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Original Research

Differential Expression of Rab5 and Rab7 Small GTPase Proteins in Placental Tissues From Pregnancies Affected by Maternal Coronavirus Disease 2019

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ABSTRACT

Purpose: The majority of pregnancies affected by maternal coronavirus disease 2019 (COVID-19) do not result in fetal transmission. However, several studies have identified parenchymal changes in their placental tissues, suggesting a placental response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the maternal–fetal interface. Although many COVID-19 placental studies have focused on the expression of the canonical SARS-CoV-2 entry proteins angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2, further characterization of subcellular molecules involved in viral trafficking have not yet been investigated in these tissues. Of interest are Rab proteins, a family of small GTPase proteins that direct intracellular transport between different endocytic organelles. Rab5 and Rab7 in particular have previously been implicated in HIV and cytomegalovirus invasion of placental trophoblast cells *in vitro*; the localization of these molecules has not been fully characterized within the human maternal–fetal interface, however, or within placental tissues from SARS-CoV-2–infected pregnancies.

Methods: Using fluorescent immunohistochemistry, Rab5 and Rab7 placental localization and comparative fluorescence intensity were explored in a cohort of placental tissues from pregnancies affected by maternal COVID-19 disease (COVID, n = 15) compared with contemporary control subjects (Control, n = 10). Fluorescence intensity was quantified by using corrected total cell fluorescence values.

Findings: Within placental villi, Rab5 was consistently localized in syncytiotrophoblast and cytotrophoblast cells. Rab5 had significantly higher mean (SEM) fluorescence intensity in the COVID cohort (Control, 1.96 [0.16]; COVID, 2.62 [0.09]; $P = 0.0014$). In contrast, although Rab7 was also localized within placental villous syncytiotrophoblast and cytotrophoblast cells, mean (SEM) Rab7 fluorescence intensity was significantly downregulated in COVID vs Control placentas (Control, 35.9 [4.1]; COVID, 20.1 [0.52]; $P = 0.0001$).

Implications: This differential expression of Rab5 and Rab7 suggests that placental endocytic pathways may be altered at the maternal–fetal interface in pregnancies affected by maternal SARS-CoV-2

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infection. As key molecules governing intracellular vesicle transport, including viral trafficking, Rab GTPase proteins may be of interest for ongoing studies examining placental responses to COVID-19 in pregnancy. (*Clin Ther*. 2021;43:308–318) © 2021 Elsevier Inc.

Keywords: COVID-19, placenta, Rab GTPase, SARS-CoV-2.

INTRODUCTION

With >10 million confirmed cases and several hundred thousand deaths reported worldwide, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the novel infectious agent responsible for coronavirus disease 2019 (COVID-19), remains a significant global health burden.[1] Of particular interest is the study of COVID-19 in pregnancy. With prolonged maternal–fetal exposures being a physiological necessity for healthy gestation, viral transmission of SARS-CoV-2 in pregnancy cannot be combated through normal quarantine measures. However, low rates of fetal transmission (1%–4%) have been consistently reported throughout the pandemic.[2,3] As the main exchange interface between mother and fetus in pregnancy, the placenta seems to be creating a physiological barrier against fetal infection of SARS-CoV-2. However, the mechanisms governing the lack of fetal transmission remain unclear. The present article reviews the current literature on COVID-19 in pregnancy and placental responses to SARS-CoV-2 infection. We also share novel data from a pilot study examining the expression of selected proteins involved in endocytic vesicle trafficking in a cohort of placental tissues from pregnancies affected by maternal COVID-19, highlighting a potential new direction for the study of SARS-CoV-2 responses at the maternal–fetal interface.

Although pregnant women have been estimated to comprise only 9% of SARS-CoV-2–positive cases among women of reproductive age in the United States,[4] they seem to be particularly susceptible to complications of COVID-19 disease, with reports of increased disease severity and higher rates of hospitalization compared with other adult populations.[5,6] Regarding perinatal clinical signs in newborns of mothers with positive SARS-CoV-2 test results, studies have reported mixed results. Interestingly, infants appear to be asymptomatic with low rates of positive SARS-CoV-2 test results. In those who have tested positive, there have been reports of complications, including preterm birth, respiratory distress syndrome, meconium-stained amniotic fluid, fever, tachycardia, and tachypnea in case series.[7–10] In other clinical literature investigating SARS-CoV-2–negative infants born to SARS-CoV-2–positive mothers, clinical findings such as chronic in utero fetal distress, chorioamnionitis, preterm birth, dyspnea, thrombocytopenia, vomiting, fever, tachycardia, and low birth weight have been reported.[11–13] However, the majority of the literature has reported normal findings (eg, APGAR scores, birth weight) in neonates born to SARS-CoV-2–positive mothers.[7,12,14–18]

Despite a significant increase in research on COVID-19 disease in pregnancy in 2020, questions remain about the risk for transmission of SARS-CoV-2 from mother to fetus (ie, vertical transmission). Before the current pandemic, coronavirus was not associated with vertical transmission. This was true for both SARS-CoV-1 and Middle East respiratory syndrome-coronavirus, which were responsible for major outbreaks in 2002 and 2012.[19,20] Selected studies to date have suggested vertical transmission of SARS-CoV-2 based on positive immunoglobulin M antibody laboratory test results in 3 neonates; however, the sensitivity and specificity of these serologic tests have been questioned.[12,21,22] Other case series and reports have further identified positive results for SARS-CoV-2 from infants' pharyngeal swabs by real time reverse transcription polymerase chain reaction (real time RT-PCR).[7,8,10,23] In 1 patient, placental biopsy specimens from a stillbirth tested positive immediately after expulsion and at 24 h.[24] Another study reported SARS-CoV-2–positive samples from placental or membrane swabs in 3 pregnant patients, but results of neonatal swabs were all negative.[25] Patanè et al[23] reported positive RT-PCR test results for 2 of 22 mother–neonate dyads, as well as both of their placentas. Nevertheless, the majority of COVID-19 studies in neonatal settings have found no evidence of viral RNA in pharyngeal swabs, blood, feces, or urine.[7,8,10–13,16–18,25,26] Furthermore, breast milk and vaginal secretions have yielded negative SARS-CoV-2 results.[16,27]

