Potential SARS-CoV-2 interactions with proteins involved in trophoblast functions – An *in-silico* study

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A B S T R A C T

Background: Though a large number of pregnant females have been affected by COVID-19, there is a dearth of information on the effects of SARS-CoV-2 infection on trophoblast function. We explored *in silico*, the potential interactions between SARS-CoV-2 proteins and proteins involved in the key functions of placenta.
Methods: Human proteins interacting with SARS-CoV-2 proteins were identified by Gordon et al. (2020). Genes that are upregulated in trophoblast sub-types and stages were obtained by gene-expression data from NCBI-GEO and by text-mining. Genes altered in pathological states like pre-eclampsia and gestational diabetes mellitus were also identified. Genes crucial in placental functions thus identified were compared to the SARS-CoV-2 inter-actome for overlaps. Proteins recurring across multiple study scenarios were analyzed using text mining and network analysis for their biological functions.
Results: The entry receptors for SARS-CoV-2 – ACE2 and TMPRSS2 are expressed in placenta. Other proteins that interact with SARS-CoV-2 like LOX, Fibulins-2 and 5, NUP98, GDF15, RBX1, CUL3, HMOX1, PLAT, MFGE8, and MRPs are vital in placental functions like trophoblast invasion and migration, syncytium formation, differentiation, and implantation. TLE3, expressed across first trimester placental tissues and cell lines, is involved in formation of placental vasculature, and is important in SARS-CoV (2003) budding and exit from the cells by COPI vesicles.
Conclusion: SARS-CoV-2 can potentially interact with proteins having crucial roles in the placental function. Whether these potential interactions identified *in silico* have effects on trophoblast functions in biological settings needs to be addressed by further in vitro and clinical studies.

1. Background

COVID-19, the viral respiratory disease caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) was first reported in Wuhan, Hubei province, China, in December 2019 [1,2]. A large number of the affected individuals, indeed more than 50% of COVID-19 patients, belong to the reproductive age group and about 47% of the affected individuals are females [3]. Thus, a large number of females in the reproductive age group have already been infected with SARS-CoV-2, and more are at potential risk of infection, given the R0 of about 2.28 of COVID-19 [4].

A characterization of pregnant females who were hospitalized for delivery during the COVID-19 outbreak showed that 15.4% of these women were positive for SARS-CoV-2, and among these, 87.9% were asymptomatic [5]. The major consensus is that there is no vertical transmission of SARS-CoV-2, like the predecessor coronaviruses causing

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SARS and MERS [6–9], though certain systematic reviews and case reports do not completely rule out such a possibility [10–12]. SARS-CoV-2 infection in the third trimester of pregnancy was found to be associated with an increased risk of preterm delivery and intrauterine fetal distress and the requirement for Cesarean sections [13]. During SARS of 2003, pregnant females who presented with the disease during the first trimester had an adverse outcome, with 57% of pregnancies ending in spontaneous miscarriage, and 4 out of 5 patients who presented with the disease progressing into preterm delivery [14]. The reasons behind this adverse outcome in pregnancy in females infected with SARS-CoV remains enigmatic. At present, there is limited data on the effect of first trimester SARS-CoV-2 infection on the pregnancy outcome. Initial reports suggest that COVID-19 does not increase the risk of spontaneous abortions [15], though placenta can be infected by SARS-CoV-2, as evident by in situ RNA hybridisation, electron microscopy [16–20], and detection of SARS-CoV-2 spike protein in placental villi in COVID-19 positive pregnancies [12,21].

Since the placenta executes and orchestrates fetal growth-related pathways, placental dysfunction has deleterious effects on the outcome of pregnancy. While certain reports suggest that SARS-CoV-2 infection is not associated with specific histopathological alterations, there are evidences of gross pathological alterations nonetheless [16]. Another study on the effect of SARS-CoV-2 on placenta in 16 pregnant females with COVID-19 revealed that five of the placentas were small for gestational age; there was histological evidence of decidual arteriopathy and maternal vascular malperfusion in 12 of 16 pregnancies [22]. Other vascular abnormalities in placenta included fetal vascular malformations and malperfusion in 10 out of 20 pregnancies [23]. Similar findings were observed in some other pregnancies with COVID-19 [24–26]. Since there is evidence of compromised placental function in pregnancy, we studied the interaction between SARS-CoV-2 and the proteins that are associated with important placental functions like invasion, differentiation, maturation, arterial remodelling etc. We used an *in-silico* based approach, employing the SARS-CoV-2 human interactome and differential expression analysis of genes associated with critical placental functions, to predict the effects on SARS-CoV-2 infection on placental functions.

2. Materials and methods

2.1. SARS-CoV-2 human interactome

Gordon et al. identified 332 proteins that can interact with 26 of the 29 SARS-CoV-2 proteins, using an affinity-purification (following cloning of viral proteins and their expression in HEK293T cells) and mass spectrometry approach [27]. These proteins are listed online (and in Supplementary Table 1).

2.2. Identification of proteins involved in crucial functions of placenta

Datasets from NCBI-GEO involving studies on placenta were analyzed for differential gene expression using GEO-2R. The datasets

Table 1
Summary of datasets used to study placental functions and pathology.

Dataset	Condition	Figure No.
GSE9984 GSE28551	Term placenta vs First trimester placenta	Fig. 1
GSE9773 GSE130339	Villous vs Extravillous trophoblasts Day 7 (differentiated) vs day 0 (undifferentiated) trophoblasts	Fig. 2
GSE20510 GSE20510	JEG3 vs HTR-8/SVneo cell lines JEG3 vs SGHPL5 cell lines	Fig. 4
GSE66273 GSE48424	Pre-eclampsia vs Control placenta	Fig. 5

used and the samples for differential expression are summarised in Table 1. The datasets were chosen to identify genes that are upregulated during various physiological processes and states involving the placenta – including invasion of trophoblasts, trophoblast differentiation, and disease conditions associated with pregnancy. The significant differentially expressed genes were identified by a cut-off adjusted p-value of 0.05, calculated using Benjamini & Hochberg false discovery rate method. From the significant differentially expressed genes, those with a $\log_2$ fold change of ≥ 1 and ≤ -1 were considered significant upregulated and downregulated, respectively.

