served as a transfection marker. Cell populations that potentially detected are as follows: living cells, which were not stained by Annexin V FITC or PI; early apoptotic cells, stained with Annexin V-FITC (green cells), but not with PI; cells in a late stage of apoptosis, stained with both Annexin V-FITC and PI (green and red cells); and primary necrotic cells, stained with PI only (red cells).

Apoptosis analysis by flow cytometry

The percentage of apoptotic cells was determined by flow cytometry using Annexin V-FITC/PI staining. Briefly, transfected cells were trypsinized, washed twice with PBS and resuspended in 100 μ l of $1 \times$ binding buffer (10 mM HEPES/NaOH, pH 7.4, 0.14 M NaCl, 2.5 mM CaCl_2) per 10^6 cells. Cell suspensions were mixed with 5 μ l Annexin V-FITC and 10 μ l PI, incubated for 10 min in the dark at room temperature. After staining, 400 μ l of binding buffer was added and the stained cells were analyzed on a flow cytometer (BD) with CellQuest software. For maximum sensitivity, cells were analyzed within 30 min after the staining. Results were shown as the percentage of live cells (Annexin V⁻/PI⁻), early apoptotic cells (Annexin V⁺/PI⁻), late apoptotic cells (Annexin V⁺/PI⁺), and dead cells (Annexin V⁻/PI⁺).

Statistical analysis

Results are expressed as mean \pm SD. Statistical comparisons were made by using an unpaired two-tailed Student's *t*-test. $P < 0.05$ was considered as statistically significant.

Results

Transcriptional activity of OAS promoter is increased in the HCV core-positive hepatocytes

To examine the transcriptional activity of the OAS gene promoter, 240 bp fragment (-157 to +82) was placed in the upstream of the firefly luciferase gene in the pGL3-Basic vector. The resultant plasmid pGL3-OAS-Luci was transiently transfected into the QSG7701/core and QSG7701 cells, and the firefly luciferase activity obtained was compared with those from the pGL3-Basic vector alone and the pGL3-control plasmid. As shown in **Fig. 1(A)**, the pGL3-OAS-Luci transfection resulted in no or little luciferase activity in the normal liver cell line QSG7701. In marked contrast, this OAS promoter construct exhibited significant

