Primary Human Trophoblasts Expressing Four Essential Receptors of Hepatitis C Virus Entry but Resistance to Infection in Vitro

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Abstract

Background: Vertical transmission (VT) is generally considered to be the leading route of pediatric acquisition of Hepatitis C Virus (HCV). However, the mechanism of HCV VT has never been satisfactorily illustrated. Moreover, CD81, scavenger receptor class B type I (SR-B1), claudin 1 (CLDN1) and occludin (OCLN) were four necessary receptors for HCV cell infection. The expression of the essential receptors may explain the reason of HCV VT.

Objective: To investigate the probable mechanism of HCV VT.

Methods: We isolated trophoblasts from human term placental samples with approval from the Institutional Review Board at the Tongji Hospital of Tongji University and cultured the cells in vitro. Flow cytometry and Western blotting were used to detect the expression of the essential receptors for HCV infection on trophoblasts. Then we generated HCV pseudoparticle (HCVpp) and cell culture-produced HCV (HCVcc) to infect HEK-293T cells and trophoblasts. At 72 h post-infection, HCVpp and HCVcc were determined in the infected cells.

Results: Trophoblasts expressed CD81, SR-B1, CLDN1 and OCLN, which were essential for HCV cell entry. However, trophoblasts were not infected by HCVpp or HCVcc.

Conclusion: Primary human trophoblasts possess the receptors necessary for HCV infection but resistant to infection in vitro.

Keywords: Trophoblast; Receptor; Hepatitis C virus; Vertical transmission

Introduction

Hepatitis C virus (HCV) is an enveloped single-stranded RNA (ssRNA) virus. It is a well-known origin of adult liver morbidity and mortality, but its problem in children is often overlooked [1]. As we all know, the risk factors most frequently cited as accounting for the bulk of HCV transmission worldwide are blood transfusions from unscreened donors, injection drug use, unsafe therapeutic injections, and other health-care related procedures [2]. Although some studies suggested that many American children acquired HCV from infected blood or blood products, the leading route of pediatric acquisition of HCV in most parts of the world is vertical transmission [1, 3-5]. And the prevalence of vertical transmission (VT) of HCV is estimated at approximately 5% (3%-10%) [5]. Despite an increased understanding of the risk factors involved in HCV transmission; thus far, little is known about the mechanism of HCV VT.

Studies have presented conclusive evidence indicating that HCV infection is initiated by interactions between the E2 glycoprotein in HCV and several cell surface molecules [6]. Furthermore, advances in the development of in vitro system to study the HCV life cycle have demonstrated an essential role for tetraspanin CD81 [7-11], scavenger receptor class B type I (SR-B1) [12-14], claudin 1 (CLDN1) [15] and tight-junction protein occludin (OCLN) [16] in virus entry. CD81 and SR-B1 bind HCV encoded E1E2 glycoproteins with high affinity. The CD81 tetraspanin is first identified as an HCV envelope glycoprotein E2-binding receptor and shown to be required for HCV entry into target cells [7]. SR-B1 acts at similar time points than CD81 forming part of the receptor complex required for HCV entry into the target cell [9]. And The HCV envelope glycoproteins do not directly interact with CLDN1, but CLDN1 interacts with CD81 and thereby plays an important role during post-binding steps of the HCV entry process [10,15,17]. OCLN have also been demonstrated to participate in post-binding steps of the HCV entry process [16].

The placenta is a hematopoietic organ mainly consisting of placental epithelium (trophoblasts), and it serves as a barrier against maternal-fetal transmission. Nutrients, certain drugs, and hormones, as well as viruses such as HCV, are some of the substances that can pass through the placental barrier to reach the fetus. Trophoblasts are the first line of defense against virus infection. Therefore, this study aimed to investigate the mechanism of HCV VT by isolation and In vitro culture of trophoblasts.

Here, trophoblasts obtained from placentas after delivery via Caesarian section were cultured in vitro. Flow cytometry and Western blotting were used to detect the essential receptors of HCV infection on the trophoblasts. However, HCVpp and HCVcc infection of trophoblasts in vitro could not be observed and thus it could not be verified whether HCV is capable of entering and infecting primary human trophoblasts.