Genes/proteins involved in critical placental functions or that are differentially expressed in placental pathology were also obtained by text mining, and literature search of studies involving such analysis – the genes that are upregulated in cytotrophoblasts, syncytiotrophoblasts and migratory trophoblasts were obtained from a study by West et al. [28], and the differentially expressed genes in gestational diabetes mellitus from a study by Radaelli et al. [29].

2.3. Identification of Proteins Involved in Placental Functions that can Bind to SARS-CoV-2

For each dataset, the significant upregulated and downregulated proteins that were identified was compared to the list of proteins interacting with SARS-CoV-2. The lists were compared using Venny 2.1.0 [30] to identify the overlapping proteins. The functions of the proteins that were recurring across multiple study scenarios were further analyzed using text mining and network analysis using GeneMANIA [31] and/or STRING [32] to evaluate their interactome and possible biological function.

The complete approach to the discovery of the proteins involved in placental functions that can bind to SARS-CoV-2 is given in Supplementary Figure 1.

3. Results

3.1. SARS-CoV-2 interacting proteins that are upregulated in first trimester and term placentas

Analysis of two different datasets, GSE9984 and GSE28551, were performed to identify the SARS-CoV-2 interacting proteins that are upregulated in first-trimester placenta and term placenta. The data sets revealed that 3 proteins (GSE9984) and 14 proteins (GSE28551), upregulated in first-trimester placenta interacting with SARS-CoV-2 in the two queried data sets (Fig. 1a and b). We further observed 21 proteins (GSE9984) and 19 proteins (GSE28551) that were upregulated in term placenta in these two data sets (Fig. 1a and b) that could interact with SARS-CoV-2. We then identified the candidate proteins in the first trimester (3 and 14) and term placenta (21 and 19) that were common in the two data sets. Our results yielded LOX as a common candidate in the term placenta and SCARB1 and PRKAR2B in the first trimester that could potentially interact with SARS-CoV-2 (Fig. 1c).

LOX (lysyl oxidase) upregulated in term placenta can interact with SARS-CoV-2 orf8. Downregulation of LOX has been implicated in impaired trophoblast migration and invasion [33]. The interaction network of LOX is shown in Fig. 1d. LOX is also highly expressed in the mesenchymal cells of placenta [34]; since LOX is involved in maturation of collagen, it has been shown to be associated with remodelling of vasculature in various tissues including placenta and lungs [33,35]. Knock-down of LOX suppressed trophoblast migration, an essential process in spiral artery remodelling [33].

SCARB1 and PRKAR2B were the two common first trimester proteins from both the datasets. Scavenger receptor class B type 1 or SCARB1 functions as the receptor for HDL and is important in HDL uptake by trophoblasts [36], and along with CD81, it forms the entry receptor for hepatitis C [37], which is an RNA virus-like SARS-CoV-2. It interacts with SARS-CoV-2 nsp7. PRKAR2B or cAMP-dependent protein kinase
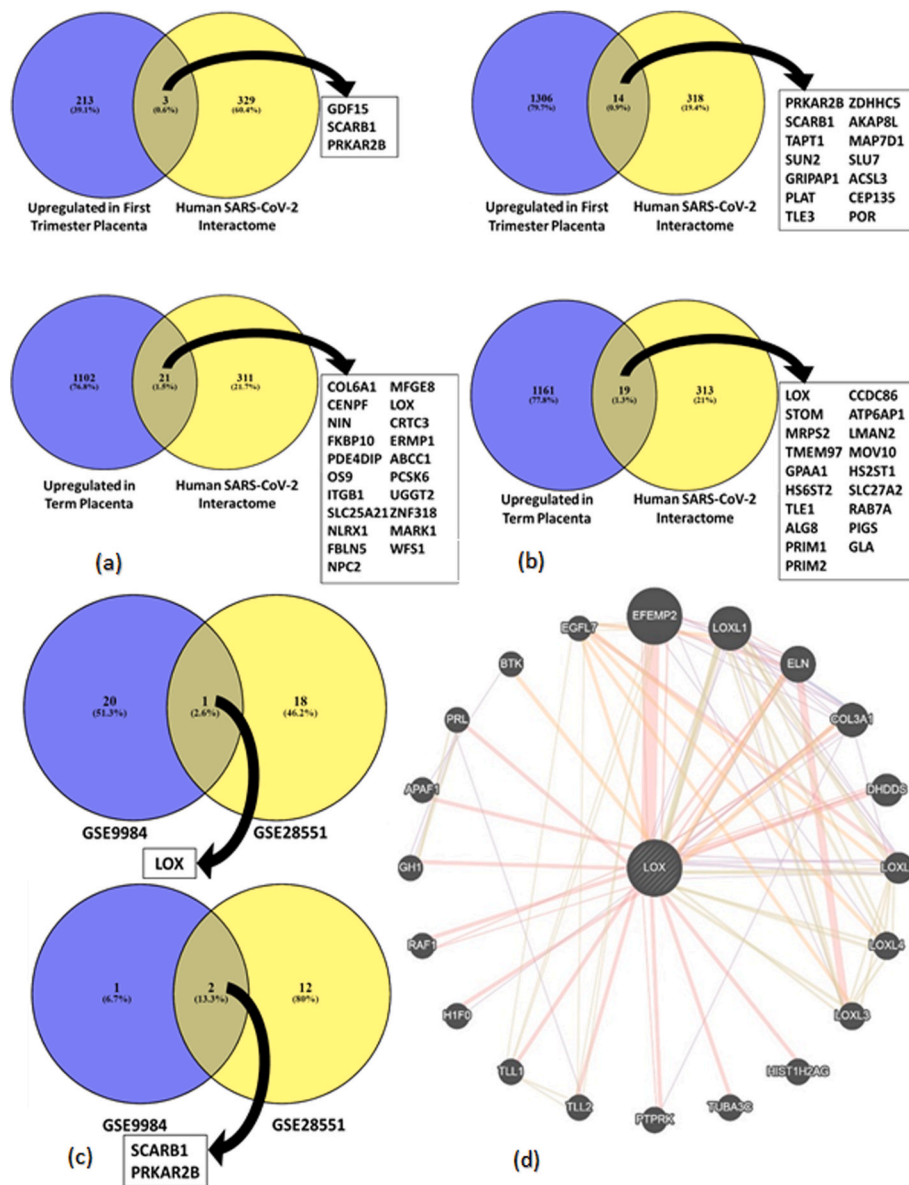
Fig. 1. SARS-CoV-2 Interacting Proteins that are Upregulated in First Trimester Placenta and Term Placenta. Fig. 1a and b shows data from GSE9984 and GSE28551, respectively. Fig. 1c shows the overlapping proteins in term placenta (left) and first trimester placenta (right) from GSE9984 and GSE28551, that interact with SARS-CoV-2. Fig. 1d shows the LOX interaction network. The expansions of all the protein names and the SARS-CoV-2 proteins interacting with them are given in Supplementary Table 2.

