

Short Report

The Effect of Herpes Simplex Virus Virion Host Shutoff Gene- a New Suicide Gene- on Tumor Cells

Hossein Bannazadeh Baghi, Taravat Bamdad* and Hoorieh Soleimanjahi

Dept. of Virology, School of Medical Sciences, Tarbiat Modares University, Tehran, Iran

Received 31 December 2008; revised 9 June 2009; accepted 10 June 2009

ABSTRACT

Background: The herpes simplex virus (HSV) UL41 gene product, virion host shutoff (Vhs) protein, mediates the rapid degradation of both viral and cellular mRNA. This ability suggests that Vhs protein can be used as a suicide gene in cancer gene therapy applications. The recent reports have shown that the degradation of cellular mRNA during herpes simplex infection is selective. RNA containing AU-rich elements (ARE) in their 3' untranslated ends are the targets for the Vhs protein. RNA that are not subject to Vhs protein-dependent degradation are up-regulated during HSV infection. ARE are frequently found in mRNA that encode proto-oncogenes, nuclear transcription factors, and cytokines. In many human cancers, the AU-rich stretch of proto-oncogenes and regulatory genes has impaired. **Methods:** To investigate whether Vhs protein might be useful for inhibition of tumor cell proliferation, a eukaryotic expression vector containing Vhs protein gene was constructed. Cell degradation and RNA content of HeLa and MRC-5 tumor cells after transfection with the constructed vector were studied. **Results:** The results showed a strong inhibitory activity in proliferation of transfected tumor cells and a sharp decrease in their RNA content. **Conclusion:** These data suggest that Vhs protein can be considered as a candidate for suicide cancer gene therapy. *Iran. Biomed. J. 13 (3): 185-189, 2009*

Keywords: Herpes simplex virus (HSV), Virion host shut off (Vhs), Tumor, RNA degradation

INTRODUCTION

Upon infection of cells with herpes simplex virus (HSV) the host protein synthesis is rapidly suppressed [1]. One of the best characterized viral factors that mediate mRNA degradation is virion host shut off (Vhs) protein, a 58-kDa phosphoprotein product of UL41 gene [2, 3]. Vhs is packaged to the virus as a tegument protein and released into the cytoplasm of infected cells, where it causes instability and degradation in cellular and viral mRNA [4, 5].

Vhs mediates the degradation of both viral and cellular mRNA, while cellular rRNA and tRNA are unaffected [6]. Within 6 h of infection with HSV-1, cellular mRNA are degraded, while this time decreases to 2 h in HSV-2 infected cells. The

activity of HSV-2 Vhs is 40 folds stronger than that of HSV-1 [7]. Vhs acts as an RNase either alone or in conjunction with eukaryotic translation initiation factor 4H [2, 8, 9]. The Vhs preferentially initiates mRNA degradation near the 5' end of RNA where the translation factors exist, although it cleaves mRNA at other locations through the molecule [10]. In the recent studies, it has been shown that not only the Vhs-dependent mRNA degradation is selective but also the Vhs protein can stabilize or delay the degradation of some mRNA. The AU-rich element (ARE), an adenylate uridylylate-rich element of the sequence AUUUA, is the major determinant of RNA instability which is located within the 3' UTR of the mRNA. In normal cells, ARE containing mRNA degrade more rapidly through interaction of ARE with different ARE

*Corresponding Author; Tel. (+98-21) 8288 4525; Fax: (+98-21) 8801 3030; E-mail: bamdad_T@modares.ac.ir

binding proteins. Vhs has been shown to facilitate the degradation of ARE containing mRNA [11]. In contrast, the RNA not containing ARE are stabilized in the presence of Vhs. ARE containing genes include a number of mRNA that produce cytokines and cell proliferation regulatory proteins. In many human cancers, ARE stretch of proto-oncogenes and regulatory genes has impaired, a phenomenon which confers the oncogenicity to these cells [12]. Regarding the ability of Vhs in mRNA degradation, it can be considered as a suicide gene for molecular cancer therapy.

Along with this aim, a preliminary study is needed to determine if Vhs can cause mRNA degradation in different tumor cell lines in which ARE stretch is impaired, or it more stabilizes the oncogene mRNA.

Human breast adenocarcinoma (MCF-7) and HeLa cells are two examples of well characterized tumor cell lines which are extremely used as model systems. It has been proven that oncogenes like c-Jun and cell cycle regulatory genes are over-expressed in both cells [13, 14] that can be related to the loss of ARE sequence in their mRNA.

