IFITM proteins inhibit placental syncytiotrophoblast formation and promote fetal demise

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Title: IFITM proteins inhibit placental syncytiotrophoblast formation and promote fetal demise

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Abstract:

A critical step of placental development is the fusion of trophoblast cells into a multinucleated syncytiotrophoblast (ST) layer. Fusion is mediated by Syncytins, proteins deriving from ancestral endogenous retroviral envelopes. Elevated levels of type-I Interferons (IFN) during pregnancy are associated with intrauterine growth retardation, preterm birth, and fetal demise, but the mechanisms are not well understood. Using cultures of human trophoblasts or mouse cells, we show that IFN-induced transmembrane proteins (IFITMs), a family of restriction factors blocking the entry step of many viruses, impair ST formation and inhibit Syncytin-mediated fusion. Moreover, the IFN inducer Poly-IC promotes fetal resorption and placental abnormalities in wild-type, but not in *Ifitm*-deleted mice. Thus, excessive levels of IFITMs may mediate pregnancy complications observed during congenital infections and other IFN-induced pathologies.

Main Text:

The placenta is the primary maternal-fetal barrier, achieving metabolic exchanges, hormone production as well as protection from pathogens and the maternal immune system. The placental tissue stems from embryonic cytotrophoblasts. The structural and functional unit of human placenta is the chorionic villous, that includes proliferative mononuclear villous cytotrophoblasts (VCT) at the basement and the syncytiotrophoblast (ST) layer at the surface. The multinuclear ST arises from the differentiation and fusion of VCT. In humans, an abnormal ST is observed in pregnancy pathologies including intrauterine growth retardation (IUGR), preeclampsia, lupus, and Down syndrome (*1-3*). In contrast to the human placenta, the mouse placenta has a labyrinthic structure and exhibits two ST layers that separate fetal capillaries from maternal blood (*4*). In gestating mice, IFN-β triggered by Zika or other viruses, or by administration of Poly-IC, promotes IUGR and fetal demise (*2, 5-7*). IFN-β

signalling leads to abnormal labyrinth and ST structures, with the presence of many unfused cells and reduced fetal blood vessels (*7*).

Cytotrophoblast fusion is mediated by envelope glycoprotein (env)-derived genes of endogenous retroviruses (ERV) that have been domesticated by mammals (*3*). These genes were termed syncytins based on their fusogenic capacity. The capture of syncytin genes, sometimes as pairs (Syncytin-1 and -2 in humans and Syncytins-A and -B in mice) (*3, 8, 9*), occurred independently from different ERV in diverse mammalian lineages 10 to 85 million years ago. Knocking out Syncytin-A or both Syncytin-A/B genes leads to fetal growth restriction and mid-gestational lethality (*8, 9*).

The immune-related interferon-induced transmembrane proteins IFITM1, IFITM2, and IFITM3 are restriction factors that protect uninfected cells from viral infection. They block the entry into host cells of many enveloped viruses by inhibiting virus–cell fusion at the hemifusion stage (*10-12*). They act by altering the biophysical properties or cholesterol content of the cellular membranes in which they are found (*11-13*). IFITM modify the rigidity and/or curvature of the membranes without evidence of a direction interaction with fusogenic viral envelopes (*11, 12*). Due to different sorting motifs, IFITM1 mostly resides at the plasma membrane, whereas IFITM2/3 accumulate in the endo-lysosomal compartment after transiting at the cell surface. IFITM proteins are expressed at various basal levels in different cell types and are upregulated by IFNs and other cytokines (*11*). Besides their antiviral activity, the physiological cellular functions of IFITM proteins remain poorly characterized. Transgenic mice in which the cluster of *Ifitm* genes is knocked out (thereafter IfitmDel mice) are more sensitive to various viral infections (*12*), but exhibit no overt abnormalities apart from being a little over-weight (*14*). Not much is known about the impact of IFITMs on fusion events mediated by cellular proteins or by ERV-derived Env proteins. Here, we examined whether IFITMs impair Syncytin-mediated fusion and thus impact fetal development.