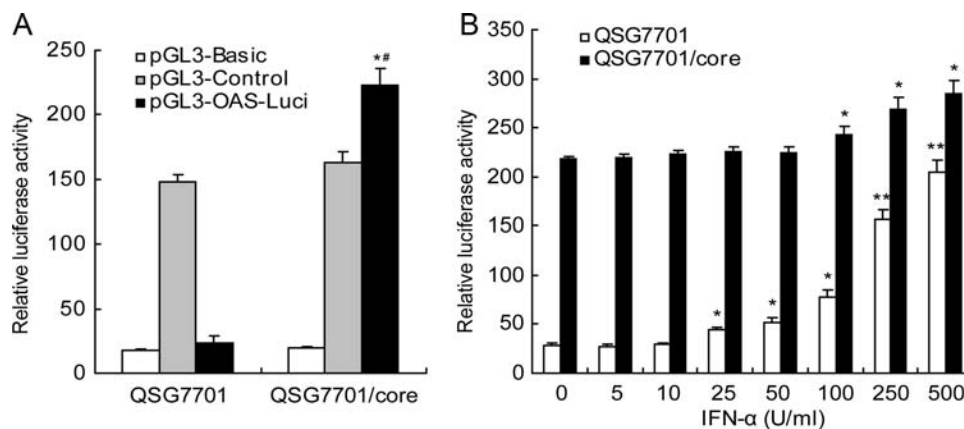


Figure 1 Transcriptional activity of the OAS promoter in QSG7701/core and QSG7701 cells. The HCV core-positive cell line QSG7701/core and normal liver cell line QSG7701 were transiently transfected with pGL3-OAS-Luci (bearing the OAS promoter). Luciferase assay was carried out 48 h after transfection. The transfection efficiency was normalized to β -galactosidase plasmid. The data were shown as mean \pm SD from three independent experiments. (A) The transcriptional activity of OAS promoter was significantly increased in the presence of HCV core protein. The pGL3-Basic (without any promoter sequences) and pGL3-control (bearing the SV40 promoter) were used as controls. * $P < 0.01$ compared with same group; # $P < 0.01$ compared with other group. (B) IFN- α affected the transcriptional activity of the OAS promoter in a dose-dependent manner. The transfected cells were treated with various concentrations of IFN- α for 12 h. The untreated cells were used as the control. * $P < 0.05$, ** $P < 0.01$ versus control.

transcriptional activity in the QSG7701/core cells that express the HCV core protein ($P < 0.01$). The relative luciferase activity in the QSG7701/core cells was 6 folds greater than that in QSG7701 cells ($P < 0.01$). These results suggested that HCV core protein transcriptionally activated the OAS gene promoter.

To determine the transcriptional activity of OAS promoter response to IFN, the transfected cells were treated with various concentrations of IFN- α for 12 h. As shown in **Fig. 1(B)**, in the QSG7701 cells transfected with pGL3-OAS-Luci, activation of OAS promoter was observed at a concentration of 25 U/ml and increased in a dose-dependent manner ($P < 0.05$). In the QSG7701/core cells, the activation of OAS promoter by core protein was enhanced by treatment with IFN- α at concentrations higher than 100 U/ml ($P < 0.01$). Altogether, these results indicated that the endogenous IFN level must be carefully considered to avoid undesired side effects, when the OAS promoter-driven gene expression system was applied *in vivo*.

Expression of re-caspase-3 driven by OAS promoter is catalytically active in the HCV core-positive hepatocytes

To examine the enzymatic activity of the re-caspase-3, a colorimetric peptide substrate (Ac-DEVD-pNA) was used. As shown in **Fig. 2**, both QSG7701/core and QSG7701 cells transfected with the pcDNA3.1-re-

caspase3 construct displayed significant higher caspase-3 activity compared with controls ($P < 0.01$), indicating that the recombinant re-caspase-3 is catalytically active independent of the upstream caspases. A marked increase of caspase-3 enzyme activity was observed in QSG7701/core cells which had been transfected with the pGL3-OAS-re-caspase-3 ($P < 0.01$), whereas the QSG7701 cells transfected with the same construct did not show higher activity than the control. The incidence of caspase-3 expression with the OAS promoter was similar to that with the CMV-promoter, but the pGL3-OAS-re-caspase-3 system can specifically induce the expression of active caspase-3 in the HCV core-positive cells.

Expression of re-caspase-3 driven by OAS promoter induces morphological changes in the HCV core-positive hepatocytes

Apoptosis is known to induce several morphological and biochemical changes in the cell. One of these changes is the exposure of phosphatidylserine (PS) on the surface of the cell membrane during the early stage of apoptosis. Annexin V is known to bind specifically to PS; therefore, fluorescein isothiocyanate (FITC)-conjugated Annexin V was used to detect early apoptosis. PI stains DNA after the disruption of plasma membrane at the late stage of apoptosis. Cells stained with Annexin V (green cells) indicated early apoptotic cells and PI-stained cells