In the present study, cell death and mRNA content of MCF-7 and HeLa cell line were analyzed following expression of Vhs in each cell line.

MATERIALS AND METHODS

Cells. MCF-7 (NCBI C135) and HeLa (NCBI C115) cell lines were obtained from national cell bank of Iran. Vero cells were used for propagation of HSV-2. Cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum.

Virus. Wild HSV-2 was isolated from a patient and confirmed by indirect immunofluorescent assay using HSV-2 specific monoclonal antibody [15].

Viral DNA extraction. HSV-2 inoculated cells were incubated in 5% CO₂ at 37°C. The virus was harvested when 70-80% of the cells showed cytopathic effect. The virus suspension was treated with DNase (1 mg/ml) at 37°C for 1h and then concentrated by polyethylene glycol 6000 (8% w/v) and sodium chloride (10% v/v). The pegylated virus suspension was incubated at 4°C overnight and then centrifuged at 8000 ×g for 1 h. The pellet was re-suspended in lysis buffer (Tris-HCl, 10 mM; EDTA, 12 mM; NaCl, 10 mM; SDS, 1.2% and protease K, 250 mg/ml) and incubated at 56°C for 2 h. The

mixture was subjected to standard phenol-chloroform DNA extraction method. Briefly, the equal volume of phenol-chloroform was added to the mixture and the aqueous phase was separated and DNA was precipitated with ethanol and NaCl.

Plasmid construction. A 1448-bp fragment encoding Vhs gene was amplified using the following primers: sense Vhs (5' agc tct aga tat ggg tct gtt tgg cat gat g 3') and anti-sense Vhs (5' agc tct tag act act cgt ccc aga att tag cc 3'). *Xba*I restriction sites were included at the 5' ends of primers. The PCR amplification was carried out using 25 µl of reaction mixture containing 0.5-1 µg of isolated viral DNA, 0.5 p mol of each primer, 0.2 m mol/L dNTP, PCR buffer 10×, 0.75 µl of MgCl₂, 1.5 mM and 0.2 µl of Taq DNA polymerase. The pvhsDNA3 plasmid which expresses Vhs in eukaryotic cells was constructed by the insertion of PCR product into the *Xba*I site of the pcDNA3 vector (Invitrogen, Burlington, Canada). The ligated clones were screened and the right orientation of the inserted fragment was verified by *Xho*I. The fidelity of the Vhs gene was determined by DNA sequence analysis and compared with standard sequence of Vhs.

Transfection and cell analysis. MCF-7 and HeLa cells were grown in 6-well plates and transfected with pvhsDNA3 plasmid using Lipofectamin 2000 (Invitrogen, Burlington, Canada) according to the manufacturer's protocol. The pcDNA3 plasmid was also transfected into the cells as negative controls. Cell morphological changes were observed microscopically at 24-48 h and 72 h post transfection. RNA degradation in transfected cells was measured by semi-quantitative RT-PCR. Total RNA of transfected cells was extracted 36 h after transfection using the guanidine isothiocyanate based RNX-plus solution (Cinnagen, Tehran, Iran). β2 microglobulin mRNA was considered as an indicator for total mRNA. β2 microglobulin mRNA content in transfected and non transfected cells was measured by a semi-quantitative method and compared with each other. In semi-quantitative method, content of a specified mRNA which changes during the experiment is compared with a constant amount of RNA. To compare the amount of β2 microglobulin mRNA with a known concentration of RNA in semi-quantitative RT-PCR test, 1 µg of entrovirus RNA was added to each sample before RNA extraction. Using the following primers: β2F: TCTGGGTTTCATCCATCC, β2 R:

TACCTG TGGAGCAACCTG, entro F: CTGAATG CGGC TAATCC, entro R: TGTCACCATAAGCA GCCA), a multiplex RT-PCR was done as previously described [16].

Semi-quantitative analysis. PCR products were run on 2% agarose gel and photographed on top of a 280-nm UV light box after ethidium bromid staining. The gel images were digitally captured with a CCD camera and analyzed with the National Institutes of Health Imager beta version 2 program. $\beta 2$ microglobulin mRNA content of each sample was measured semi-quantitatively by the ratio of band densitometry of $\beta 2$ microglobulin to enterovirus bands.