We first investigated whether IFITMs inhibit cell fusion mediated by exogenously-expressed human Syncytins. We generated 293T cells carrying a GFP-Split complementation system, in which

two cells separately produce half of the reporter protein, generating a signal only upon fusion (Fig. 1A). The extent of fusion was quantified by measuring the GFP-positive area (Fig. 1A). Transfection of Syncytin-1 induced the appearance of multinucleated GFP+ cells. Fusion was significantly decreased when Syncytin-1 was co-transfected with FLAG-tagged IFITM1, 2, or 3, but not with a control plasmid (Fig. 1A). The extent of inhibition of fusion varied between individual IFITMs. The IFITM3Δ1-21 and IFITM3Δub mutants, which lack endocytic and ubiquitination motifs, respectively, and accumulate in the cell (*15, 16*), were more active at inhibiting fusion than WT IFITM3. In contrast, an IFITM3Δpalm mutant, whose levels are reduced (*15, 16*), was inactive. The co-expression of three IFITMs strongly inhibited fusion (Fig. 1A). IFITMs similarly inhibited fusion mediated by Syncytin-2 (Fig. S1A). IFITMs did not decrease the levels of Syncytin-1 in transfected cells (Fig. S1B). To distinguish the effect of IFITMs in Syncytin-1+ (donor) cells from that in target cells (expressing SLC1A5, the Syncytin-1 receptor), we used an HIV Tat and LTR-luc transactivation co-culture system, where cell fusion generates luciferase activity (*13*). IFITMs inhibited fusion when present in either donor and/or target cells (Fig. S1C).

We next examined the effect of IFITMs on endogenous Syncytins. In trophoblast-like BeWo human choriocarcinoma cells, addition of the adenylate cyclase activator forskolin triggers Syncytin-1 production and syncytium formation (*17*). To quantify fusion, we generated BeWo cells carrying two complementation systems, based on either β-galactosidase-α/ω (β-Gal-α/ω) (Fig. 1B) or GFP-Split (Fig. S2C). These cells were then transduced with lentiviral vectors to express a control protein or FLAG-tagged IFITM1-3 (Fig. S2A and D). Forskolin triggered fusion and production of β-Gal, detected in fixed cells and quantified in cell lysates (Fig. 1 B and Fig. S2B). The fusion efficacy was significantly reduced by IFITMs. The presence of IFITMs did not modify BeWo cell growth or alter their ability to produce syncytia-independent β-Gal (Fig. S3A-D). Similar results were observed with the GFP-based system. A video-microscopy analysis showed delayed and reduced fusion with each IFITM (Fig. S2C-E, Movie S1).

We next tested the capacity of endogenous IFITMs to block fusion in mouse embryonic fibroblasts (MEFs) derived from WT or IfitmDel mice (*14*). To this aim, MEFs were co-transfected with Syncytin-A and GFP plasmids (Fig. 1C, Fig. S4A). Syncytin-A induced numerous and large GFP+ syncytia (with up to 20 nuclei) in IfitmDel MEFs, and fewer and smaller syncytia in WT MEFs. Transduction of IfitmDel MEFs with a murine Ifitm3 vector (Fig. S4B) restored resistance to fusion (Fig. 1C, Fig. S4A). That WT MEFs were poorly sensitive to fusion was likely due to high basal IFITM3 levels (Fig. S4B).

We then evaluated the effect of type-I IFN and IFITMs in primary human trophoblasts. We first asked whether IFITMs were upregulated by type-I IFN in human placental explants. It has been reported that in human mid-term placental chorionic villi explants, addition of IFN-β, but not IFN-λ, leads to defects including cellular damage, decreased production of human chorionic gonadotropin (hCG), and appearance of syncytial knots (*7*). In such explants, IFN-β upregulated hundreds of Interferon Stimulated Genes (ISGs) including IFITM1 (*7*). We took advantage of this large set of published RNA data to investigate the expression of IFITMs, Syncytins, and their receptors. IFITMs were upregulated by IFN-β, when compared to mock and IFN-λ treated explants, whereas Syncytins and their receptors were not modulated (Fig. S5).

These observations were made in whole placental explants where non-trophoblastic cells are also present. We thus studied the impact of IFN-β on primary VCT isolated from 8 term human placentas. VCT can be cultured up to three days and spontaneously differentiate in multinucleated ST (*18*). Treating VCT with IFN-β (100 or 1000 IU/mL for 48 h) strongly upregulated IFITM1 and 2/3 proteins, as shown by immunofluorescence and western blot (Fig. S6A, C). IFN-β slightly upregulated RNA levels of Syncytin-1 but did not impact those of Syncytin-2 and Syncytin receptors (Fig. S6D). We next quantified VCT fusion, using a method based on the detection of the transcription factor GATA3 by immunofluorescence and its down-regulation upon ST differentiation (*18*). Cells were also stained for E-cadherin, to visualize plasma membranes. IFN-β inhibited VCT fusion, to the same extent than

GW9662, a PPARγ antagonist known to block this process (*18*) (Fig. 2A,B). As it acts independently of IFN signalling, GW9662 did not induce IFITM (Fig. S6A,C). The effect of IFN-ß on ST differentiation was supported by a decreased release of hCG, secreted by ST but not by VCT (Fig. 2C). The reduction of fusion was not due to a cytotoxic effect of IFN-β, since levels of an apoptosis marker (cCK18), were not augmented (Fig. S6B, C).