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Materials and Methods

Trophoblast isolation and culture

Trophoblasts were isolated from human term placental samples with approval from the Institutional Review Board at the Tongji Hospital (affiliated to the Tongji University). Human term placentas were obtained from normal pregnant women after they underwent Cesarean section. Trophoblasts were purified according to the method of Kliman et al. [18]. After delivery of the infant, the placenta was removed and placental lobules were cut, stored in an aseptic bottle containing 4°C normal saline, and immediately brought to the laboratory for the experiment. The placental lobules were transferred to a beaker and rinsed thoroughly in 4°C normal saline under aseptic conditions. The tissues were cut into 1-3 mm³ sections and digested with 0.125% trypsin (Sigma) and 0.01% DNase I (Sigma) in warmed Dulbecco's modified Eagle's medium (DMEM) for 30 min at 37°C. After filtration through 0.01-mm stainless steel wire mesh, cell suspensions were centrifuged at 2,200 rpm for 10 min, and the supernatants were resuspended in 5 ml DMEM. The resulting cell suspension was placed in a 5-70% Percoll (GE) gradient made up in Hanks' solution. The gradients were centrifuged at 2,550 rpm for 20 min. The 30-45% band containing the trophoblasts were removed after centrifugation, washed with DMEM, and resuspended in DMEM. The viability of the trophoblasts was assessed by trypan blue exclusion. Live cells comprised more than 90% of the cytotothrophoblast preparation. The purified trophoblasts were counted and then diluted with DMEM-HG containing 4 mM glutamine (Sigma), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 10% heat-inactivated fetal calf serum (Gibco) to achieve a concentration of 10⁶ cells/ml seeded in 24- and 96-well plates, then plated in 35-mm culture dishes. Cells were incubated in 5% CO₂ at 37°C. Culture media were changed every 24 h.

Identification of trophoblasts

The Percoll gradient-purified cells cultured in the 96-well plate were fixed using methanol for 30 min at minus 20°C. The cells were washed twice with phosphate-buffered saline (PBS), blocked with 3% bovine serum albumin (BSA) at room temperature for 1 h, and incubated with anti-cytokeratin or anti-vimentin antibodies (Invitrogen). Then, the cells were incubated with anti-rabbit IgG (Invitrogen) and stained with anti-cytokeratin or anti-vimentin antibodies (Invitrogen). Then, the cells were washed and fixed with ice-cold methanol for 20 min at minus 20°C, and stained for NS5A with monoclonal antibody (mAb) 9E10 and Alexa 488-conjugated anti-mouse IgG. Infection was quantified by enumerating NS5A-positive foci and the virus titer of focus-forming units (FFU)/ml was calculated.

Following an overnight culture, the trophoblasts, seeded in 24-well plate, were infected with HCVpp and Huh7.5.1 cells were used as positive controls, respectively. The medium was removed after 8 h, washed with 1ml PBS and 500 ul normal medium was added per well. The medium was changed every 24 h. At three days post infection, infected cells were identified by the expression of NS5A or HCV RNA. Purified cellular RNA samples (Qiagen, Hamburg, Germany) were amplified for HCV RNA (Primer Design Ltd, Southampton, UK) in a single-tube reverse-transcription polymerase chain reaction in accordance with manufacturer’s guidelines (CellsDirect kit; Invitrogen) and fluorescence monitored in an MxPro 3000 real-time polymerase chain reaction machine (Stratagene, La Jolla, CA). In all reactions the house keeping gene glyceraldehyde-3-phosphate dehydrogenase was included as an internal endogenous control for amplification efficiency and for RNA quantification (primer-limited endogenous control; ABI, Carlsbad, CA).
Results

Trophoblast isolation and culture

Trypan blue staining of the gradient-purified cells showed that more than 90% of the cells were viable. Negative vimentin staining and positive cytokeratin staining were observed, verifying that the collected cells were human trophoblasts. The purified trophoblasts contained very little contamination of endothelial cells or fibroblasts, as evidenced by the anti-vimentin antibody staining (Figure 1).