Pathology evaluations of placentas from pregnancies affected by maternal COVID-19 have reported a range of placental findings. These reports have included tissue

cohorts showing minimal changes as well as various placental abnormalities, including features of maternal vascular malperfusion, increased intervillous thrombi, diffuse perivillous fibrin and inflammatory infiltrates, and increased incidence of chorangiosis compared with controls.[24,26,28–30] Other COVID-19 placental case series also observed low-grade fetal vascular malperfusion, maternal vascular malperfusion, villitis, and fetal vascular thrombosis.[31,32]

The COVID-19 pregnancy literature to date has largely focused on various clinical manifestations, epidemiology, and placental pathology; few studies have fully evaluated mechanisms governing the low rates of fetal transmission and/or the placental cellular responses to maternal SARS-CoV-2 infection. The symbiotic maternal–fetal physiology required for pregnancy maintenance and normal fetal development necessitates close and prolonged exposures between mother and fetus throughout gestation. However, despite these exposures, vertical transmission between mother and fetus remains a heterogeneous process, with a broad variability in maternal infections that result in true fetal pathology.[33] In viral infections in which vertical transmission is prevented, the placenta can be a key boundary providing physiological viral blockade.

Placenta villi are the main anatomical interface between mother and infant during pregnancy (Figure 1A) and are composed of placental cell layers covering a central core of fetal blood vessels.[34] These placental cells, termed villous trophoblasts, are subdivided into syncytiotrophoblast cells (sTBs) that are fed by an underlying layer of cytotrophoblast cells (cTBs) (Figure 1B). sTBs form a monolayer on the outer aspect of the placental villous, which lies in direct contact with maternal blood and is juxtaposed with cTBs and fetal blood vessels. Trafficking between the sTB layer, cTBs, and fetal blood vessels thus comprises the main cellular exchange interface between mother and fetus during pregnancy. Thus, it is important to evaluate the more sub-anatomical aspects of placental tissues to evaluate where a potential SARS-CoV-2 blockade could be occurring at the maternal–fetal interface and identify areas for future investigation.

In ongoing evaluations of COVID-19 in pregnancy, there has been varied evidence of SARS-CoV-2 entry within the placental compartment.[23–25,28,30,35–40] When SARS-CoV-2 has been identified within placental tissues, multiple studies have found localization within both sTB and cTB placental villous subtypes.[30,35] Although the reported variation of SARS-CoV-2 in placental tissues could be due to differences in patient populations and/or experimental techniques, recent meta-analyses have estimated an overall range of 7%–21% of placental tissues showing evidence of SARS-CoV-2 placental invasion in the current literature.[39,40] Interestingly,

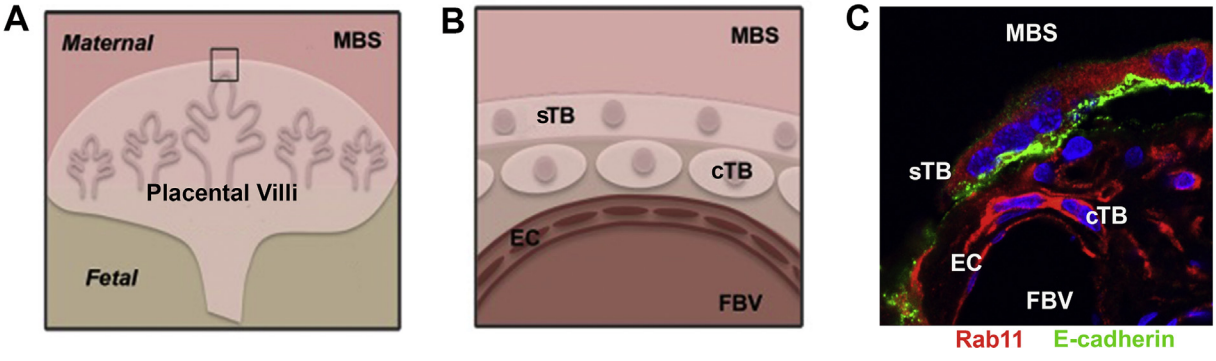

Figure 1. Viral entry viewpoint of the maternal–fetal interface. (A) Schematic of human placental villi. (B) Enlarged view of placental villous anatomy. (C) Rab 11 expression in human placental villous. Red, Rab11; Green, E-cadherin; Blue, 4′,6-diamidino-2-phenylindole (DAPI) nuclear stain. cTB = cytotrophoblast cell; EC = endothelial cell; FBV = fetal blood vessel; MBS = maternal blood space; sTB = syncytiotrophoblast cell. Modified from Taglauer et al[49]

these estimates are still fourfold to fivefold higher than the reported percentages of fetal transmission (1%–4%),[2,3] suggesting that even if SARS-CoV-2 gains access to the placental compartment, other mechanisms may still be in place preventing its subsequent transmission to the developing fetus.