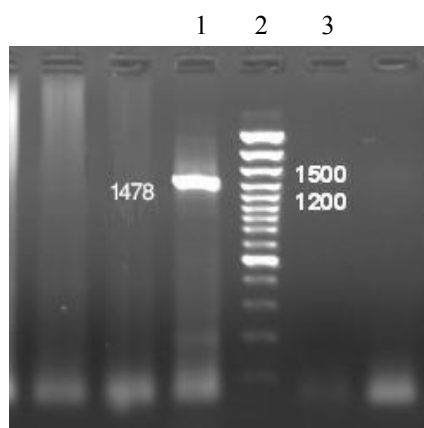


Fig. 1. Agarose gel electrophoresis of the *vhs* gene after PCR amplification. Lane 1, *Vhs* gene; lane 2, DNA size marker and lane 3, control.

RESULTS

Construction of *pvhsDNA3* plasmid and its confirmation. Total viral DNA was extracted from HSV-2 infected cells. The *Vhs* gene was amplified using the designed primers (Fig. 1). The PCR product was digested with *Xba*I enzyme to produce the appropriate ends. The *pvhsDNA3* plasmid was constructed by insertion of *Vhs* gene into the *Xba*I site of *pcDNA3* and confirmed by colony PCR, restriction enzymes and sequence analysis.

Effects of *Vhs* on MCF-7 and HeLa cell lines.

a) Morphological changes. The effect of *pvhsDNA3* on destruction of the transfected cells was studied at 36-48 and 96 h post transfection. Cell degradation was more rapid and stronger in HeLa cells comparing to MCF-7 cells (Fig. 2).

b) mRNA degradation. Quantitative analysis of band densitometry on RT-PCR products (Fig. 3) in MCF-7 and HeLa transfected cells showed a sharp decrease in mRNA content of *Vhs* transfected cells comparing to controls (P value = 0.04953). The ratio of $\beta 2$ microglobulin RNA of control cells to enterovirus RNA was obtained 1.3 ± 0.471 , while the ratio for MCF-7 and HeLa transfected cells was 0.332 ± 0.116 and 0.301 ± 0.102 , respectively (Fig. 4). The differences between mRNA content of control and *Vhs* transfected cells are statistically significant.

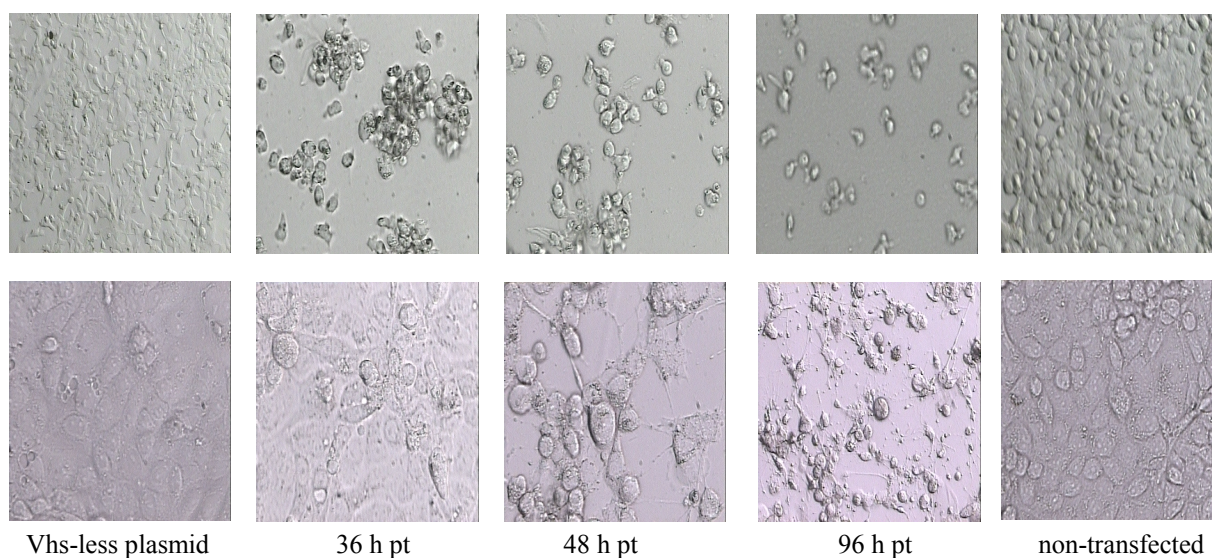


Fig. 2. The effect of *Vhs* on HeLa (above) and MCF-7 (below) cells at different indicated time points. The increasing rate of morphological changes and cell death by time are observed in both cell lines. pt, post transfection.