Initially, trophoblasts showed individual growth when cultured, but they appeared to form aggregates consistently. After 5 days of culture, syncyta were formed.

Expression of CD81, SR-B1, CLDN1, and OCLN on trophoblasts

The expressions of CD81, SR-B1, CLDN1, and OCLN were detected with Western blotting (Figure 2) and flow cytometry analysis (Figure 3), suggesting the possibility of HCV infection of trophoblasts.

In vitro HCVpp infection of trophoblasts

Huh7.5.1 cells and trophoblasts were infected with HCVpp. At 72 h after infection, Huh7.5.1 cells and trophoblasts had infections rates of 30% and 0%, respectively. The infection rates of trophoblasts were much lower than those of Huh7.5.1 cells, indicating that the trophoblasts cultured in vitro may be resistant to infection with HCVpp (Figure 4).

In vitro HCVcc infection of trophoblasts

Huh7.5.1 cells and trophoblasts were incubated with HCVcc for 8 h and 3 days after infection, NS5A and HCV RNA level were quantified (Figure 5A and B). There were no detectable NS5A and HCV RNA in trophoblasts three days post-infection, demonstrating trophoblasts resistance to HCVcc infection in vitro.

Discussion

HCV infection is recognized as a disease of global importance. It is considered to be a major health and economic burden in adults and in children in both developing and developed countries. And infection in the uterus is a significant route of vertical HCV transmission [1, 3-5]. However, the exact mechanism of HCV VT is not very clear.
The interaction of HCV with its cellular receptor initiates a chain of dynamic events that enables its entry into the trophoblasts, then to the fetus. CD81, SR-B1, CLDN1, and OCLN are considered essential for HCV cell entry [6]. CD81 and SR-B1 are the classical receptors of HCV and work together in the initial stage of HCV infection. They are considered vital for the infection of HCV [7,11]. OCLN and CLDN1 are tight junction proteins, and a previous study has demonstrated that the expression of these proteins is indispensable for the invasion of HCV [16]. This study focused on these four HCV receptors and attempted to investigate the mechanism of HCV VT.

The placenta serves as a barrier between the mother and fetus, and it prevents the transmission of certain drugs and infectious agents. The placental epithelium mainly consists of trophoblasts; therefore, isolating and culturing trophoblasts is vital for researching the mechanism through which infectious agents crossing the placenta barrier infect the fetus [21]. Here, trophoblasts were isolated successfully according to the method of Kliman et al. as described previously [18].

Trophoblasts have always been the focus for the study of HCV infection in vitro to investigate VT. Nevertheless, little evidence has been reported about the possibility of HCV infecting trophoblasts through receptors or about the mechanism by which this occurs. Our study is the first to detect the expressions of the HCV receptors CD81, SR-B1, CLDN1, and OCLN on trophoblasts.

Through a series of experiments, the expressions of four essential receptors in primary human trophoblasts were verified, suggesting the possibility of HCV infection of trophoblasts. However, green fluorescence was not observed in trophoblasts infected with HCVpp and hNSA and HCV RNA were not detectable in trophoblasts infected with HCVcc. A possible explanation is that the expressions of the four receptors on trophoblasts cultured in vitro were considerably less than those in vivo, as a result of which the trophoblasts were not susceptible to HCV infection in vitro. Moreover, it is possible that other factors involved in HCV entry were not expressed by primary trophoblasts in vitro. In addition, the exact timing of vertical HCV transmission is unknown. Both intrauterine and intrapartum infections are possible [22-24]. The intrauterine infection maybe happens at an early or middle or late stage of pregnancy [25, 26] to date little is known about that. Consequently, there is no enough evidence to explain the exact mechanism of HCV VT. Further experiment and inquiry is required.

In this study, HCV infection of primary human trophoblasts could not be simulated in vitro using HCVpp and HCVcc. Nevertheless, our study is the first to verify that primary human trophoblasts possess the receptors necessary for HCV infection. Further studies should be performed to obtain additional insights into HCV VT.

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