Short Communication

Eukaryotic expression of the core gene of hepatitis C virus genotype 1a

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Abstract

Background: Worldwide, hepatitis C virus (HCV) infection is a serious public health disease unlike hepatitis A and B, however there is currently no vaccine against HCV available. Thus, extensive studies are under way to design new and effective treatments against HCV. Core protein is a component of HCV particle which is the first antigen recognized by the immune system. Beside protective properties of core protein, anti–core antibodies can be used to monitor the disease progress. The purpose of the present study was to isolate and clone the core (C) gene from HCV genotype 1a in an attempt to construct a recombinant vector and subsequently evaluate its expression in a cell culture system.

Materials and Methods: RNA genome of HCV genotype 1a was extracted from the blood of an infected patient. Complementary DNA (cDNA) was synthesized. HCV 1a core gene was amplified by PCR using specific primers and it was cloned into a eukaryotic expression vector. Huh7.5 cells were transfected by the designed recombinant vector and the cellular expression of the core gene was confirmed by RT-PCR.

Results: Recombinant pcDNA3.1 (+) vector containing the HCV core gene with approximate size of 576bp was successfully designed. RT-PCR was used to confirm the expression of core antigen in Huh7.5 cell line.

Conclusion: The results showed that the core gene was successfully isolated from HCV genotype 1a and was cloned into the eukaryotic expression vector. This recombinant vector effectively replicated in Huh7.5 cell line, and its protective and therapeutic effects can be examined in further investigations.

Keywords: Hepatitis C virus; cDNA; pCDNA3.1 vector; PCR

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Introduction

Hepatitis C virus (HCV) is an enveloped, single stranded positive sense RNA virus that belongs to Flaviviridae family. HCV infection afflicts more than 170 million people worldwide. In the majority of patients with HCV acute infection, the disease progresses to the chronic form with severe complications such as liver cirrhosis and carcinoma that leads to global death of thousands of people. The prevalence of infection varies in different regions of the world. In some areas, like Egypt, the prevalence is as high as about 22% (1, 2).

It is estimated that around 2 million people in Europe are infected with HCV. The prevalence of HCV infection is higher in Africa compared to that of America and Europe (1, 2). Injecting drug users and infected blood recipients are more at risk. So far, at
least Seven genotype (1, 2, 3, 4, 5, 6 &7) and several subtypes (a, b, c, d, etc) of HCV have been defined. These genotypes have different global distributions, disease progression, and response status to antiviral treatments (3). The course of medical treatments in patients with genotype 2 or 3 is 24 weeks, while patients with genotype 4, 1 need 48 weeks of treatment. Among these, genotype 1 strains are less responsive to interferon therapy. HCV genome encodes at least 10 proteins. The core protein and the envelope glycoproteins E1 and E2 are the structural proteins while the others are non-structural proteins (4, 5).

HCV core protein is a multifunctional protein which has roles in RNA replication, virion self-assembly, and the production of HCV-like particles. Envelope proteins including the core, E1 and E2 of HCV are seemingly the main targets for inducing antibodies against HCV. These antibodies can be used as therapeutic, diagnostic and other downstream purposes (6, 7).

IC41 is a new vaccine based on a synthetic core, NS3, and NS4 peptides that stimulated efficiently the immune system in volunteers in phase 2 clinical trials. The results indicated the importance of core protein in development of new generations of vaccine (8).

Several other studies have shown that the detection of core antigen is a cost effective (compared to the highly cost routine test of RNA quantification) alternative way to the diagnosis and monitoring the HCV infection because in more than 50 % of infected individuals, the anti-core antibodies are present. The measurement of HCV-core antigen can be a reliable indicator for active infection (9, 10).

In present study, a recombinant vector that expresses the highly conserved HCV core gene was constructed and its expression was confirmed in a Huh7.5 cell line. This vector can be used for future studies such as attempts on its purification, vaccine design and diagnostic tests.

**Methods**

**RNA extraction and cDNA synthesis.** A blood sample was obtained from a patient infected with HCV genotype 1a. The procedure for RNA extraction was performed according to the manufacturer’s recommendations (Qiagen, USA). Purified RNA was used to synthesis the cDNA, according to manufacturer’s recommendations (Pars Tous, Iran).

**Polymerase chain reaction (PCR) for isolation of core gene.** A pair of specific primers was designated by Gene Runner (Version 3.05, Hastings Software Inc.) and DNAMAN (version 5.2, LynnonBiosoft) software.

The full-length of core gene was amplified by polymerase chain reaction (PCR) from cDNA genome. 5'-TAGACCAAGCTTCATGAGCACGAATCCTAAAcct-3 as forward primer contained HindIII restriction site and 5'-ATACATGGATCCttactaGGCCGA(A/G)CAGAGG GTGG-3 as reverse primer contained BamHI restriction site and stop codon were used for amplification of core gene. Under lined letters in forward and reverse primers represent HindIII and BamHI restriction sites.