SARS-CoV-2 cellular entry proteins have also been investigated in placental tissues. It has been well established that the SARS-CoV-2 spike protein and the host cell proteins angiotensin-converting enzyme 2 (ACE2) and transmembrane serine protease 2 are required for cell entry and processing.[41,42] Studies examining historical single-cell molecular datasets have reported varying mRNA expression patterns of ACE2 and transmembrane serine protease 2 in placental cell subtypes throughout gestation.[38,43,44] Subsequent histologic evaluations have identified the presence of both receptors in villous tissues from term control and COVID-19 placental samples, with a comparative predominance of ACE2.[30,35]

Although the majority of studies examining SARS-CoV-2 infection in the placenta have focused on the presence of viral contents and cell surface entry molecules, there has been an overall lack of more detailed exploration on other intracellular molecules, which could determine viral transmission in trophoblast cells and subsequent entry to the fetal circulation (Figure 1B). Of particular interest are pathways involved in endocytosis, which is a common host cell entry mechanism for many viruses. Indeed, a recent review emphasized the importance of examining endocytic pathways in studies on COVID-19, highlighting endocytosis as a high-yield area to identify cell biological mechanism(s) governing SARS-CoV-2 infection.[45]

Central to the process of endocytosis are Rab proteins, a family of small GTPase molecules that are master regulators of endocytic vesicle trafficking and organelle dynamics in eukaryotic cells. Rab5, Rab7, and Rab11 in particular are involved in viral cell entry/egress in a variety of cell types, and their cellular expression can be altered by cytokine-dependent regulation.[46,47] Rab5 and Rab7 regulate the biogenesis of early endosomes and endo-lysosomal trafficking, respectively, whereas Rab11 is critical for endocytic recycling to the plasma membrane.[47,48] Our previous study found that Rab11 is prominently expressed within trophoblast cells and fetal blood vessels in normal human placental tissues (Figure 1C).[49] Rab 5 and Rab7 have been implicated in HIV and cytomegalovirus entry into trophoblast cells *in vitro*, but the localization of these molecules has not been fully characterized within primary human placental tissues.[50,51] Given their previously described role in viral entry into trophoblast cells, we hypothesized that evaluation of Rab5 and Rab7 in placental tissues affected by maternal COVID-19 would yield important information on the intracellular trophoblast responses to SARS-CoV-2 in pregnancy. To address this hypothesis, we conducted a comparative evaluation of Rab5 and Rab7 expression in a selected cohort of placental tissues from pregnancies affected by maternal COVID-19 disease compared with contemporary controls.

MATERIALS AND METHODS

Sample Collection

This study was conducted at Boston Medical Center, Boston, Massachusetts, with samples obtained between April and May 2020 during a period of peak COVID-19 admissions. At that time, universal testing for SARS- CoV-2 by PCR of nasopharyngeal swabs was instituted at our hospital for all women admitted in labor. Placental samples were collected from women who tested positive at the time of delivery along with contemporary control subjects who tested negative at the time of delivery who delivered in the same time period. Control subjects were also matched for gestational age at delivery. Within the COVID-19 placental cohort, a subset of five COVID-19 placental tissues were specifically selected from pregnancies with evidence of fetal transmission (ie, positive infant SARS-CoV-2 PCR testing from nasopharyngeal swabs within 24 h of delivery).[35] Tissues were collected with approval from the Boston University Medical Campus Institutional Review Board, with an informed consent waiver specific to this study.

Tissue Processing

Shortly after delivery, placental tissues were placed in 4% buffered formalin followed by grossing, staging, and histologic evaluation by board-certified pathologists at Boston Medical Center. The remaining formalin-fixed placental tissues were then dissected into full thickness placental biopsy specimens (including decidua, villous tissue, and chorionic plate)

and soaked in 18% sucrose for 1 week, which was then followed by embedding in Tissue-Tek O.C.T. solution (ThermoFisher, Waltham, MA) and freezing at −80 °C. Tissue blocks were then cryo-sectioned at 10 μm thickness for subsequent immunostaining.

Immunohistochemistry

Placental tissue sections were first subjected to antigen retrieval with 10 mM sodium citrate, 0.05% Tween 20, pH 6, boiling for 20 min, followed by a series of washes with phosphate-buffered saline 0.05% with Tween 20 (PBS-T). Slides were then incubated for 1 h at room temperature in PBS with permeabilization solution 0.2% Triton X-100 along with 10% blocking serum of host species for all correlate secondary antibodies (MilliporeSigma, Burlington, Massachusetts).

Rab5/Rab7 Co-labeling

Slides were then incubated at 4 °C overnight with the following primary antibodies at 1:100 diluted in PBS-T: Rab5A (mouse anti-human, 66,339; Proteintech, Rosemont, Illinois) or Rab7 (rabbit anti-human Ab137029; Abcam, Cambridge, United Kingdom). After a series of washes, slides were incubated with the following corresponding secondary antibodies (Abcam) at a dilution of 1:500: Rab5A, donkey anti-mouse Alexa Fluor 594; and Rab7, donkey anti-rabbit Alexa Fluor 647.

Cytokeratin 7 and Rab Co-labeling

Rab5 and Rab7 primary and secondary staining was performed as described earlier. Following a series of washes after secondary antibody labeling, an Alexa Fluor 488—conjugated anti—cytokeratin-7 primary antibody (mouse anti-human, ab185048) was added to slides at 1:100 diluted in PBS-T. For control staining of placental tissues, slides were incubated with only secondary antibodies (without primary antibodies). After a final series of washes, slides were cover-slipped with Prolong Gold with 4′,6-diamidino-2-phenylindole (ThermoFisher). To ensure consistency for comparative analysis, all cohort slides (COVID-19 and control) were stained together in bulk and imaged within 24—48 h of staining.

Microscopy

Immunofluorescent images were captured by using a Nikon deconvolution wide-field epifluorescence microscope using NIS-Elements Software (Nikon, Tokyo, Japan) with slide cohorts blinded at time of image acquisition. Within the villous compartment, 8 randomized images were captured at 200× magnification, using standardized exposure times. Images were further processed post-acquisition via Fiji software package for ImageJ (US National Institutes of Health, Bethesda, Maryland; https://imagej.nih.gov.ij/).