type II-beta regulatory subunit interacts with SARS-CoV-2 nsp13.

3.2. SARS-CoV-2 Interacting Proteins that are Upregulated in Villous and Extravillous Trophoblasts, and during Villous Trophoblast Differentiation

Considering the importance of both villous and extravillous lineages of trophoblast in placentation and fetal development [38] we investigated if SARS-CoV-2 interacting proteins are crucial during this differentiation program. We analyzed the villous and extravillous data set (GSE9773) (Fig. 2a and b). The extravillous trophoblast (EVT) populations are highly motile and exhibit a migratory phenotype, which enables them to leave the villous core and enter the maternal endometrium. As seen in the intersecting Venn (Fig. 2a), GSE9773 identified 5 common entries between villous cytotrophoblasts and SARS-CoV-2 interactome. COL6A1 (collagen type VI α1 chain), identified as one target, is reported to play a critical role in cell migration [39] and cancer metastasis [40].

Another target, FBLN5 (fibulin-5) is a member of the fibulin family that alters cell adhesive and invasive properties and is expressed in human villous cytotrophoblasts as reported by Winship et al. [41] Derived from decidua and EVT, it regulates EVT invasion and

placentation. Gauster et al. [42] based on cell culture experiments with the villous trophoblast-derived placental fusogenic cell line BeWo, showed that fibulin-5 expression was downregulated during functional differentiation and intercellular fusion. BeWo under hypoxia showed a reduced tendency to fuse, along with an increase in FBLN5 expression. Moore et al. [43] localized major microfibrillar networks in amnion. Other targets NPC2 (along with NPC1) are required for egress of lysosomal cholesterol, by which cholesterol is removed from the late endocytic pathway [44]. Placenta is an active steroidogenic tissue; this pathway is therefore of paramount importance, which can be perturbed upon interaction with SARS-CoV-2. We then identified the overlapping proteins between EVT and SARS-CoV-2 interactome, which yielded eight interacting members (GSE9984) (Fig. 2b). FAR2 is abundantly expressed in placenta and trophoblast (Supplemenatry Fig. 2) [45], but currently, its function is unknown.

We next looked into the role of STOM (Stomatin) gene as one of the candidates in our analysis. Chen et al. [46] found that STOM can induce trophoblast fusion and hence could play a role in syncytiotrophoblast formation. Similarly, NUP98 plays a role in the nuclear pore complex (NPC) assembly and/or maintenance. NUP98, along with NUP96, are involved in bi-directional transport across the NPC. NUP98 seems
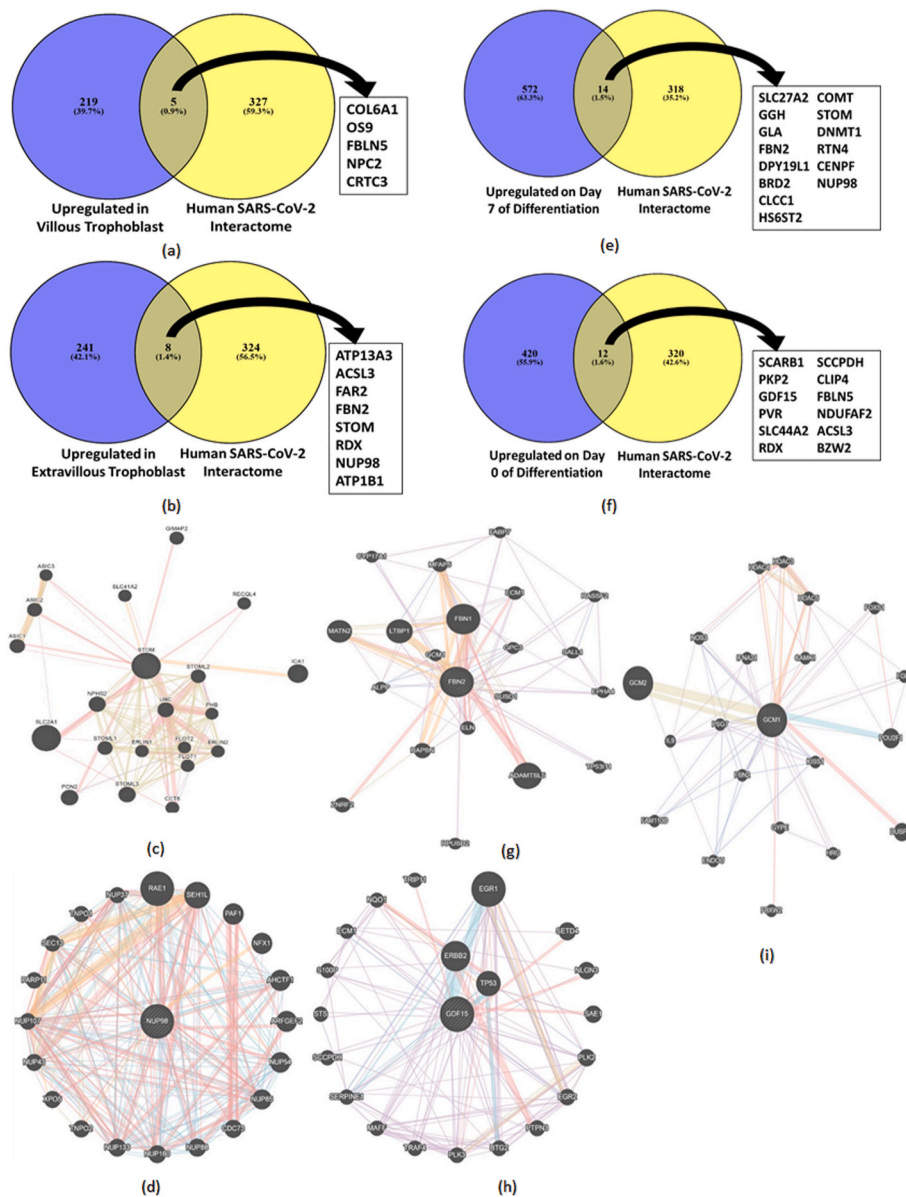
Fig. 2. SARS-CoV-2 Interacting Proteins that are Upregulated in Villous Trophoblasts (Fig. 2a) and Extravillous Trophoblasts (Fig. 2b). The data was obtained by analysing GSE9773. Fig. 2c and d shows GeneMANIA network showing STOM and NUP98, respectively, as hub of a complex interactome. Several cell cycle genes associated with NUP98. Fig. 2e and f shows SARS-CoV-2 Interacting Proteins that are Upregulated on Day 0 and Day 7 of Villous Trophoblast Differentiation, respectively. The data was obtained by analyzing GSE130339. Fig. 2g and h depict GeneMANIA network showing FBN and GCM1 interactome. GeneMANIA network showing GDF15 interactome is given in Fig. 2i. The expansions of all the gene names are given in Supplementary Table 2.

