Paper #:
O-040

High Expression Level of Hepatitis C Virus E1 and NS3 Genes under the Control of the Adenoviral Major Late Promoter

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Abstract

Hepatitis C virus (HCV) is the major causative agent of the liver disease which can progress to liver cirrhosis and hepatocellular carcinoma. It is estimated that nearly 200 million people are infected with HCV worldwide. Given the lack of an effective therapy or vaccine until now, the development of an efficient vaccine is urgently required. The high and prolonged expression of antigens is a major requirement for the efficacy of DNA vaccines. The HCV glycoprotein E1 is a viral envelope protein that is involved in virus entry into liver cells. Also, E1 is known to induce high neutralizing antibodies against HCV. The non-structural protein NS3 plays a major role in viral replication and has been shown to induce both humoral and cellular immunity. Therefore E1 and NS3 represent attractive targets for DNA-based vaccines.

In this study, we constructed a number of plasmids encoding the NS3 and E1 proteins under the control of the adenoviral major late promoter (MLP). These plasmids also contain the adenoviral tripartite leader sequence (TPL), which enhances mRNA translation. In this poster we will be reporting on the in vitro evaluation of gene expression levels of these constructs in various cell lines. We also evaluated the effect of wild type adenovirus infection on the transcription of the transgenes from MLP.

Introduction

- Hepatitis C virus (HCV) is a small, enveloped, positive single-stranded RNA virus that belongs to the family Flaviviridae (1). HCV is a major global health problem, as it infects large populations throughout the world. In the majority of the cases the infection becomes chronic, which can progress to liver cirrhosis and hepatocellular carcinoma (2). No vaccine or effective therapy has been available up until this point. The high mutation rate of the virus and the lack of a suitable animal model are the main difficulties facing vaccine development.
- DNA vaccines expressing HCV antigens provide a promising approach. To achieve high levels of transgene expression, careful selection of the promoter element driving the foreign gene is therefore important.
- The adenoviral major late promoter (MLP) is an extremely strong promoter which controls the transcription of the viral major late transcription unit. MLP is active during both the early and late phases of adenoviral infection, however after viral DNA replication MLP transcription activity is greatly enhanced resulting in abundant expression of the late proteins (3). The presence of TPL sequence in the 5' end of mRNA transcribed from MLP enhances the selective translation of viral genes (4, 5)
- In this study we evaluated the transcriptional activity of the MLP using 2 plasmids encoding the HCV glycoprotein E1 and the HCV non-structural protein NS3, and analyzed their activity in 2 different cell lines: human embryonic kidney 293 cells (HEK 293) and Chinese hamster ovary cells (CHO).
- In order to evaluate the activity of the promoter alone and during the adenoviral infection, transfected CHO cells were superinfected with wild type dl309 adenovirus. The level of mRNA isolated from transfected and superinfected cells were analysed and compared over time.

Methods

- HCV E1 and NS3 genes were amplified from FL-Neo con1 cells (kind gift from Dr. Rice, Apath, L.L.C., St. Louis, MO, USA), a human hepatoma cell line stably replicating the HCV subgenomic replicon con1.
- Plasmids encoding NS3 and E1 under the adenoviral MLP combined with TPL were constructed. The plasmids were purified using Norgen's Plasmid DNA Maxiprep Kit (Endotoxin-Free) (Norgen Biotek Corp, Thorold, ON, Canada).
- The plasmids were transfected in 293 and CHO cells using Lipofectamine 2000 (Invitrogen, Burlington, ON, Canada) in antibiotic-free media, which was changed 6 hours post-transfection.
- The transfected CHO cells were superinfected 12 hours later with wild type dl309 adenovirus at a multiplicity of infection of 1 (MOI 1). This is compared to CHO cells transfected only with the plasmids.
- Transfected and superinfected cells were harvested at different time points: 0, 1, 2, 3, 4, and 5 days after transfection of 293 cells and 0, 0.5, 1.5, 2.5, 3.5 and 4.5 days post transfection (0-4 days post superinfection) in CHO cells.
- RNA and DNA were extracted from all cells using Norgen's RNA/DNA/Protein Purification Kit (Norgen Biotek Corp, Thorold, ON, Canada). Isolated RNA was treated with DNAse Turbo (Ambion, Austin, TX, USA) to digest any contaminating DNA. The RNA samples were then purified using Norgen's RNA Cleanup and Concentration Kit (Norgen Biotek Corp, Thorold, ON, Canada).
- Gene expression was analyzed by Real-time qRT-PCR using IQ SYBR-Green Master Mix (BioRad, Inc, Hercules, CA). qPCR of the DNA samples was performed to investigate the plasmid stability over time. Equal amounts of DNA and RNA were used as the templates in the qPCR and qRT-PCR reactions.