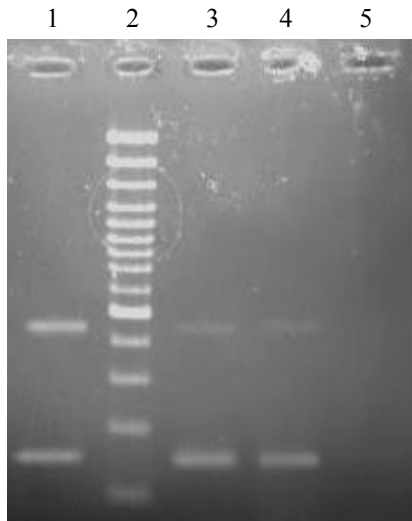


Fig. 3. Agarose gel electrophoresis of the entrovirus (the below band) and $\beta 2$ microglobulin (the above band) cDNA before and after transfection. Lane 1, before transfection; lane 2, size marker; lane 3, MCF-7 after transfection; lane 4, HeLa after transfection and lane 5, negative control.

DISCUSSION

The Vhs protein is known to accelerate the degradation of host and viral mRNA. Regarding the Vhs effect on cells, it can be used as an effective anti-cancer gene if its expression is controlled by a suitable promoter. In contrast to the previous studies, the recent reports have demonstrated that Vhs degrade mRNA in a selective manner [6, 17]. The mRNA which contain ARE in their 3' untranslated domains are targets for Vhs. In many cancer cells in which a proto-oncogene is activated ARE is impaired. The impaired mRNA not only cannot be decayed by Vhs but also they may become more stabilized [11].

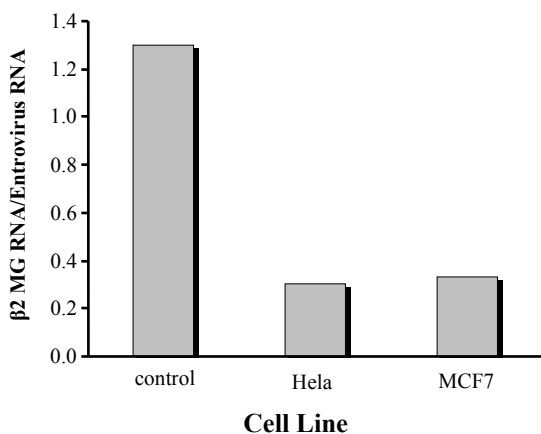


Fig 4. mRNA content of Vhs transfected cells 36 h post infection.

This study was initiated to investigate the effect of Vhs on tumor cells. The Vhs gene was amplified from HSV-2 genome and cloned into the pcDNA3 plasmid. The pcDNA3 plasmid contains a very strong cytomegalovirus promoter for protein expression in eukaryotic cells. MCF-7 and HeLa cells were used as models for cancer cells. In both of the cells, different proto-oncogenes are activated. Regardless to the effect of Vhs on specific oncogenes in tumor cells, it was shown that tumor cells can be destroyed by Vhs and total mRNA content decreases by Vhs. Previous reports show that Vhs reduces expression of a reporter plasmid [18] or replication of HIV in transfected cells [19]. It was also suggested that Vhs could be used as a suicide gene for adenovirus virotherapy of cancer [20].

Our study showed that the Vhs can effectively induce morphological changes and cell death in both tumor cell lines. Although the exact mechanism of Vhs on different mRNA in individual tumor cell lines needs a precise study to evaluate interaction of each oncogene with Vhs, our data suggests that even though Vhs stabilize ARE impaired mRNA, the activity of Vhs is too strong to destroy other mRNA in tumor cells. Apart from the well known anticancer potential of genetically engineered HSV mutated in ICP34.5 for treatment of malignant diseases, the Vhs gene of HSV may have the potential to be added to the list of suicide genes for gene therapy purposes.

REFERENCES

1. Strelow, L.I. and Leib, D.A. (1995) Role of the virion host shutoff (Vhs) of herpes simplex virus type 1 in latency and pathogenesis. *J. Virol.* 69 (11): 6779-6786.
2. Everly, D.N. and Read, G.S. (2002) mRNA degradation by the virion host shutoff (Vhs) protein of herpes simplex virus: genetic and biochemical evidence that Vhs is a nuclease. *J. Virol.* 76 (17): 8560-8571.
3. Strelow, L.I. and Leib, D.A. (1996) Analysis of conserved domains of UL41 of herpes simplex virus type 1 in virion host shutoff and pathogenesis. *J. Virol.* 70 (8): 5665-5667.
4. Smith, T.J., Morrison, L.A. and Leib, D.A. (2002) Pathogenesis of herpes simplex virus type 2 virion host shutoff (Vhs) mutants. *J. Virol.* 76 (5): 2054-2061.
5. Zelus, B.D., Stewart, R.S. and Ross, J. (1996) The virion host shutoff protein of herpes simplex virus

