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Restriction of SARS-CoV-2 replication in the human placenta

Kazuhide Takada a, Sayaka Shimodai-Yamada b, Mayumi Suzuki b, Quang Duy Trinh a, Chika Takano a, Kaori Kawakami c, Mikiko Asai-Sato c, Atsushi Komatsu c, Aya Okahashi d, Nobuhiko Nagano d, Toshiya Misawa e, Kyohei Yamaguchi f, Tadaki Suzuki g, Kei Kawana c, Ichiro Morioka d, Hideto Yamada b, Satoshi Hayakawa a, Hiroyuki Hao b,*, Shihoko Komine-Aizawa a,*

a Division of Microbiology, Department of Pathology and Microbiology, Nihon University School of Medicine, Japan
b Division of Human Pathology, Department of Pathology and Microbiology, Nihon University School of Medicine, Japan
c Department of Obstetrics and Gynecology, Nihon University School of Medicine, Japan
d Department of Pediatrics and Child Health, Nihon University School of Medicine, Japan
e Department of Obstetrics and Gynecology, Nagoya Ekisaikai Hospital, Japan
f Department of Obstetrics and Gynecology, National Hospital Organization, Mie Chuo Medical Center, Japan
g Department of Pathology, National Institute of Infectious Diseases, Japan
h Center for Recurrent Pregnancy Loss, Teine Keijinkai Hospital Department of Pathology, National Institute of Infectious Diseases, Tokyo, Japan

A B S T R A C T

Although SARS-CoV-2 can infect human placental tissue, vertical transmission is rare. Therefore, the placenta may function as a barrier to inhibit viral transmission to the foetus, though the mechanisms remain unclear. In this study, we confirmed the presence of the SARS-CoV-2 genome in human placental tissue by in situ hybridization with antisense probes targeting the spike protein; tissue staining was much lower when using sense probes for the spike protein. To the best of our knowledge, this is the first evidence directly indicating inefficient viral replication in the SARS-CoV-2-infected placenta. Additional studies are required to reveal the detailed mechanisms.

1. Introduction

Accumulative studies have reported that SARS-CoV-2 can infect placental tissue, especially the syncytiotrophoblast, during pregnancy. However, vertical transmission of the virus is unlikely to occur [1–5]. Therefore, the placental barrier might be effective in inhibiting viral transmission to the foetus, though the mechanisms are still unknown [6]. Inefficient viral infection and replication in the placenta have already been suggested by ex vivo and in vitro studies [7,8]. To date, however, compelling evidence of inefficient viral replication in the placenta of SARS-CoV-2-infected women is still lacking.

Some studies employed an in situ hybridization (ISH) technique to detect the SARS-CoV-2 genome in the placenta [5,8–20]. All these studies used “antisense” probes to detect the viral genome, which confirmed only the presence of SARS-CoV-2 in the tissue. Although other studies, such as [21], have also used the ISH technique, we could not find the details of the probes used. SARS-CoV-2 is a positive-sense, single-stranded RNA virus, and a negative-sense strand is generated during the replication process [4,22]. The negative-sense strand of SARS-CoV-2 can be detected by a “sense” probe [23]. We found only one study that used both antisense and sense probes to detect SARS-CoV-2 in the placenta [24]; however, it lacked detailed descriptions. To confirm the presence and replication of the SARS-CoV-2 genome, we performed ISH on serial sections of the placenta using antisense and sense probes.