Quantitative Image Analysis

Using ImageJ software, image area and integrated density were measured for each immunofluorescent 200× image (n = 8 randomized areas per slide). Mean fluorescence values of 5 random background readings per image cohort were also measured. Measurements were obtained of 2 blinded reviewers (Y.B. and E.T.). From these values, a raw corrected total cell fluorescence (CTCF) was calculated per published protocols: CTCF = integrated density − (area of selected image × average mean background fluorescence).[52—54] Average CTCF was also calculated from 3 secondary-only, negative control slides (background CTCF). Final CTCF values for each target were then used to generate a final CTCF value as a ratio of target CTCF/secondary-only control CTCF.

Statistical Analysis

All statistical analyses were performed by using Prism 7 software (GraphPad, San Diego, California). CTCF values were compared by using independent sample *t* tests for normally distributed, continuous data. Differences were considered significant at $P < 0.05$.

RESULTS

Demographic characteristics of our placental cohort were as previously published by Taglauer et al[35] for the 15 COVID-19 and 10 control mother—infant dyads. Because Rab5 and Rab7 have only been previously described in trophoblast cells *in vitro*, we first evaluated their *in situ* localization in our cohort of control placental tissues. Both Rab5 and Rab7 were identified in placental villi, and co-staining with cytokeratin 7, a pan trophoblast marker, revealed localization in villous sTB and cTB cell subpopulations (Figures 2A and 2B). Placental tissues were then evaluated for comparative Rab5 and Rab7

expression in COVID-19 versus control tissues by using dual immunofluorescence followed by analysis of CTCF values. In both tissue cohorts, Rab5 expression was found throughout sTBs as well as the underlying cTB cell layers, with a small but significant increase in overall fluorescence intensity among COVID-19 placentas (Figures 3A and 3E; Figure 4A). In contrast, there was a striking decrease in Rab7 intensity in villous tissues among all COVID-19 placentas evaluated (Figures 3B and 3F). This resulted in a statistically significant decrease in the Rab7 fluorescence intensity (CTCF) values in COVID-19 placentas (Figure 4B).

Overlay of Rab5 and Rab7 imaging further illustrated the co-localization of Rab5 and Rab7 in the sTB layer of control tissues and the predominance of Rab5 expression in the sTB layer of COVID-19 placental villi (Figures 3C, 3G, 3D, and 3H). Subanalysis of COVI-19 placental tissues (n = 5) from selected pregnancies with evidence of fetal transmission[35] showed a pattern of Rab5 and Rab7 consistent with the rest of the COVID-19 placental cohort (data not shown). Overall, these results identify Rab5 and Rab7 localization within placental villi, a key anatomical area of the maternal–fetal interface. Furthermore, they illustrate altered

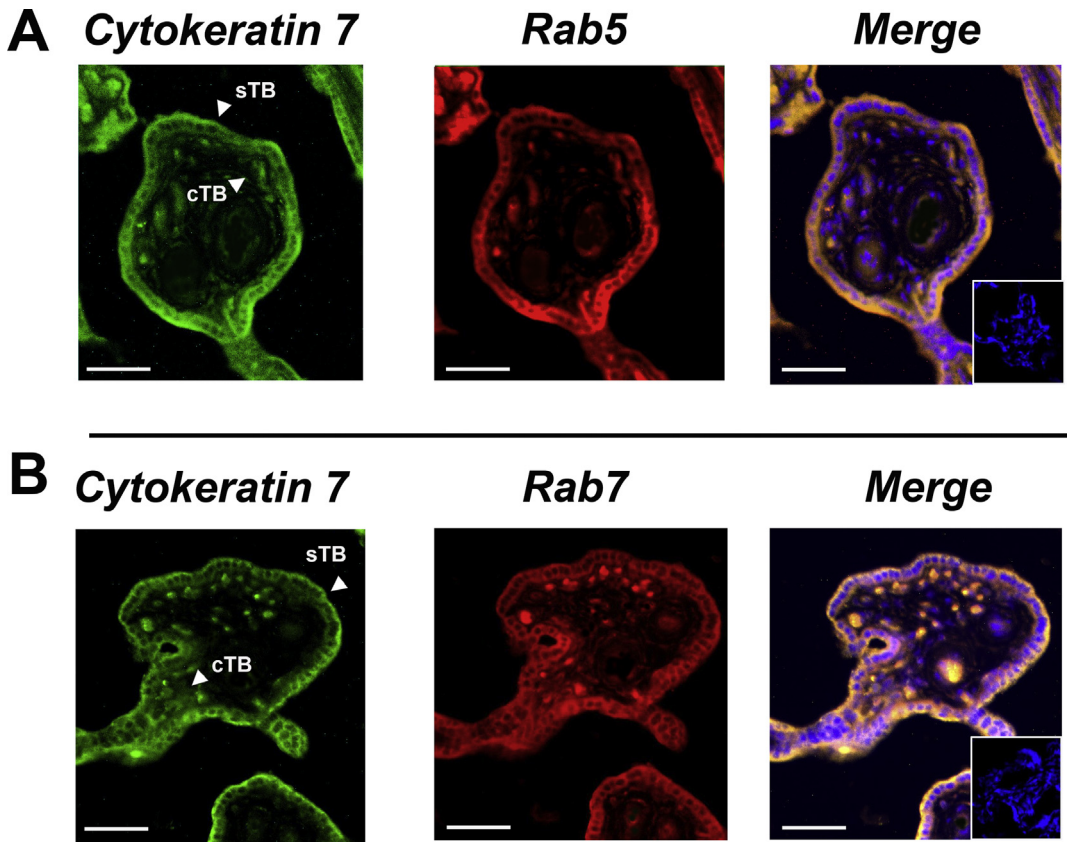

Figure 2. Rab5 and Rab7 trophoblast localization in placental villi. Evaluation of pan trophoblast marker cytokeratin 7 along with Rab5 and Rab7 expression in healthy term placental tissues (n = 10). (A) Representative images of co-staining with cytokeratin 7 (green) and Rab5 (red). (B) Representative images of co-staining with cytokeratin 7 (green) and Rab7 (red). Blue, 4′,6-diamidino-2-phenylindole (DAPI) nuclear stain. Scale bars, 12 μm. Inset images: secondary-only negative control. cTB = cytotrophoblast cell; sTB = syncytiotrophoblast cell.