critical for embryonic stem cell development, as was associated with pregnancy loss [47]. The interaction networks of STOM and NUP98 are shown in Fig. 2c and d respectively. Thus, we conclude that several key proteins related to trophoblast fusion, invasion, and genome maintenance seem to be part of SARS-CoV-2 interactome.

Our observation was also reinforced when we analyzed the next data gene set (GSE130339). This gene data set associated with early and latter day 7 trophoblast differentiation (Fig. 2e and f). STOM and NUP98 were also found to be enriched here. Of particular interest is FBN2. A recent report published by Yu Y et al. [48] mentions that FBN1 encodes asprosin, a glucogenic hormone, following furin cleavage of the C-terminus of profibrillin. They identified a peptide hormone, placensin encoded by FBN2 in trophoblasts of human placenta. Placensin secretion by immortalized human trophoblastic HTR-8/SVneo cells is accompanied by an increase in matrix metalloproteinase-9 (MMP9) expression, thereby promoting cell invasion. FBN2 also seems to interact with GCM1 (Fig. 2g and h), a critical factor for trophoblast differentiation. GCM1 is a master regulator of trophoblast differentiation and seems to be associated with several HDAC complex and is possible that this alters the epigenetic landscape in the cell, an event needed prior to differentiation and lineage commitment.

Of interest is GDF15 present in Day 0 differentiation. Recently Turco et al. [49], using placental organoids derived from early placental villi, found that these cultures organize into villous-like structures and secretes placental-specific peptides and hormones, including human chorionic gonadotropin (hCG), growth differentiation factor 15 (GDF15). This gene encodes a secreted ligand of TGF superfamily, which binds to various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. GDF15 is involved in trophoblast development where it controls apoptosis and differentiation [50]. GDF15 interactome showed p53 and ERBB2 as close neighbors (Fig. 2i).

3.3. SARS-CoV-2 Interacting Proteins that are Upregulated in Cytotrophoblasts, Syncytiotrophoblasts and Migrating Trophoblasts

We did the data mining for SARS-CoV-2 interactome with genes highly expressed in cytotrophoblasts, syncytiotrophoblasts, and migrating trophoblasts (Fig. 3a, b and 3c, respectively). A significant number of genes upregulated in cytotrophoblasts were found to overlap
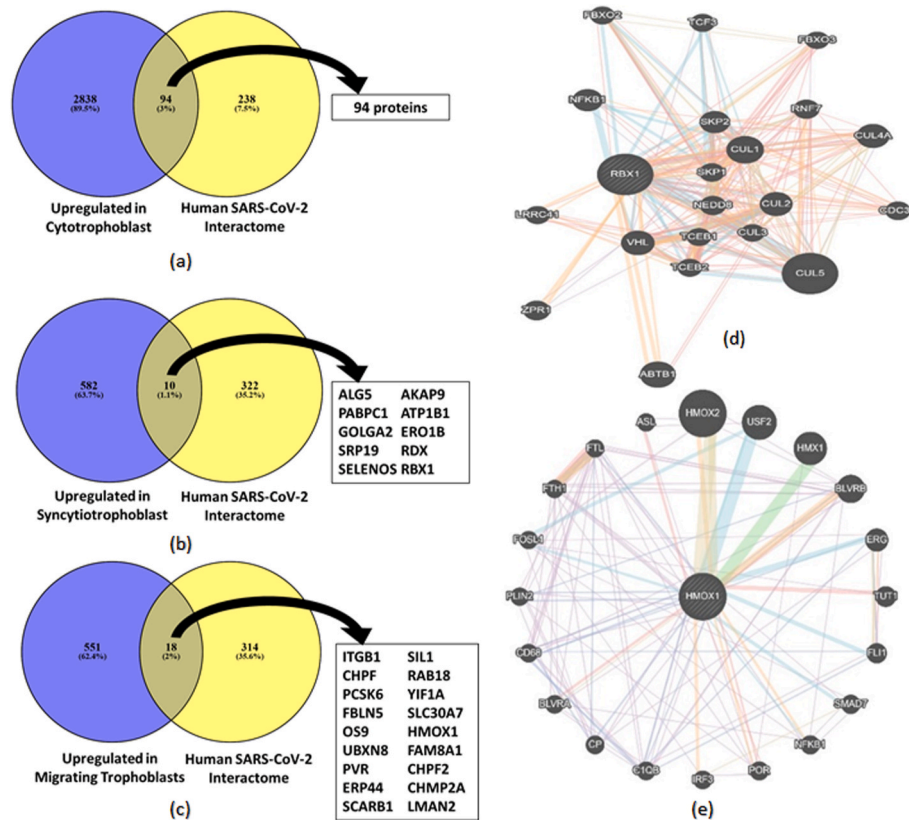
Fig. 3. SARS-CoV-2 Interacting Proteins that are Upregulated in Cytotrophoblasts, Syncytiotrophoblasts, and Migrating Trophoblasts (Fig. 3a, b and 3c, respectively). Fig. 3d shows RBX1 CUL3 complex which is predominantly expressed in invasive EVT. Fig. 3e shows HMOX1 interactome. Several of the interacting partners play important roles in trophoblast invasion. The expansions of all the gene names are given in Supplementary Table 2.