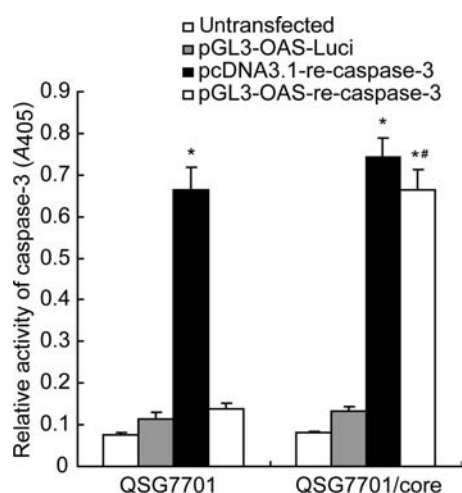


Figure 2 Caspase-3 activity in QSG7701 and QSG7701/core cells after transfection with re-caspase-3 expression vector under the OAS promoter (pGL3-OAS-re-caspase-3) and the CMV promoter (pcDNA3.1-re-caspase-3). The pGL3-OAS-Luci plasmid was used as a negative control. Transfection efficiency was normalized by co-transfection of β -galactosidase reporter plasmid. The results illustrated that the re-caspase-3 has the enzymatic activity and the OAS promoter-driven re-caspase-3 induces the expression of active caspase-3 only in the HCV core-positive cells. * $P < 0.01$ compared with same group; ** $P < 0.01$ compared with other group. The results are expressed as absorbance at 405 nm (A_{405}) and the data were shown as mean \pm SD from three independent experiments.

(red cells) indicated late apoptotic/necrotic cells. Cells were considered to be apoptotic, if they were either Annexin V⁺/PI⁻ (early apoptotic) or Annexin V⁺/PI⁺ (late apoptotic).

To determine whether the pGL3-OAS-re-caspase-3 construct induced apoptosis only in HCV core-positive liver cells, QSG7701/core and QSG7701 cells were transiently transfected with pGL3-OAS-Luci, pGL3-OAS-re-caspase-3, and pcDNA3.1-re-caspase-3. As shown in **Fig. 3**, both QSG7701/core and QSG7701 cells underwent apoptosis after transfection with the pcDNA3.1-re-caspase-3 construct, whereas cells transfected with pGL3-OAS-Luci did not show any signs of apoptosis. QSG7701/core cells that had been transfected with the pGL3-OAS-re-caspase-3 displayed an increased number of early or late apoptotic cells. In contrast, the QSG7701 cells transfected with pGL3-OAS-re-caspase-3 did not undergo apoptosis. Morphologically, the apoptotic cells became shrinking and deformation, and cells showed condensation of cytoplasm and nuclear, nuclei were fragmented and filled with compacted chromatin. These results indicated that pGL3-OAS-re-caspase-3 construct induced apoptosis in HCV core-positive liver cells.

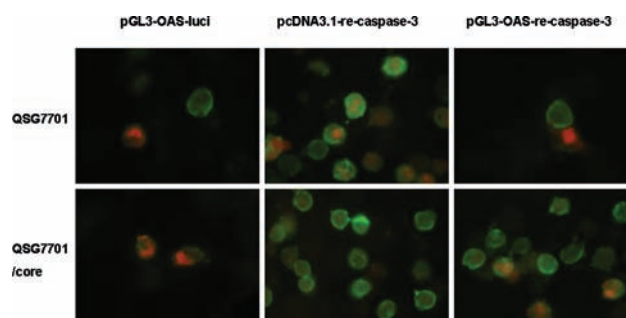


Figure 3 Apoptosis was visualized by fluorescent microscopy using an Annexin V/PI double staining. QSG7701/core and QSG7701 cells were transiently transfected with pGL3-OAS-re-caspase-3 or pcDNA3.1-re-caspase-3 and incubated for 48 h. Then cells were stained with 5 μ l Annexin V-FITC and 10 μ l PI and examined under a fluorescence microscope. The pGL3-OAS-Luci was used as a negative control. Cells stained with Annexin V (green cells) indicated early apoptotic cells and PI-stained cells (red cells) indicated late apoptotic/necrotic cells. Cells were considered to be apoptotic if they were either Annexin V⁺/PI⁻ (early apoptotic) or Annexin V⁺/PI⁺ (late apoptotic). The images are representative of three separate experiments. Magnification, $\times 200$.