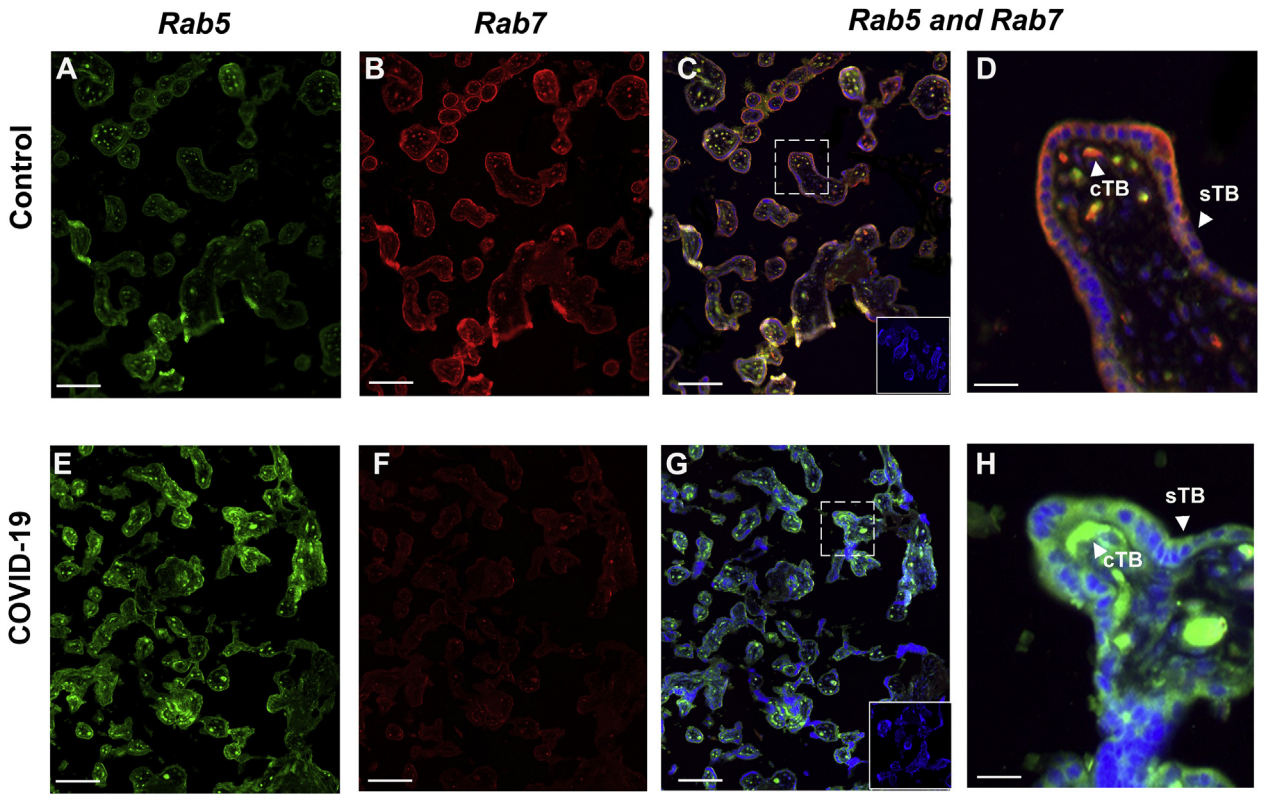

Figure 3. Rab5 and Rab7 expression in control versus coronavirus disease 2019 (COVID-19) placental villous tissues. (A–D) Representative images of Rab5 and Rab7 co-labeling in control tissues (ie, placental tissues from pregnant women who were severe acute respiratory syndrome coronavirus 2 negative upon admission screening (n = 10). (E–H) Representative images of Rab5 and Rab7 co-labeling in COVID-19 tissues (ie, placental tissues from pregnant women who were severe acute respiratory syndrome coronavirus 2 positive upon admission screening (n = 15). (C and G) Inset images, secondary-only negative control. (D and H) Enlarged placental image of dashed white boxes in panels C and G, respectively. Green, Rab5; Red, Rab7; Blue, 4′,6-diamidino-2-phenylindole (DAPI) nuclear stain. Scale bars: panels A through C and panels E through G, 50 μm; panels D and H, 10 μm cTB = cytotrophoblast cell; sTB = syncytiotrophoblast cell.

expression of both proteins in placental tissues affected by maternal COVID-19 disease.