with SARS-CoV-2 interactome (approx. 94, also see Supplementary Table 3), implying that SARS-CoV-2 can strongly influence cyto-trobhoblast stem cells. We observed about ten overlapping proteins related to syncytiotrophoblast formation. Of special interest is RBX1, a ring box 1 protein which is found to be abundantly expressed in placenta (Fig. 3d). Cullin 3 (CUL3), is a scaffold protein that assembles into a large number of ubiquitin ligase complexes, similar to SKP1-Cullin 1-F-box protein complex. CUL3 associates with Bric-a-brac-Tramtrack-Broad (BTB) complex and RBX1 to form a BTB-CUL3-RBX1 (BCR) ubiquitin ligase complex. CUL3 was found to be highly expressed in the invasive EVTs of human placenta villi from normal pregnant women, and the expression of CUL3 in the less invasive EVTs from PE patients was significantly lower [51]. CUL3 also promoted the invasion and migra-tion of human trophoblast cells in the human EVT cell line HTR8/SVneo as well as in placental explants. These results show that RBX1 interac-tion with SARS-CoV-2 proteins is crucial for EVT invasion.

Further, we found 18 genes related to migratory trophoblast over-lapped with SARS-CoV-2 interactome. Of particular interest is HMOX1, which produces a signaling molecule, carbon monoxide (CO). Bilban et al. [52] reported that modulation of HMOX1 expression in loss-of-function as well as gain-of-function cell models (BeWo and HTR8/SVneo, respectively) demonstrated a reciprocal relationship of HMOX1 expression with trophoblast migration. Also, HMOX1 expres-sion led to an increase in peroxisome proliferator-activated receptor (PPAR) gamma. The HMOX1 interactome is depicted in Fig. 3e.

3.4. SARS-CoV-2 interacting proteins that are upregulated in placental cell lines

We next explored that to what extent SARS-CoV-2 interacting pro-teins overlap with those derived from the cell lines (Fig. 4). Our analysis

interrogated gene set data from HTR8/SVneo, SGHPL5, and JEG3 cell lines, three of the most popular trophoblast cell lines, using the dataset GSE20510. Our results show 18 and 43 overlapping proteins between HTR8/SVneo and JEG3 cell lines, respectively, and 8 and 42 overlapping proteins between SGHPL5 and JEG3 cell lines, respectively. 5 proteins overlapped between the representative early placental cell lines – SGHPL5 and JEG3 (Supplementary Figure 3). We next looked for the overlaps between the genes upregulated in the early placental cell lines – HTR8/SVneo and SGHPL5 – and early placenta and the human SARS-CoV-2 interactome, using datasets GSE20510 and GSE28551. The result showed the commanality of transducin-like enhancer protein 3 (TLE3) (Fig. 4e).