2. Methods

2.1. Subjects

This study was approved by the ethical committees of the Nihon University School of Medicine (P20-22-0, and P20-29-0, and RK-200512-9), the Mie Chuo Medical Center (MCERB-202106), and the Nagoya Ekisaikai Hospital (2021–042). All patients were informed and signed an informed consent form. Nasopharyngeal swabs from the patients were used to diagnose SARS-CoV-2 infection, and 15 placental tissues were obtained from 13 mothers (two had twin pregnancies). Each
In situ hybridizations of SARS-CoV-2 spike protein mRNA on serial sections of infected human placenta. Placental tissues were obtained from pregnant women with COVID-19. Images are representative of each case. Lung tissue of a SARS-CoV-2-infected mouse served as a positive control. Placentas obtained from uninfected pregnant women, as well as a PCR-negative placenta from a patient with COVID-19, were used as negative controls. An antisense probe for the positive-sense mRNA of the spike protein was used to detect the distribution of the virus. A sense probe for the negative-sense mRNA was used to detect the intermediate process of viral replication. Note that the reaction of the sense probe was very weak on the SARS-CoV-2-infected placenta compared with the reaction of the antisense probe on a serial section, indicating that viral replication was restricted in the placentas of cases 1 and 3. Magnification: 400x. Bars: 20 μM.
sample was obtained from the center of the placenta. The placental tissues were fixed with formalin and embedded in paraffin. The sample was obtained from the center of the placenta. The placental tissues, especially in the syncytiotrophoblast, but not evident that the reaction of the antisense probe was strong in the PCR-positive probe (Fig. 1). The results obtained with the placental tissues showed the reaction of the sense probe was weaker than that of the antisense probes were detected at the same site on serial sections, although

Table 1

| Case | RT-qPCR | ISH (anti-sense) | ISH (sense) |
|------|---------|-----------------|-------------|
| 1-1  | +       | +               | –           |
| 1-2  | +       | +               | –           |
| 2    | +       | +               | –           |
| 3-1  | +       | +               | –           |
| 3-2  | +       | +               | –           |

2.2. Reverse transcription–quantitative polymerase chain reaction (RT-qPCR)

Viral RNA was extracted from the placental tissue by using ReliaPrep RNA Miniprep Systems (Promega, Madison, WI, United States) according to the manufacturer’s instructions. The presence of SARS-CoV-2 was detected by RT-qPCR amplification of SARS-CoV-2 genes using primer/probe N2 (TaKaRa, Shiga, Japan) and One Step PrimeScript III RT-qPCR Mix (TaKaRa). Amplification was performed by a QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, United States) according to the manufacturer’s instructions.

2.3. In situ hybridization (ISH)

Three PCR-positive samples (cases 1 to 3; two were twin pregnancies) were used for the ISH study. These patients delivered during the second trimester of pregnancy (8–15 days after the diagnosis of COVID-19). In case 1, the symptoms of COVID-19 were mild, but HELLP syndrome occurred just before the delivery. Cases 2 and 3 were asymptomatic.

We selected these three cases of COVID-19 with placental infection for ISH. ISH for the genome of the SARS-CoV-2 spike protein was performed using RNAscope Target Probe - V-nCoV2019-S (Cat No. 848561), RNAscope Target Probe - V-nCoV2019-S-sense (Cat No. 845701), and RNAscope 2.5 HD Reagent Kit-Brown (Cat No. 322300) (Advanced Cell Diagnostic, Hayward, CA, United States), according to the manufacturer’s instructions. Six-micrometre sections of formalin-fixed, paraffin-embedded tissue were used. Sections were counterstained with haematotoxin. Lung tissue of a SARS-CoV-2-infected mouse served as a positive control. Tissues obtained from an uninfected case and a PCR-negative placenta from a patient with COVID-19 (Fig. 1) were used as negative controls.

3. Results

In the murine lung positive control, the reactions of the antisense and sense probes were detected at the same site on serial sections, although the reaction of the sense probe was weaker than that of the antisense probe (Fig. 1). The results obtained with the placental tissues showed that the reaction of the antisense probe was strong in the PCR-positive placental tissues, especially in the syncytiotrophoblast, but not evident in the decidua in all three independent cases. However, in cases 1 and 3, the reaction of the sense probe was very weak in serial sections from paraffin-embedded sections (Fig. 1). Neither the antisense nor the sense probe reacted with the tissues obtained from the uninfected case and with the PCR-negative placenta from the patient with COVID-19 (Fig. 1). The RT-qPCR and ISH results are summarized in Table 1.
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