DISCUSSION

This study highlights Rab GTPases as placental targets of interest for ongoing analysis of COVID-19 in pregnancy. The differential expression of Rab5 and Rab7 in COVID-19 placental tissues suggests trophoblast-specific molecular alterations in response to SARS-CoV-2 at the maternal–fetal interface. Although these results require ongoing validation, they are consistent with prior research examining Rab5 and Rab7 expression in host–cell pathogen interactions. Bacterial component muramyl dipeptide (found in both gram-positive and gram-negative bacteria) has been shown to have opposing effects on Rab5 and Rab7 expression in macrophages, corresponding with pathogen lysosomal degradation.[55] Furthermore, although mechanisms dictating Rab protein expression are multifactorial, some key themes emerge from their regulation in

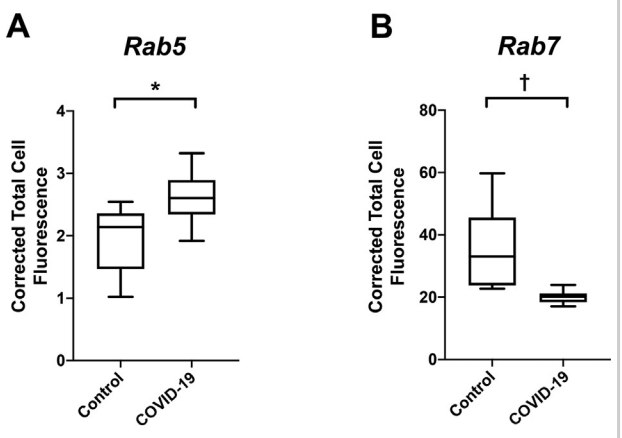

Figure 4. Rab5 and Rab7 differential expression in coronavirus disease 2019 (COVID-19) placental tissues. Graphical analysis of corrected total cell fluorescence values from Rab5 (A) and Rab7 (B) image analysis. Placental tissue cohorts are as described in Figure 3. $^{*}P < 0.0014$. $^{\dagger}P = 0.0001$.

response to infection.[46] Rab5 and Rab7 seem to be governed by distinct signaling pathways in response to cytokine production.[56] Rab5 can be upregulated in response to proinflammatory cytokines such as interleukin-6 (IL-6) via activation of the extracellular signal–regulated kinase pathway. In contrast, Rab7 can be altered by an IL-12–dependent, p38 mitogen-activated protein kinase–directed pathway. Because altered levels of both IL-6 and IL-12 are among the systemic cytokine responses associated with COVID-19 disease,[57] intrauterine cytokine changes could be one of the determinants driving altered expression of Rab5 and Rab7 in these placental tissues. However, as the pregnancy-specific cytokine profiles of maternal COVID-19 infection have not been well defined, ongoing studies will be needed to identify factors regulating Rab5 and Rab7 differential expression in placental tissues and the potential role of these proteins in the SARS-CoV-2 response at the maternal–fetal interface.

The current study has several limitations. First, it was a pilot analysis of histologic findings in a selected cohort of fixed placental tissues. Although differences were observed between groups, these initial data require ongoing validation with comparative evaluation of mRNA and protein expression in greater numbers of freshly preserved samples. In addition, these tissues were collected from pregnancies during a specific time frame during a peak of admissions and illness severity in the early stages of the COVID-19 pandemic. Ongoing evaluation of placental tissues from COVID-19–positive pregnancies over a broader time frame and clinical heterogeneity will be informative to capture a more complete picture of Rab5 and Rab7 expression changes. Finally, Rab protein expression was only evaluated in pregnancies with maternal COVID-19 in the third trimester. Analysis across first-, second-, and third-trimester maternal SARS-CoV-2 infections will be informative to evaluate how the placental expression of these proteins changes relative to the gestational timing of maternal COVID-19.

Overall, this work highlights the importance of investigating additional subcellular pathways to more fully understand the placental response to COVID-19 in pregnancy. Because Rab5 regulates early endosome processes and Rab7 directs early to late endosomal transport,[58] future evaluation of endosomal subtype markers will also be a key area of investigation. Comparative analysis of early endosome antigen 1, a marker of early endosomes, and CD63, a late endosome and multivesicular body marker, could be informative to identify whether downregulation of Rab7 in COVID-19 placental tissues results in retention of the virus in early endosomes or trapping within late endosomes and multivesicular bodies. It is important to note that within our COVID-19 placental cohort, Rab5 upregulation and Rab7 downregulation was noted among all tissues, including those selected from pregnancies with evidence of SARS-CoV-2 fetal transmission.

CONCLUSIONS

Although the current study was not powered to identify true correlations with these expression changes and fetal transmission, our findings do suggest that mechanisms in addition to altered Rab GTPase expression could be mediating the physiological placental blockade of SARS-CoV-2 in pregnancy. This would be entirely expected as

maternal–fetal trafficking is a highly regulated and multifactorial process throughout pregnancy. Continued evaluation of larger COVID-19 placental cohorts using multivariate analyses such as RNA-sequencing and spatial transcriptomic approaches will be highly informative to more fully characterize the physiological placental response to COVID-19 in pregnancy and potentially identify therapeutic targets for other organ systems to help combat systemic SARS-CoV-2–mediated disease.

DISCLOSURES

The authors have indicated that they have no conflicts of interest regarding the content of this article. The funding agencies sponsoring this work had no role in the study design, the collection, analysis or interpretation of data, in the writing of the manuscript or the decision to submit the manuscript for publication.

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Mr. Benarroch, Dr. Wachman, and Dr. Taglauer participated in the literature review, study design, study execution, data collection, data analysis, and manuscript preparation and study funding. Dr. Juttukonda participated in literature review and manuscript preparation. Mr. Boateng participated in technical support. Drs. Sabharwal and Yarrington contributed clinical cohort identification and tracking. Dr. Khan contributed to data analysis, literature review, and manuscript preparation. All authors have reviewed and approved the final submitted article.