Results

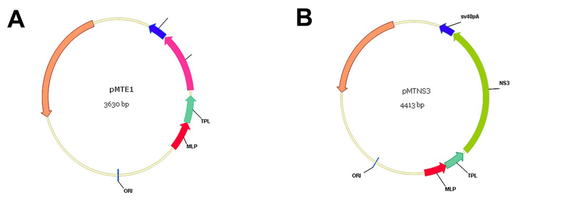

Figure 1. Schematic diagram showing the constructed plasmids. Panel A shows the plasmid pMTE1, which contains the HCV envelope protein E1. Panel B shows the plasmid PMTNS3, which contains the HCV non-structural protein NS3. Both plasmids contain the adenoviral Major late promoter (MLP), and the tripartite leader sequence (TLP) is cloned downstream of the promoter. Also, the poly A signal from simian virus 40 (SV40) was cloned downstream of the transgene.

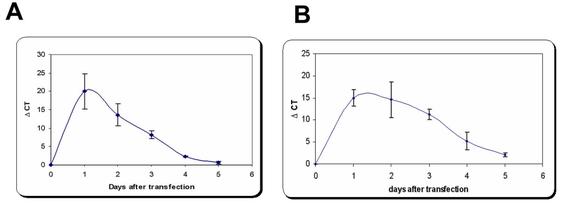

Figure 2. mRNA level of E1(**Panel A**) and NS3 (**Panel B**) in 293 cells over 0-5 days after transfection. The quantification was performed by qRT-PCR using standard curves of the plasmids with known concentrations. The graphs show plots of ΔCT against days 0 – 5 after transfection. ΔCT is the difference between CT values of -RT and RT. The higher ΔCT is, the higher the mRNA level. In both cases the mRNA levels were highest at 1 day post transfection.

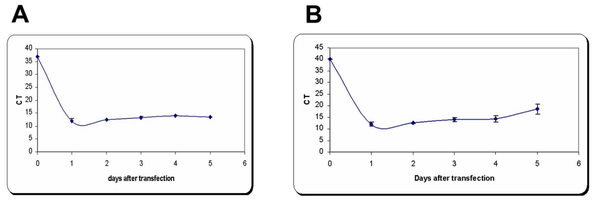

Figure 3: The plasmid stability of E1(**Panel A**) and NS3 (**Panel B**) transfected in 293 cells. Plasmid stability was determined by q-PCR using the standard curve method. The graphs show plots of CT against days after transfection. From observing Figure 3 it can be seen that the stability of both plasmids decreased over time.

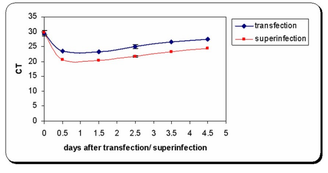

Figure 4: mRNA level of E1 in CHO cells over 0-4.5 days after transfection (0-4 days after superinfection). The quantification was done by qRT-PCR using a standard curve of the plasmid with known concentrations. The graph shows a plot of CT against days after transfection. The mRNA level increased after adenovirus superinfection.

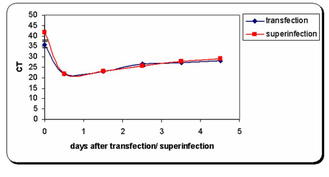

Figure 5. The plasmid stability of E1 in transfected and superinfected CHO cells. Quantification was done by q-PCR using a standard curve for known plasmid concentrations. The graph shows a plot of CT against time after transfection/ superinfection. From observing the results in Figure 5 it can be seen that the level of plasmid DNA decreased over time.

Conclusions

- Our results showed that in both cell lines, CHO and 293 cells, the expression of the E1 and NS3 genes as measured by the mRNA level was highest at 24h post-transfection and decreased gradually until the 5th day after transfection.
- q-PCR of the DNA isolated from the transfected cells indicated that the plasmid copy number decreased with time. This explains the decrease in expression.
- In CHO cells super infected with dl309 adenovirus we observed a significant increase in the mRNA level compared to the cells transfected with the plasmids alone.
- These findings proved that viral infection is required for high expression from the adenoviral MLP. Superinfection with dl309 virus transactivated MLP and therefore increased transgene transcription.
- Superinfection with adenovirus did not affect plasmid stability. DNA levels were the same in the transfected and superinfected cells.

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Acknowledgements

The authors would like to thank the Egyptian Government and the Egyptian Cultural & Educational Bureau in Canada for their financial support of this project. The authors would also like to thank the staff of Norgen Biotek for their technical assistance and Pam Roberts for her help in preparing this poster.

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