Expression of re-caspase-3 driven by OAS promoter selectively induces apoptosis in the HCV core-positive hepatocytes

On the basis of the morphological changes induced by re-caspase3 system in HCV core-positive cells, the percentage of apoptotic cells was quantitated by flow cytometry analysis using Annexin V/PI double staining. As shown in **Fig. 4**, an increased apoptotic percentage was observed in QSG7701/core cells after transfection with pGL3-OAS-re-caspase-3 (~19% Annexin V⁺/PI⁻, 27% Annexin V⁺/PI⁺) compared with the control ($P < 0.01$). In contrast, very small apoptotic percentage was detected in QSG7701 cells transfected with the same construct, without significant difference compared with the control. Both QSG7701/core and QSG7701 cells underwent apoptosis after the transfection with the pcDNA3.1-re-caspase-3 construct ($P < 0.01$). These results indicated that the re-caspase-3 system driven by OAS promoter (pGL3-OAS-re-caspase-3) induced apoptosis in QSG7701/core cells significantly and specifically.

Discussion

Since the discovery of HCV in 1989 [17], many significant progresses have been made in the development of HCV therapy. Major research efforts have focused on the identification of agents that inhibit specific steps in

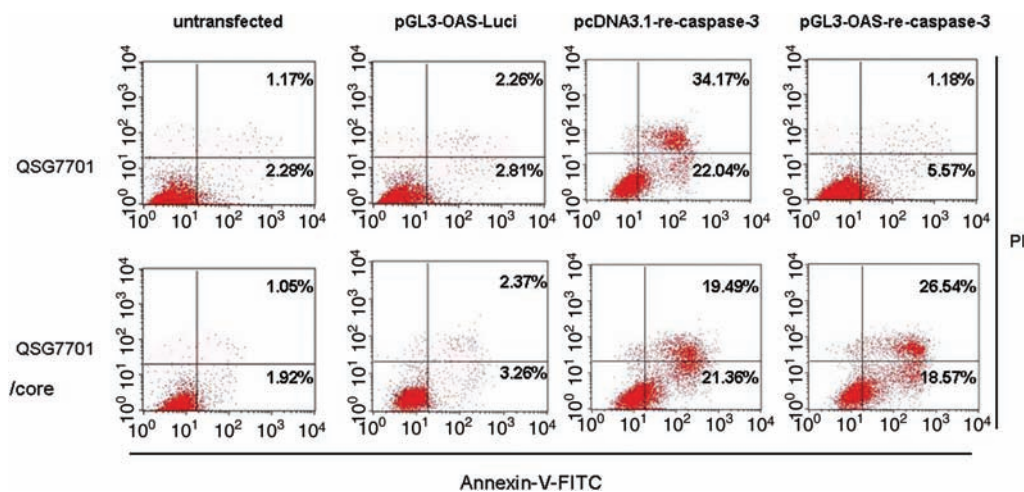


Figure 4 Quantitative analysis of apoptosis induced by re-caspase-3 system QSG7701/core and QSG7701 cells were transiently transfected with pGL3-OAS-re-caspase-3 or pcDNA3.1-re-caspase-3 and incubated for 48 h. Then cells were stained with 5 μ l Annexin V-FITC and 10 μ l PI and analyzed by flow cytometry. The numbers at the bottom right quadrant of each dot plot represent the percentage of cells in early apoptosis (Annexin V⁺/PI⁻). Numbers at the top right quadrant represent the percentage of cells in late apoptosis and/or secondary necrosis (Annexin V⁺/PI⁺). The re-caspase-3 system driven by OAS promoter (pGL3-OAS-re-caspase-3) induced apoptosis in QSG7701/core cells significantly and specifically. The experiments were performed three times with similar results and the data are representative of three separate experiments.

the life cycle of the virus. These ‘HCV-targeted drugs’ include small-molecule inhibitors of the HCV enzymes and nucleic acid-based agents that attack the viral RNA [18–20]. Nevertheless, there are considerable barriers to the development of anti-HCV therapeutics, which include the persistence of the virus, the genetic diversity during replication in the host, the development of drug-resistant virus mutants, and the lack of reproducible infectious culture systems and small-animal models for HCV replication and pathogenesis [21,22].