TLE3 is a transcriptional co-repressor. In murine models, TLE3 known out was associated with abnormal placental development with a severe defect in the vasculature and intrauterine death. TLE3 has also involved in Notch-3 mediated signaling and is involved in the devel-opment of trophoblast giant cells lining maternal blood spaces in the mouse placenta [53]. The interaction network of TLE3 using STRING shows its association with various proteins involved in the transcrip-tional regulation like Histone deacetylase 1 (HDAC1), CREB-binding protein (CREBBP), C-terminal-binding protein 1 (CTBP1) and Fork-head box protein A1 (FOXA1) (Fig. 4f). GeneMANIA of TLE3 inter-actome (Fig. 4g) showed strong associations with COPB1 and COPZ1. These are components of coat protein I (COPI) complex which is involved in intracellular protein trafficking. They are also involved in the binding of SARS-CoV (cause of SARS of 2003) spike protein (S protein) to the membrane protein (M-protein), a process required for viral entry into the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC), budding and viral shedding [54]. This also im-plies that inhibition of COPI complex using specific pharmacological agents may prevent the virus infection cycle, at least as observed with
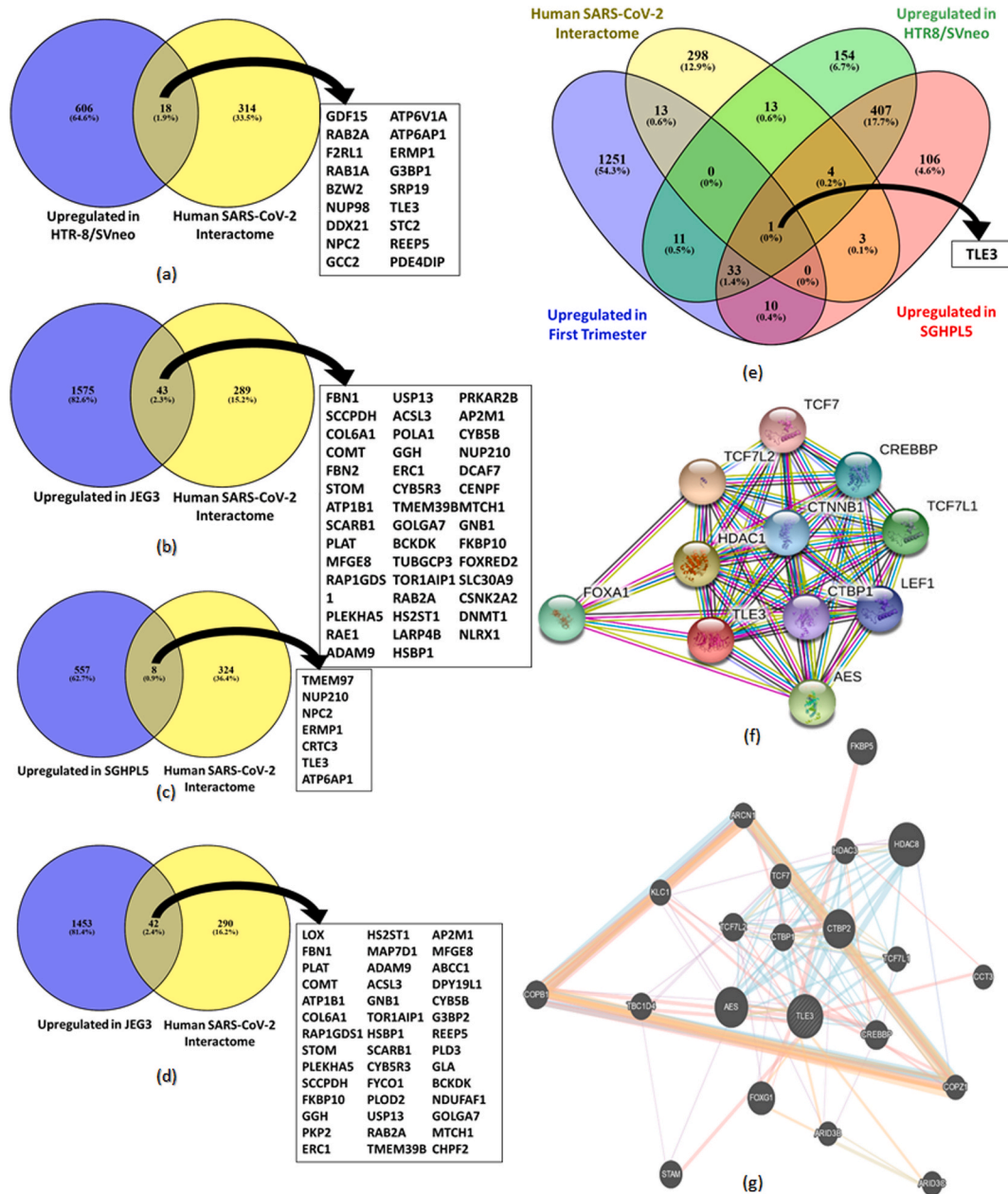
Fig. 4. SARS-CoV-2 Interacting Proteins that are Upregulated in Different Placental Cell Lines. Fig. 4a and c shows genes upregulated in HTR-8/SVneo and SGHPL5 cells, respectively, in comparison with JEG3 cells (Fig. 4b and d). The dataset used was GSE20510. Fig. 4e shows combined analysis of datasets GSE20510 and GSE28551 involving genes that are upregulated in first trimester placenta and surrogates used in vitro studies for first-trimester placenta – HTR8/SVneo and SGHPL-5 cell lines, and the human SARS-CoV-2 interactome. STRING (Fig. 4f) and GeneMANIA (Fig. 4g) analysis show TLE3 interacting proteins. The expansions of all the gene names are given in Supplementary Table 2.

the influenza virus [55].

We further explored on ARCNI, as one of the potential TLE3 interacting partners as evident from GeneMANIA (Fig. 4g and Supplementary Figure 4). Results show ARCN1 as a hub, interacting with a large family of COP proteins (COPA, COPB1, COPB2, COPG1, COPG2, COPE, COPZ1) as well as Golgi components (GOLPH3, GOLPH3L). As discussed above, COP plays an important role in virus entry, assembly, and transmission, thereby indicating that these trophoblast cell lines may suffice as a suitable models to study the viral infection cycle.