The PCR reaction mixture for the amplification of core gene contained 10 ng DNA sample, 2.5 mM MgCl2, 0.5 mM each dNTPs, 10 pmol of each primer, 1 unit Taq polymerase (Sinaclon, Iran). The PCR program was as follows: initial denaturation stage of 95°C for 5 min, followed by 35 cycles at elongation stage(95°C for 1 min, 58°C for 1 min, 72°C for 1 min), and a final extension of 72°C for 20 min (11, 12).

**Cloning of the core gene into an expression plasmid.** Isolated PCR products, along with pCDNA3.1+ vector, were digested with HindIII and BamHI restriction enzymes. The digested vector and PCR products were fused together as described previously (13). Competent E. coli bacteria strain JM109 were prepared based on Sambrook protocols (14) and were then transformed by the recombinant vector. Colony PCR, using the above-mentioned primers, and the sequencing method were used to confirm the accuracy of the cloning procedure.

**Cells.** Huh-7.5 cells (American Type Culture Collection, USA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) enriched with 10% fetal bovine serum (Invitrogen, USA), 0.2% sodium bicarbonate (Sigma, USA), 100U/ml penicillin (Invitrogen, USA), and 100µg/ml
streptomycin (Invitrogen, USA) at 37°C in a 5% CO2 atmosphere (12).

Transfection. DMRIE-C reagent (Invitrogen, USA) was used for the transfection of Huh-7.5 cells. The transfection was performed as described previously (12). Briefly, 3 × 105 Huh-7.5 cells were cultured in a 6-well tissue culture microplate containing 2 ml of complete DMEM media and the microplate was incubated at 37°C under 5% CO2 condition. After 24h, the medium was discarded and was replaced with 2 ml of Opti-media (Invitrogen, USA). In the next step, the microplate was incubated at 37°C under 5% CO2 condition for 30 minute. The transfection mixture was prepared according to the manufacturer’s instructions (Invitrogen, USA) and the transfection was performed. Seventy-two hours post transfection, the culture media and the transfected cells were collected in order to confirm the expression of recombinant plasmid containing HCV core gene by RT-PCR.

RT-PCR. To confirm the in vitro expression of recombinant plasmid containing HCV core gene, 72h after transfection, the cells were treated with 0.5 ml trypsin (Invitrogen, USA) incubated for 10 min and the cells were collected for RNA extraction. RNAXplus (CinnaGen, Iran) was used for the total RNA extraction as described previously (15, 16), and DNase I (Fermentas, Germany) was used to eliminate the transfected plasmids. Purified RNA was used for cDNA synthesis using the cDNA synthesis kit and oligo-dT primer (Pars Tous, Iran) as template in the PCR reaction. PCR was performed as described above.

Results

Core gene was isolated from HCV genome by using specific primers and then cloned into pCDNA3.1+vector (Figure 1).

Huh7.5 cell line was transfected with the recombinant vector encoding the Core antigen of HCV genotype 1a. The gene expression was confirmed by RT-PCR (Figure 2).

Discussion

Hepatitis C virus (HCV) is a dangerous human pathogen that causes chronic liver disease and is a serious public health problem throughout the world. It is estimated that about 3% of the world population is infected with HCV and most of infected individual unable to resolve the virus so HCV is the leading cause of hepatocellular carcinoma (HCC) around the world. The infection is more prevalent in the
developing countries with about 1.5 million deaths per year and intravenous drug use and blood transfusion are the main routes of acquisition the infection. Therefore it is necessary to develop new therapeutic approaches to cure or at least to prevent further progression to chronicity (3, 17, 18). HCV has at least 6 different genotypes and 30 subtypes. Some HCV subtypes such as 1a, 1b, 2a, 2b are spread all over the world. High mutation rates in the HCV genome, which generates new genotypes and sub genotypes, lack of suitable cell culture systems and proper animal model has hampered the progress in the development of effective vaccines and drugs (2, 19-21).

The genetic diversity in glycoproteins E2 and E1 are very high, while in genetic regions associated with NS5B, core NS5A genes is more stable. So far, effective immune responses in clearing the HCV infection have not been clearly identified. However, many studies have shown that both CD4 and CD8 T cells have important role in eliminating the virus during infection. Disability of immune cells to produce IFN-γ, reduction in the production of antiviral cytokines, dysfunction of dendritic cells, development of suppressor T cells, along with further viral mutations paves the way for the virus to evade the immune system and progress to chronic infection (22-24).