The present study demonstrates that treatment with the OAS promoter-driven re-caspase-3 expression system (pGL3-OAS-re-caspase-3) is an effective and promising gene therapy for HCV core-positive hepatocytes. Caspase-3 is a member of the executioner caspases, and overexpression of caspase-3 alone does not induce apoptosis because it must be activated by upstream initiator caspases. Re-caspase-3 is constitutively active, and it does not depend on any upstream components. Although re-caspase-3 has been used in targeted cells in cancer therapy, our study is the first demonstration that such a constitutively active caspase can be used in HCV treatment.

Strict HCV-specific expression of re-caspase-3 gene is mandatory because inappropriate transgene expression can result in non-specific toxicity. One attractive approach to this problem is to use promoter elements to control gene expression tightly at the transcriptional level. Previous reports found that HCV core protein

could specifically activate the OAS gene promoter in human hepatocyte cells. These findings suggested that the OAS promoter could be a powerful novel candidate for targeted gene therapy of HCV. We therefore cloned the OAS promoter and introduced it to the upstream of re-caspase-3 gene. Our results showed that the pGL3-OAS-re-caspase-3 vector induced apoptosis only in HCV core-positive cells, whereas normal liver cells did not undergo apoptosis.

The major challenge of this strategy is that other potential factors may influence the transcriptional activity of OAS promoter, among which the most important is IFN. We demonstrated that the activation of OAS promoter response to IFN- α stimuli was in a dose-dependent manner, and the low dose (in the present study <10 U/ml) did not affect OAS promoter-driven gene expression. Multiple studies have provided molecular evidence that there is a clear absence or only a low level of IFN gene expression in hepatocytes of patients with chronic HCV infection [23]. Therefore, the OAS promoter-driven re-caspase-3 system may become an attractive strategy for chronic hepatitis C therapy, especially for those who are IFN resistant or lack of endogenous IFN.

Another challenge is that the expression level of HCV core protein may affect the activity of OAS promoter. Naganuma *et al.* [15] have demonstrated that the core protein activated OAS promoter in a dose-dependent manner. This result was achieved by transient

transfection with different doses of core-expressing vector (ranging from 0.5 to 4 μ g). However, the OAS promoter-driven expression vector must enter the cells first and then interact with intracellular HCV core protein. So it is rather difficult to assess the core expressing level in a single cell. Since HCV core protein can be detected by immunohistochemistry in liver biopsy samples, we speculate that it is sufficient to activate OAS promoter in the patients infected with HCV.

As the present study is based on the *in vitro* cell cultures stably expressing HCV core protein, further experiments using animal models with HCV infection are necessary to determine whether this approach could be applied for the treatment of patients with hepatitis C. Currently, experimental animals for the study of HCV are limited only to chimpanzees and mice transplanted with human hepatocytes [24,25]. It is difficult to employ chimpanzees as an experimental model for both ethical and economic reasons. Therefore, we are going to establish a mouse model transplanted with human liver fragments to evaluate the efficacy of antiviral effect of re-caspase-3 system *in vivo*.