3.5. SARS-CoV-2 Interacting Proteins that are Altered in Pre-eclampsia and Gestational Diabetes Mellitus

So far, we saw that a significant number of genes associated with

trophoblast invasion, differentiation, and migration that could potentially interact with SARS-CoV-2. This prompted us to speculate the status of engagement between SARS-CoV-2 and females with preeclampsia (PE). The datasets for PE included GSE66273, where gestational age for PE was 31.0 (30.9–34.0) weeks, and controls were 31.2 (29.3–33.2) weeks, and GSE48424, where gestational age for PE was 34 (31–35) weeks, and for controls was (33–37) weeks. Our analysis of the dataset of genes overexpressed in preeclampsia using GSE66273 revealed MFGE8 (milk fat globule-epidermal growth factor-factor 8) as one of the targets that was also upregulated in PE (Fig. 5a). MFGE8 protein (Fig. 5e) is highly expressed in human chorionic villi at all trimesters of gestation and in murine implantation sites [56]. MFGE8 performs an important role in physiological conditions during menstrual endometrium remodelling and implantation, and dysfunctions of its expression may be
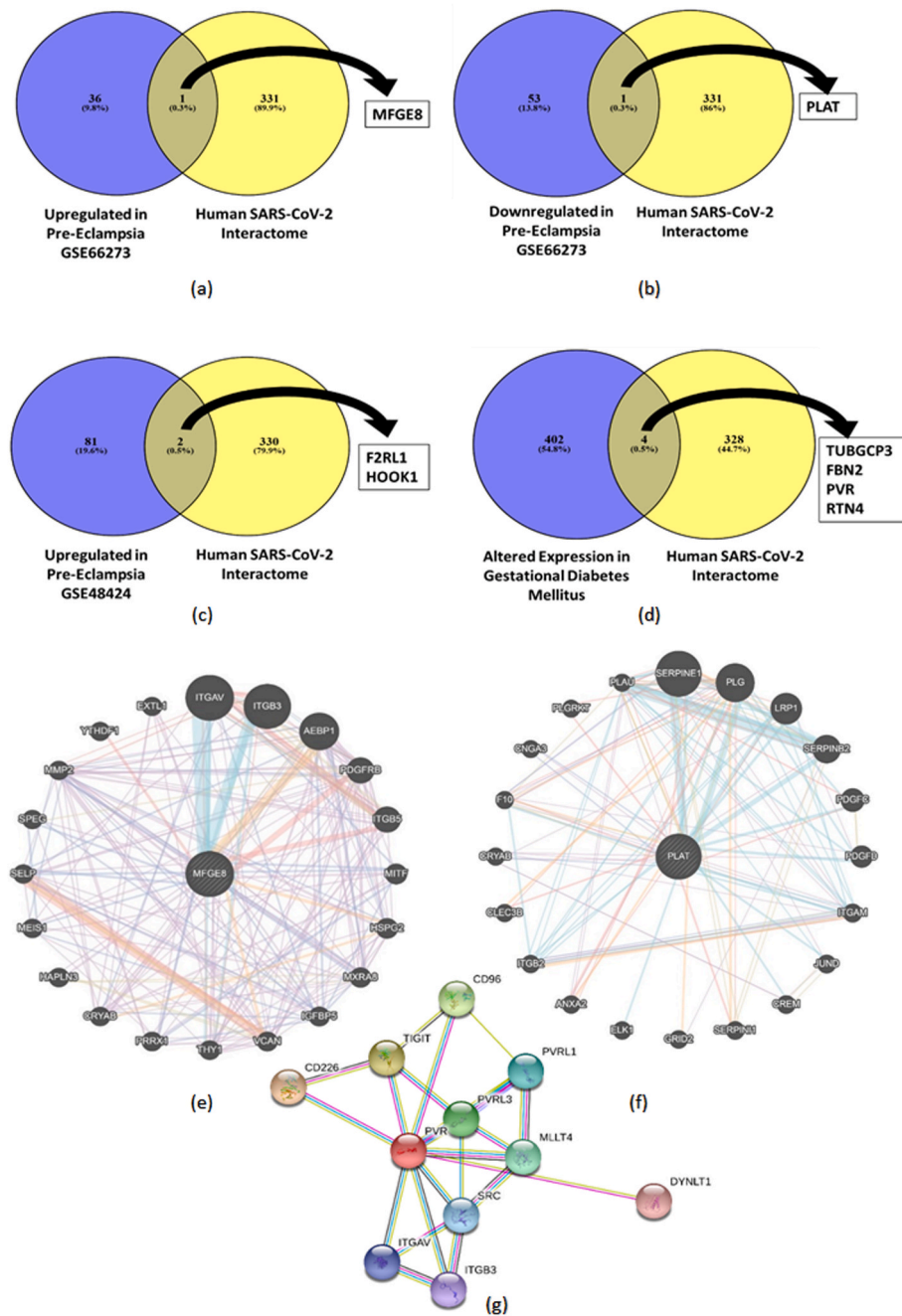
Fig. 5. SARS-CoV-2 Interacting Proteins that are Altered in Pre-eclampsia (Fig. 5 a, b, and c) and Gestational Diabetes Mellitus (Fig. 5d). Fig. 5e and f shows GeneMANIA protein interaction networks of MFGE8 and PLAT, respectively. Fig. 5g shows STRING protein interaction network of PVR (CD155). The expansions of all the gene names are given in Supplementary Table 2.

associated with endometrial pathological conditions. Further, Schmitz, et al. [57] investigated that MFGE8 and its receptor integrin αvβ3 play an important role in the attachment of trophoblast cells to the endometrial epithelium. Yu et al. [58] reported MFGE8 as a master regulator for TGFbeta1 in orchestrating EMT, an event that seems to be dysfunctional in PE. Of particular interest is that MMP2 (72 kDa MMP) as one of the interacting partners for MFGE8 as identified in the gene interaction model (Fig. 5e). MMP2 has been strongly associated with the onset of PE [59]. Therefore, we can conclude that SARS-CoV-2 interaction with MFGE8 could have potentially serious consequences in the onset of PE.

We next investigated the genes down-regulated in PE overlapping with SARS-CoV-2 interactome and identified PLAT as one member (Fig. 5b). PLAT (Plasminogen Activator Tissue Type) interaction

network comprises (Fig. 5f) of several serine proteases and their inhibitors - SERPINE1 (also called PAI-1, Plasminogen Activator Inhibitor), PLAU (Plasminogen Urokinase Activator), PLG (Plasminogen). One of the PLAT interacting partners, LRP1 (LDL receptor Related Protein 1) is reported to be poorly expressed in preeclamptic group placental in SGA (Small for Gestational Age) delivering mothers [60] which explains the atherosclerotic phenotype of preeclamptic placentas. Several of the members of this interaction network (SERPINE1, LRP1, PDGFD, JunD) seems to be associated with preeclampsia and PIH [61,62].

Analysis of GSE48424 (Figure 5c) showed one of the overlapping proteins upregulated in PE and human SARS-CoV-2 as F2LR1. F2LR1 or coagulation factor II (thrombin) receptor-like 1, also known as Protease-activated receptor 2. PAR2 expression was found to be absent in normal

placental endothelial cells but overexpressed in placental endothelial cells of pre-eclampsia patients. Since this is an extracellular receptor, it will be intriguing to know whether pre-eclampsia placenta will be at risk for SARS-CoV-2 binding compared to normal pregnancies [63].

