Multiplication and Isolation of Hepatitis C Virus in the NGUK-1 Rat Neurinoma Neural Cells

L. Yu. Vergun, P. M. Perechrestenko, L. I. Kondakova*, and A. S. Khalansky

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Hepatitis C virus (HCV) strains were isolated from human sera *in vitro*. NGUK-1 rat neurinoma neural cells [1] were selected as the experimental model of HCV. The cells were infected after attaining the confluence. The virus caused no cytopathic effect during its multiplication. After monolayer infection (24-96 h), culture fluid samples were tested for HCV RNA by classical PCR with electrophoretic detection of the reaction products and by quantitative real time PCR. All samples from infected culture were positive, control samples (intact cultures) were negative. Coefficient of correlations in quantitative PCR was 0.99. The results are reliable, conform to the normal values for this series of the test system, and indicate that HCV is replicated in continuous NGUK-1 cells. This *in vitro* model can be used for isolation of HCV.

Key Words: *hepatitis C virus; continuous NGUK-1 cell culture; polymerase chain reaction*

According to modern classification, hepatitis C virus (HCV) is a member of the *Flaviviridae* family, *Hepacivirus* genus [6]. The majority of representatives of this family cause no cytopathic effect during culturing under laboratory conditions, which impedes *in vitro* diagnosis. Modern molecular methods for virus identification detect only the presence of protein or nucleic acid in the sample, but the viable virus. Positive EIA and/or PCR are also indicative of possible infection [10]. NASBA methods are not yet widely used. The only experimental model most fully reproducing HCV infection is infection of chimpanzee [7,11], which is in fact unavailable. It was reported that HepG2 human hepatoma cells maintain genome replication and even assembly of virus-like particles [7]. The virus was isolated on continuous cells of renal origin (BHK-21, Vero, HAK, PS) and primary brain cells of suckling mice [4,5]. The major proteins of HCV structural region are expressed in vv/HCV1-906 mammalian cells [12]. HuH-7 cells containing HCV replicon RNA were used for isolation of total cell RNA, RNA amplification of HCV replicon in reverse transcription (RT) PCR, and antiviral activity of some drugs was evaluated on this model [13]. Virus indication in these cases can be carried out by the PCR or enzyme immunoassay and immunofluorescent methods [5].

We studied the possibility of using NGUK-1 cells for HCV isolation and its detection by biomolecular method.

MATERIALS AND METHODS

The study was carried out on NGUK-1 rat neurinoma neural continuous cell strain derived and maintained at Institute of Human Morphology, Russian Academy of Medical Sciences. The sensitivity of this cell strain to viruses of different groups was described. For example, togaviruses and rhabdoviruses rapidly accumulate in NGUK-1 cells, the reproduction is not associated with apparent cytopathic effect [1]. Prion agents of scrapie and Kreutzfeld—Jakob disease also replicate in the culture [8,9].

The cells were cultured in glass and plastic flasks or microtitration plates (M 96; Sarstedt AG & Co.) at

Institute of Hematology and Transfusiology, Academy of Medical Sciences of Ukraine, Kiev; *Institute of Human Morphology, Moscow, Russia. ***Address for correspondence:*** vergunl@mail.ru. L. Yu. Vergun

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37°C in DMEM with 10 mM glutamine, 5-10% FCS (PERBIO), and 50 μg/ml gentamicin. The cell monolayer was removed with trypsin:versen (1:9) mixture, precipitated by centrifugation at 2500-3000 rpm, 10 min), the cell precipitate was carefully pipetted in 1 ml nutrient medium, and diluted 1:3-1:5.

The cells were infected by adding non-diluted sterile human sera positive in PCR (AmpliSense HCV-240/BKO-440) onto newly formed monolayer in a minimum volume sufficient for covering the cells and incubated for 30 min at 37°C, rocking the plates periodically. The serum was then removed, the monolayer was washed in DMEM, nutrient medium with 5% FCS was then added and the preparations were cultured at 37°C in a CO_2 incubator at 5% CO_2.

Culture fluid was collected after 24-96 h for testing by qualitative and quantitative PCR (AmpliSense HCV-240/BKO-440, AmpliSense HCV-Monitor-FRT). Culture fluid from flasks or wells with intact cells served as the control.

The PCR was carried out according to instructions for the test systems. The results of qualitative PCR were detected by electrophoresis. Quantitative PCR was carried out on a Rotor-Gene 3000 device (Corbett Research) and Rotor Gene 6.0 software for RT, PCR amplification, and detection of PCR amplification products. The data were analyzed using software attached to the device for RT-PCR with hybridization fluorescent detection in the real time mode.

RESULTS

The virus was isolated from sera collected from HCV patients hospitalized in infectious wards of Clinical Hospital No. 15 (Kiev). Serum samples were positive in PCR and according to EIA data contained specific antibodies in different concentrations (anti-HCV; Table 1).

Fourteen sera from 9 HCV patients were tested. No cytopathic effect was detected during cell culturing after infection. The selected samples of culture medium were tested by classical PCR with electrophoretic detection of the reaction products according to manufacturer's instruction (AmpliSense HCV-240/BKO-440) and quantitative real time PCR (AmpliSense HCV-Monitor-FRT; Table 1). Specimens of culture fluid and in one case cell lysate (No. 14) tested in quantitative PCR with the linear range of 500-50×10^6 U/ml demonstrated the levels of viral RNA accumulation ranging from 1200 to 252,800 copies/ml; *r* coefficient of correlations for calibration curve was 0.99.

The mechanism of virus tropism to various organs and tissues leading to systemic manifestations of HCV

TABLE 1. Isolation of HCV in Continuous Culture of NGUK-1 Cells

No. of serum from HCV patients positive in PCR	Level of anti-HCV in sera used for infection, opt. dens. units	Duration of accumulation, h	Electrophoretic detection	Real time RT-PCR, number of RNA copies/ml
2	2.521	96	+	—
2	2.521	60		21,600
2	2.521	67		3427
5	3.600	72		1200
6	3.600	72		52,000
7	0.468	24		20,323
12	3.092	67		2400
14	2.956	96	+	—
14	2.956	60		252,800
14	2.956	67		28,235
14 (cell lysate)	2.956	72		3604
42	0.179	24		32,400
87	0.753	48	+	—
87	0.753	60		6800
87	0.753	96	+	—
83+87	0.618+0.753	60		14,999

Note. Dash: not tested.

infection remains not quite clear [11]. HCV tropism to liver hepatocytes is a known fact, but extrahepatic replication of HCV was also demonstrated. The virus genome was detected in various human organs and tissues: bone marrow, peripheral blood monocots, spleen, muscles, lymph nodes, pancreas, kidneys, *etc.* [3,11]. High lymphotropism of the virus was noted [3]. Our results suggest also the neurotropism of HCV.

Hence, our experiments demonstrated sensitivity of NGUK-1 cells to HCV and the possibility of using these cells for isolation and immunochemical detection of HCV.

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