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References

- Keefe B. Interferon-induced depression in hepatitis C: an update. *Curr Psychiatry Rep* 2007, 9: 255–261.
- Manns MP, Wedemeyer H and Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 2006, 55: 1350–1359.
- Bertoletti A and Ferrari C. Kinetics of the immune response during HBV and HCV infection. *Hepatology* 2003, 3: 4–13.
- Neumann-Haefelin C, Blum HE, Chisari FV and Thimme R. T cell response in hepatitis C virus infection. *J Clin Virol* 2005, 32: 75–85.
- Edwards KM, Davis JE, Browne KA, Sutton VR and Trapani JA. Anti-viral strategies of cytotoxic T lymphocytes are manifested through a variety of granule-bound pathways of apoptosis induction. *Immunol Cell Biol* 1999, 77: 76–89.
- Gale M, Jr and Foy EM. Evasion of intracellular host defence by hepatitis C virus. *Nature* 2005, 436: 939–945.
- Hiramatsu N, Hayashi N, Haruna Y, Kasahara A, Fusamoto H, Mori C and Fuke I, *et al.* Immunohistochemical detection of hepatitis C virus-infected hepatocytes in chronic liver disease with monoclonal antibodies to core, envelope and NS3 regions of the hepatitis C virus genome. *Hepatology* 1992, 16: 306–311.
- Lanford R and Bigger C. Advances in model systems for hepatitis C virus research. *Virology* 2002, 293: 1–9.
- Thorberry NA and Lazebnik Y. Caspase: enemies within. *Science* 1998, 281: 1312–1316.
- Shariat SF, Desai S, Song W, Khan T, Zhao J, Nguyen C and Foster BA, *et al.* Adenovirus-mediated transfer of inducible caspase: a novel ‘death switch’ gene therapeutic approach to prostate cancer. *Cancer Res* 2001, 61: 2562–2571.
- Friedrich K, Wieder T and Von Haefen C. Overexpression of caspase-3 restores sensitivity for drug-induced apoptosis in breast cancer cell lines with acquired drug resistance. *Oncogene* 2001, 20: 2749–2760.
- Shinoura N, Muramatsu Y, Yoshida Y, Asai A, Kirino T and Hamada H. Adenovirus-mediated transfer of caspase-3 with Fas ligand induces drastic in U-373MG glioma cells. *Exp Cell Res* 2000, 256: 423–433.
- Yamabe K, Shimizu S, Ito T, Yoshioka Y, Nomura M, Narita M and Saito I, *et al.* Cancer gene therapy using a pro-apoptotic gene, caspase-3. *Gene Ther* 1999, 6: 1952–1959.
- Srinivasula SM, Ahmad M, MacFarlane M, Luo Z, Huang Z, Fernandes-Alnemri T and Alnemri ES. Generation of constitutively active recombinant caspases-3 and -6 by rearrangement of their subunits. *J Biol Chem* 1998, 273: 10107–10111.
- Naganuma A, Nozaki A, Tanaka T, Sugiyama K, Takagi H, Mori M and Shimotohno K, *et al.* Activation of the interferon-inducible 2',5' oligoadenylate synthetase gene by hepatitis C virus core protein. *J Virol* 2000, 74: 8744–8750.
- Li B, Feng DY, Cheng RX, He QQ, Hu ZL, Zheng H and Wen JF. The effects of hepatitis C virus core protein on biological behaviors of human hepatocytes. *Zhonghua Yi Xue Za Zhi* 2005, 85: 1243–1248.
- Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW and Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989, 244: 359–362.
- McHutchison JG, Patel K, Pockros P, Nyberg L, Pianko S, Yu RZ and Dorr FA, *et al.* A phase I trial of an antisense inhibitor of hepatitis C virus (ISIS 14803), administered to chronic hepatitis C patients. *J Hepatol* 2006, 44: 88–96.
- Jarczak D, Korf M, Beger C, Manns MP and Krüger M. Hairpin ribozymes in combination with siRNAs against highly conserved hepatitis C virus sequence inhibit RNA replication and protein translation from hepatitis C virus subgenomic replicons. *FEBS J* 2005, 272: 5910–5922.
- Kanda T, Steele R, Ray R and Ray RB. Small interfering RNA targeted to hepatitis C virus 5' non-translated region exerts potent antiviral effect. *J Virol* 2007, 81: 669–676.
- Martell M, Esteban JI, Quer J, Genesca J, Weiner A, Esteban R and Guardia J, *et al.* Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *J Virol* 1992, 66: 3225–3229.
- Simmonds P. Genetic diversity and evolution of hepatitis C virus—15 years on. *J Gen Virol* 2004, 85: 3173–3188.
- Mihm S, Frese M, Meier V, Wietzke-Braun P, Scharf JG, Bartenschlager R and Ramadori G. Interferon type I gene expression in chronic hepatitis C. *Lab Invest* 2004, 84: 1148–1159.
- Bukh J. A critical role for the chimpanzee model in the study of hepatitis C. *Hepatology* 2004, 39: 1469–1475.
- Kremsdorf D and Brezillon N. New animal models for hepatitis C viral infection and pathogenesis studies. *World J Gastroenterol* 2007, 13: 2427–2435.