The list of genes with altered expression in gestational diabetes mellitus (GDM) was obtained from a study by Radaelli et al. [29] The average gestational age in GDM was 38.5 ± 0.5 weeks and in controls was 38.9 ± 0.4 weeks. Among the overlapping genes with SARS-CoV-2 (Fig. 5d) interactome was FBN2 or Fibulin 2, which has already been mentioned previously as upregulated during extra-villous trophoblast differentiation. Another protein in the common Venn is poliovirus receptor (PVR) or CD155, which was found to interact with actin and integrin alpha-V, which are known factors affecting cell motility and migration; thus CD155 is involved in tumor cell invasion and migration, a shared property of tumor cells and trophoblast cells [64]. The STRING analysis also showed the confirmed this finding (Fig. 5g).

4. Discussion

We investigated, *in silico*, the effect of SARS-CoV-2 infection on placental functions. Several of the key genes associated with trophoblast invasion, villous vs. extravillous differentiation, as well as migratory behavior of these cells seem to be a part of SARS-CoV-2 human interactome. Our analysis also identified many novel, unreported candidate proteins that could be targeted during these infections. We also explored the differentially expressed proteomes from trophoblast cell lines and found a significant number of overlaps with SARS-CoV-2, indicating that these cells can be used as a model to study viral infection.

Since the SARS-CoV-2 interactome reported by Gordon et al. [27] does not include cell surface proteins, we also manually sifted through the list of genes upregulated in various processes and states associated with placenta to check for the entry receptors – ACE2 and TMPRSS2 – of SARS-CoV-2 [65]. Though some studies are skeptical regarding the co-expression of these proteins in term placenta [66,67], others suggest the presence of both ACE2 and TMPRSS2 in first trimester [68,69]. Our analysis showed that TMPRSS2 is up-regulated in term placenta relative to first trimester (GSE28551), while ACE2 is upregulated in extravillous trophoblasts (GSE9773), thus, further making the interaction between SARS-CoV-2 and placental tissue probable.

Analysis of placental morphology in COVID-19 pregnancies by Shanes et al. [22] revealed widespread abnormalities in the vasculature and maternal vascular malperfusion in the studied samples, although limited in number. Two of the proteins that were recurring in our *in silico* analysis, that were found to be interacting with SARS-CoV-2 and upregulated in early and term placental tissues or surrogates were TLE3 and LOX, respectively. Both these proteins play important roles in the establishment of placental vasculature and arterial remodelling, and their downregulation has been associated with abnormal placentation in animal models [53]. Interestingly, this was predicted and substantiated in the preprint version of the current article [70], which was published before the findings by Shanes et al. [22] were available. Further, Baergen and Heller [23] observed that amongst 50% of COVID-19 positive pregnant females, there was evidence of fetal vascular malperfusion or fetal vascular thrombosis, a possibility well predicted in our current study given the fact that SARS-CoV-2 interaction with EVT can alter trophoblast mediated alteration of maternal vasculature, leading to poor perfusion and hypoxia [71].

We also explored the interaction of placental proteins with SARS-CoV-2 in pathological states like preclampsia and GDM, two of the most common associated pathological states in pregnancy. We found a few interesting overlapping members, both in PE and GDM. PLAT and MFGE8 in PE seem to be associated with serine proteases and their regulators, many of which were reported to be associated with matrix remodelling, invasion, and vasculogenesis. Though certain studies hypothesize a probability for development of PE in COVID-19 pregnancies, there are no definite evidences to suggest so [72].

One limitation of the current analysis is that the human proteins interacting with SARS-CoV-2 obtained after cloning the viral proteins in HEK293T cells (which are human embryonic kidney cells) may not be exactly similar to the interactome of SARS-CoV-2 in placental cells. However, to the best of our knowledge, there are no reports available on the latter, and at this stage, the former study is unique in that manner. Further, we investigated the transcription profile in placenta and in kidney [45] and found that a majority of the proteins that are a part of the SARS-CoV-2 interactome in HEK293T cells are also represented in the placental transcriptome (Supplementary Figure 5) indicating that we could potentially see these similar interactions conserved there.

We attempted KEGG and GO pathway analysis with DAVID 6.8 [73] for functional annotation of the proteins interacting with SARS-CoV-2, that are overlapping with those involved in key roles in placental biology. However, the analysis did not suggest any significant enrichment of specific biological processes or pathways. This could be due to the already restricted number of proteins of the SARS-CoV-2 interactome, which is further narrowed down by a screen for their role in placental function.

Stefanovic [74] reported that the rate of Cesarean deliveries among COVID-19 women is unacceptably high and that while significant attention is devoted to the mother, the fetus also needs to be treated as a patient and deserves even more attention. However, there are only very few clinical reports on the outcome of COVID-19 during the first trimester of pregnancy when the trophoblast invasion and differentiation are very critical. The lack of this information should be factored in before making any rigid conclusions from our study, which is solely based on *in silico* analysis.

5. Conclusion

Our findings suggest that SARS-CoV-2 might have a profound influence on the placentation process through their effect on the trophoblast cells, as several of the viral proteins can potentially interact with host proteins that are critical components of trophoblast invasion, migration, proliferation, and differentiation processes. We, however, want to add a note of caution that our conclusion is entirely based upon *in silico* data analysis and therefore lack experimental validation. Given the robustness of the process and the individual variability, the final results may differ. It will be interesting to analyze more clinical results to substantiate our prediction. Though clinical studies are limited, following our findings, it becomes, even more, a priority to take into account the possible consequences of COVID-19 on placental functions and prepare health care professionals to adopt effective management to avoid an adverse health crisis affecting both mother and the child.

Declaration of competing interest

All the authors declare that there are no conflicts of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.placenta.2020.10.027.

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