REFERENCES

1. Map C. *COVID-19 Map*. Baltimore, MD: Johns Hopkins Coronavirus Resource Center; 2020. https://coronavirus.jhu.edu/data.
2. Duran P, Berman S, Niermeyer S, et al. COVID-19 and newborn health: systematic review. *Rev Panam Salud Publica*. 2020;44:e54.
3. Huntley BJF, Huntley ES, Di Mascio D, Chen T, Berghella V, Chauhan SP. Rates of maternal and perinatal mortality and vertical transmission in pregnancies complicated by severe acute respiratory syndrome coronavirus 2 (SARS-Co-V-2) infection: a systematic review. *Obstet Gynecol*. 2020;136:303–312.
4. Ellington S, Strid P, Tong VT, et al. Characteristics of women of reproductive age with laboratory-confirmed sARS-CoV-2 infection by pregnancy status—United States, January 22-June 7, 2020. *MMWR Morb Mortal Wkly Rep*. 2020;69:769–775.
5. Allotey J, Stallings E, Bonet M, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: living systematic review and meta-analysis. *BMJ*. 2020;370:m3320.
6. Dubey P, Reddy SY, Manuel S, Dwivedi AK. Maternal and neonatal characteristics and outcomes among COVID-19 infected women: an updated systematic review and meta-analysis. *Eur J Obstet Gynecol Reprod Biol*. 2020;252:490–501.
7. Zeng L, Xia S, Yuan W, et al. Neonatal early-onset infection with SARS-CoV-2 in 33 neonates born to mothers with COVID-19 in Wuhan, China. *JAMA Pediatr*. 2020;174:722–725.
8. Wang S, Guo L, Chen L, et al. A case report of neonatal 2019 coronavirus disease in China. *Clin Infect Dis*. 2020;71:853–857.
9. Wei M, Yuan J, Liu Y, Fu T, Yu X, Zhang ZJ. Novel coronavirus infection in hospitalized infants under 1 year of age in China. *JAMA*. 2020;323:1313–1314.
10. Kamali Aghdam M, Jafari N, Eftekhari K. Novel coronavirus in a 15-day-old neonate with clinical signs of sepsis, a case report. *Infect Dis (Lond)*. 2020;52:427–429.
11. Li N, Han L, Peng M, et al. Maternal and neonatal outcomes of pregnant women with coronavirus disease 2019 (COVID-19) pneumonia: a case-control study. *Clin Infect Dis*. 2020;71:2035–2041.
12. Dong L, Tian J, He S, et al. Possible vertical transmission of SARS-CoV-2 from an infected mother to her newborn. *JAMA*. 2020;323:1846–1848.

13. Khan S, Peng L, Siddique R, et al. Impact of COVID-19 infection on pregnancy outcomes and the risk of maternal-to-neonatal intrapartum transmission of COVID-19 during natural birth. *Infect Control Hosp Epidemiol*. 2020;41:748—750.
14. Liu Y, Chen H, Tang K, Guo Y. Clinical manifestations and outcome of SARS-CoV-2 infection during pregnancy. *J Infect*. 2020.
15. Yu N, Li W, Kang Q, et al. Clinical features and obstetric and neonatal outcomes of pregnant patients with COVID-19 in Wuhan, China: a retrospective, single-centre, descriptive study. *Lancet Infect Dis*. 2020;20:559—564.
16. Chen H, Guo J, Wang C, et al. Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. *Lancet (London, England)*. 2020;395:809—815.
17. Chen Y, Peng H, Wang L, et al. Infants born to mothers with a new coronavirus (COVID-19). *Front Pediatr*. 2020;8:104.
18. Yang H, Sun G, Tang F, et al. Clinical features and outcomes of pregnant women suspected of coronavirus disease 2019. *J Infect*. 2020;81:e40—e44.
19. Schwartz DA, Graham AL. Potential maternal and infant outcomes from (Wuhan) coronavirus 2019-nCoV infecting pregnant women: lessons from SARS, MERS, and other human coronavirus infections. *Viruses*. 2020;12:194.
20. WAPM (World Association of Perinatal Medicine) Working Group on COVID-19. Maternal and perinatal outcomes of pregnant women with SARS-COV-2 infection. *Ultrasound Obstet Gynecol*. 2020.
21. Zeng H, Xu C, Fan J, et al. Antibodies in infants born to mothers with COVID-19 pneumonia. *JAMA*. 2020;323:1848—1849.
22. Kimberlin DW, Stagno S. Can SARS-CoV-2 infection be acquired in utero?: more definitive evidence is needed. *JAMA*. 2020;323:1788—1789.
23. Patanè L, Morotti D, Giunta MR, et al. Vertical transmission of COVID-19: SARS-CoV-2 RNA on the fetal side of the placenta in pregnancies with COVID-19 positive mothers and neonates at birth. *Am J Obstet Gynecol MFM*. 2020;2:100145.
24. Baud D, Greub G, Favre G, et al. Second-trimester miscarriage in a pregnant woman with SARS-CoV-2 infection. *JAMA*. 2020;323:2198—2200.
25. Penfield CA, Brubaker SG, Limaye MA, et al. Detection of severe acute respiratory syndrome coronavirus 2 in placental and fetal membrane samples. *Am J Obstet Gynecol MFM*. 2020;2:100133.
26. Chen S, Huang B, Luo DJ, et al. Pregnancy with new coronavirus infection: clinical characteristics and placental pathological analysis of three cases. *Zhonghua Bing Li Xue Za Zhi*. 2020;49:418—423.
27. Qiu L, Liu X, Xiao M, et al. SARS-CoV-2 is not detectable in the vaginal fluid of women with severe COVID-19 infection. *Clin Infect Dis*. 2020;71:813—817.
28. Hosier H, Farhadian SF, Morotti RA, et al. SARS-CoV-2 infection of the placenta. *J Clin Invest*. 2020;130:4947—4953.
29. Shanes ED, Mithal LB, Otero S, Azad HA, Miller ES, Goldstein JA. Placental pathology in COVID-19. *Am J Clin Pathol*. 2020;154:23—32.
30. Hecht JL, Quade B, Deshpande V, et al. SARS-CoV-2 can infect the placenta and is not associated with specific placental histopathology: a series of 19 placentas from COVID-19-positive mothers. *Mod Pathol*. 2020:1—12.
31. Baergen RN, Heller DS. Placental pathology in Covid-19 positive mothers: preliminary findings. *Pediatr Dev Pathol*. 2020;23:177—180.
32. Mulvey JJ, Magro CM, Ma LX, Nuovo GJ, Baergen RN. Analysis of complement deposition and viral RNA in placentas of COVID-19 patients. *Ann Diagn Pathol*. 2020;46:151530.
33. Mor G, Aldo P, Alvero AB. The unique immunological and microbial aspects of pregnancy. *Nat Rev Immunol*. 2017;17:469—482.
34. Benirschke K, Kaufmann P. *Pathology of the Human Placenta*. 3rd ed. New York: Springer-Verlag; 1995.
35. Taglauer E, Benarroch Y, Rop K, et al. Consistent localization of SARS-CoV-2 spike glycoprotein and ACE2 over TMPRSS2 predominance in placental villi of 15 COVID-19 positive maternal-fetal dyads. *Placenta*. 2020;100:69—74.
36. Algarroba GN, Rekawek P, Vahanian SA, et al. Visualization of severe acute respiratory syndrome coronavirus 2 invading the human placenta using electron microscopy. *Am J Obstetr Gynecol*. 2020;223:275—278.
37. Facchetti F, Bugatti M, Drera E, et al. SARS-CoV2 vertical transmission with adverse effects on the newborn revealed through integrated immunohistochemical, electron microscopy and molecular analyses of placenta. *EBioMedicine*. 2020;59:102951.
38. Bloise E, Zhang J, Nakpu J, et al. Expression of severe acute respiratory syndrome coronavirus 2 cell entry genes, angiotensin-converting enzyme 2 and transmembrane protease serine 2, in the placenta across gestation and at the maternal-fetal interface in pregnancies complicated by preterm birth or preeclampsia. *Am J Obstet Gynecol*. 2020.