HCV is responsible for 40 -60% of chronic liver infections. In about 30% of the cases, the infection progresses to cirrhosis and fibrosis. Costly treatment options, adverse side effects, genetic variation in different viral strains and host factors can restrict the effectiveness of drug treatments. This necessitates the development of an effective vaccine to control infection (23, 25, 26). Among different HCV genotypes, the core antigen is much conserved and can be the proper candidate for vaccine development against HCV infection.

E1, E2, and core proteins of HCV are structural proteins that may induce the production of neutralizing antibodies. Therefore, core protein might serve as a vaccine candidate. Currently, extensive efforts are taken to establish effective vaccines against the HCV virus. The purpose of designing a vaccine against this virus can be divided into preventive vaccine or therapeutic vaccines so recognition most immunogenic antigens of the HCV antigens is very important. Several vaccine candidates, based on these antigens, are under evaluation in phase I / II human clinical trials (27, 28).

Recent studies reveal that core antigen is able to induce both humoral and cellular immune responses in natural infection. Antibodies upon core protein during the acute phase of infection is the first antibodies to appear against HCV antigens however cell mediate immunity play critical role in controlling virus replication so that generation core-specific CTL cells is correlated with lower levels of viremia and restrain infection(29). Nowadays many attempts have been devoted for the development of efficient therapies against HCV infection. Studies have shown that the vaccination with DNA vaccines could be a reliable and efficient mode of vaccination. The DNA vaccines are currently studies in this field. In a study, Ajourloo et al provided evidence that the anti-Core antibody is a suitable marker to discriminate people who have had cirrhosis from non-cirrhotic people as the titer of anti-Core antibody is significantly higher in patients with cirrhosis (30, 31).

Other similar studies indicate that the Core antigen is a valuable antigen to develop serological assays for monitoring HCV infection. Previous studies have shown that the effective immune responses against HCV are neutralizing antibodies and cell mediate immunity. Core antigen, as a structural antigen, induces neutralizing antibodies and promote T cell responses especially cytotoxic T cell (CTL). HCV has broad genetic diversity and so prepared vector in this study may become a basis for development DNA or viral-based vaccine to overcome viral immune escape both in the prophylactic or therapeutic settings (32-34).

In a study by Dueñas-Carrera et al. a DNA vaccine encoding core, E1 and E2 proteins and individually constructed and then administered to BALB / c mice. High titer of antibody and the production of IFN-γ as a marker for cell mediated immune responses in mice were showed that the DNA vaccine encodes core- E1 - E2 protein in compared to the vectors that only express E1 or E2 antigen would have much immunogenicity (35, 36).

Alvarez-Lajonchere showed that the co-
administration of core protein with DNA vaccine encoding the envelope proteins and core (CIGB-230) can stimulate and significantly increase the immune responses against the DNA vaccine.

Also Alvarez-Lajonchere showed in a phase 1 clinical trial that the intra-muscular injection of CIGB-230 vaccine in patients with chronic HCV infection who were non-responders to treatment with ribavirin and interferon gamma was very successful in such a way that after the last injection, high titers of HCV-specific antibodies and the generation of virus-specific T cells producing IFN-γ were observed. In the final course of treatment, 33.3% of patients showed cellular immune responses against core protein. This study showed that CIGB-230 vaccine is a good candidate for the treatment of patients with chronic HCV infection (37-39).

Beside the issue of vaccine, core antigen is a diagnostic marker. There are several for identifying HCV infection. For instance, anti-HCV antibodies and the level of viral replication can be studied by an enzyme immune assay (EIA) and nucleic acid testing (NAT). However, both of them have several advantages and disadvantages. For instance, false-positive or false-negative results can be achieved by EIA test and it is not possible to distinguish new infection from the past cleared infections. Nucleic acid testing (NAT) like Real time PCR has high specificity and sensitivity for quantifying HCV RNA in blood and is used to monitor the progress of the treatment but it is very costly and it is not readily available in some developing countries. These are the reasons why in recent years several tests based on core antigen have been developed to monitor HCV infection so recently a commercial test based on HCV core antigen to confirm infection has been developed. This test in compared to HCV RNA assays is more cost effective and easier to perform. (40-43).

In the current study a vector encoding the core gene of HCV was developed in order to analyze its expression in a cell culture system. This construct could be used as a basis for designing new generations of vaccines against HCV 1a genotype in further studies.

Conclusions

Due to the capacity of Core antigen in stimulating immune responses, a recombinant vector containing core gene from HCV genotype 1a was constructed and its expression was confirmed. Successful cloning provides a foundation for development of new DNA vaccines against HCV infection. Further studies are needed in order to evaluate immune responses.

Conflicts of Interest

There is no conflict of interest among authors.

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