- type 1: messenger ribonucleolytic activity *in vitro*. *J. Virol.* 70 (4): 2411-2419.
6. Esclatine, A., Taddeo, B., Evans, L. and Roizman, B. (2004) The herpes simplex virus 1 U_L41 gene-dependent destabilization of cellular RNAs is selective and may be sequence-specific. *Proc. Natl. Acad. Sci.* 101 (10): 3603-3608.
 7. Murphy, A.J., Duerst, R.J., Smith, T.J. and Morrison, L.A. (2003) Herpes simplex virus type 2 virion host shutoff protein regulates alpha/beta interferon but not adaptive immune responses during primary infection *in vivo*. *J. Virol.* 77 (17): 9337-9345.
 8. Doepker, R.C., Hsu, W.L., Saffran, H.A. and Smiley, J.R. (2004) Herpes simplex virus virion host shutoff protein is stimulated by translation initiation factor eIF4 B eIF4H. *J. Virol.* 78 (9): 4684-4699.
 9. Taddeo, B., Weiran, Z. and Roizman, B. (2006) The U_L41 protein of herpes simplex virus 1 degrades RNA by endonucleolytic cleavage in absence of other cellular or viral proteins. *Proc. Natl. Acad. Sci.* 103 (8): 2827-2832.
 10. Smiley, J.R. (2004) Herpes simplex virus virion host shutoff protein: immune evasion mediated by a viral RNase? *J. Virol.* 78 (3): 1063-1068.
 11. Esclatine, A., Taddeo, B. and Roizman, B. (2004) The U_L41 protein of herpes simplex virus mediates selective stabilization or degradation of cellular mRNAs. *Proc. Natl. Acad. Sci. PNAS.* 101(52):18165-18170.
 12. Bakheet, T., Frevel, M., Williams, B.R., Greer, W. and Khabar, K.S. (2001) ARED: human AU-rich element-containing mRNA database reveals an unexpectedly diverse functional repertoire of encoded proteins. *Nucleic Acids Res.* 29 (1): 246-254.
 13. Balczon, R. (2004) Overexpression of cyclin a in human HeLa cells induces detachment of kinetochores and spindle pole/centrosome overproduction. *Chromosoma* 110 (6): 381-392.
 14. Rinehart-Kim, J., Johnston, M., Birrer, M. and Bos, T. (2000) Alterations in the gene expression profile of MCF-7 breast tumor cells in response to c-Jun. *Int. J. Cancer* 88 (2): 180-190.
 15. Fotouhi, F., Soleimanjahi, H., Roostae, M.H. and Dalimi Asl, A. (2008) Expression of the herpes simplex virus type 2 glycoprotein d in baculovirus expression system and evaluation of its immunogenicity in Guinea pigs. *Iran. Biomed. J.* 12 (2): 59-66.
 16. Kermanian, M., Soleimanjahi, H., Arefian, E. and Bamdad, T. (2008) Enrichment of CSF samples on cell culture for enhancement of sensitivity of mumps and enterovirus detection by multiplex RT-PCR. *Diagnostic. Microbiol. Inf. Dis.* 60 (4): 375-379.
 17. Read, G.S. and Patterson, M. (2007) Packaging of the virion host shutoff (Vhs) protein of herpes simplex virus: Two forms of the Vhs polypeptide are associated with intranuclear B and C capsids, but only one is associated with enveloped virions. *J. Virol.* 81 (3): 1148-1161.
 18. Pak, AS., Everly DN., Knight, K. and Read, G.S. (1995) The virion host shutoff protein of herpes simplex virus inhibits reporter gene expression in the absence of other viral gene products. *Virology* 211 (2): 491-506.
 19. Hamouda, T., McPhee, R., Hsia, S.C., Read, G.S., Holland, T.C. and King, S.R. (1997) Inhibition of human immunodeficiency virus replication by the herpes simplex virus virion host shutoff protein. *J. Virol.* 71 (7): 5521-5527.
 20. Glenn, GM. and Chatterjee, S. (2001) Generation of adenovirus encoding the herpes simplex virus vhs gene: A novel strategy to generate adenoviruses expressing genes toxic to producer cells. *Cancer Gene Therapy* 8 (8): 566-572.