39. Kotlyar AM, Grechukhina O, Chen A, et al. Vertical transmission of coronavirus disease 2019: a systematic review and meta-analysis. *Am J Obstet Gynecol*. 2021;224:35—53. e3.
40. Sharps MC, Hayes DJL, Lee S, et al. A structured review of placental morphology and histopathological lesions associated with SARS-CoV-2 infection. *Placenta*. 2020;101:13—29.
41. Shang J, Ye G, Shi K, et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature*. 2020;581:221—224.
42. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and Is blocked by a clinically proven protease inhibitor. *Cell*. 2020;181:271—280. e278.
43. Li M, Chen L, Zhang J, Xiong C, Li X. The SARS-CoV-2 receptor ACE2 expression of maternal-fetal interface and fetal organs by single-cell transcriptome study. *PLoS One*. 2020;15, e0230295.
44. Pique-Regi R, Romero R, Tarca AL, et al. Does the human placenta express the canonical cell entry mediators for SARS-CoV-2? *eLife*. 2020;9.
45. Glebov OO. Understanding SARS-CoV-2 endocytosis for COVID-19 drug repurposing. *FEBS J*. 2020;287:3664—3671.
46. Prashar A, Schnettger L, Bernard EM, Gutierrez MG. Rab GTPases in immunity and inflammation. *Front Cell Infect Microbiol*. 2017;7:435.
47. Spearman P. Viral interactions with host cell Rab GTPases. *Small GTPases*. 2018;9:192—201.
48. Takahashi S, Kubo K, Waguri S, et al. Rab11 regulates exocytosis of recycling vesicles at the plasma membrane. *J Cell Sci*. 2012;125:4049—4057.
49. Taglauer ES, Artemiuk PA, Hanscom SR, et al. Rab11 family expression in the human placenta: localization at the maternal-fetal interface. *PLoS One*. 2017;12, e0184864.
50. Maidji E, McDonagh S, Genbacev O, Tabata T, Pereira L. Maternal antibodies enhance or prevent cytomegalovirus infection in the placenta by neonatal Fc receptor-mediated transcytosis. *Am J Pathol*. 2006;168:1210—1226.
51. Vidricaire G, Tremblay MJ. Rab5 and Rab7, but not ARF6, govern the early events of HIV-1 infection in polarized human placental cells. *J Immunol*. 2005;175:6517—6530.
52. Andrejeva G, Gowan S, Lin G, et al. De novo phosphatidylcholine synthesis is required for autophagosome membrane formation and maintenance during autophagy. *Autophagy*. 2020;16:1044—1060.
53. Kenan S, Liang H, Goodman HJ, et al. 5-Aminolevulinic acid tumor paint and photodynamic therapy for myxofibrosarcoma: an in vitro study. *J Orthop Surg Res*. 2020;15:94.
54. Zang R, Gomez Castro MF, McCune BT, et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci Immunol*. 2020;5.
55. Mukherjee K, Parashuraman S, Krishnamurthy G, et al. Diverting intracellular trafficking of Salmonella to the lysosome through activation of the late endocytic Rab7 by intracellular delivery of muramyl dipeptide. *J Cell Sci*. 2002;115:3693—3701.
56. Bhattacharya M, Ojha N, Solanki S, et al. IL-6 and IL-12 specifically regulate the expression of Rab5 and Rab7 via distinct signaling pathways. *EMBO J*. 2006;25:2878—2888.
57. Costela-Ruiz VJ, Illescas-Montes R, Puerta-Puerta JM, Ruiz C, Melguizo-Rodríguez L. SARS-CoV-2 infection: the role of cytokines in COVID-19 disease. *Cytokine Growth Factor Rev*. 2020;54:62—75.
58. Langemeyer L, Fröhlich F, Ungermann C. Rab GTPase function in endosome and lysosome biogenesis. *Trends Cell Biol*. 2018;28:957—970.

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