To further assess the effect of IFITMs in this system, VCTs were transfected with a FLAG-tagged IFITM3 or a control GFP plasmid. The poor efficiency of transfection (10% of the cells expressing IFITM or GFP) precluded precise quantification of syncytia. However, a visual examination indicated that IFITM3+ cells remained mononucleated, even in vicinity of large syncytia, whereas GFP-transfected cells were able to fuse (Fig. S7). Altogether, these results demonstrate that IFN-β induces IFITMs in VCT and that both IFN-ß and IFITMs inhibit ST formation.

We then evaluated the role of IFITM in pregnant mice. Administration of Poly-IC has been extensively used as a model of type-I IFN induction, triggering fetal growth retardation and resorption (*2, 6, 7*). We thus tested the effect of Poly-IC in gestating WT, Ifnar-/- or IfitmDel dams that have been mated to males of the same genotypes (Fig. 3A). Poly-IC was injected at E7.5, a time point that shortly precedes ST formation (about E8.5) (*4*). A dose of 200 µg, that results in resorption of almost all fetuses within 48h (*7*) was injected. With Poly-IC, WT fetuses, but neither Ifnar-/- nor IfitmDel fetuses were resorbed at E9.5 (Fig. 3 B). The fetus size was similar in Ifnar-/- and IfitmDel mice (Fig. 3 C, S8) and slightly reduced compared to untreated animals (Fig. 3C, S8). We checked that IfitmDel and WT mice similarly responded to Poly-IC, as shown by the induction of various ISGs (Irf7, Oas1a, Stat1) measured in the liver at 14h post-injection, indicative of a systemic inflammation (Fig. S9B). A local response was also observed in placental extracts of WT mice, which up-regulated ifitm1, ifitm3 and Irf7 RNAs. As expected, IfitmDel placentas did not express Ifitm genes but up-regulated Irf7 (Fig. S9 C). Of note, levels of Syncytin A and B RNAs were not significantly impacted by Poly-IC (Fig. S9C).

We next performed histological analysis of the placentas to examine the consequences of Poly-IC treatment. Placentas from WT and IfitmDel fetuses were stained for E-cadherin to label trophoblasts (Fig. 3D). As expected, ST formation was detectable in untreated placenta from both WT and IfitmDel fetoplacental units (arrows). In contrast, with Poly-IC, less ST was detected in WT placentas, whereas it was still present in IfitmDel placentas. We next triple stained placenta 14h post Poly-IC injection for IFITM3, E-cadherin, and CD31 (a marker of endothelial cells) (Fig. S9D). As expected, IFITM3 was upregulated in placentas of Poly-IC treated WT mice, but not in IfitmDel mice. IFITM3 overexpression upon Poly-IC injection was noticed in CD31 positive E-cadherin negative cells (endothelium), and CD31 negative E-cadherin positive cells (trophoblasts), demonstrating that IFITM3 is induced in trophoblasts in response to Poly-IC, consistent with *in vitro* results.

Altogether, these results strongly suggest that IFITM upregulation in trophoblasts inhibits ST formation *in vivo*, which likely contributes to type-I IFN-associated placental dysfunction and fetal demise in mice.

In summary, we have uncovered a function for the IFITM family of antiviral restriction factors. We report that IFITMs impair the fusogenic activity of Syncytins required for ST formation and maintenance. Our results provide a possible molecular explanation for placental dysfunctions associated with IFN-mediated disorders, such as IUGR and "TORCH" infections (Toxoplasmosis, Other, Rubella, Cytomegalovirus, and Herpes) (*19*). In addition to infection, genetic and auto-immune interferonopathies such as Aicardi-Goutières syndrome and systemic lupus erythematosus (SLE) are associated with pregnancy complications (*2*). High serum IFN level is a marker of poor pregnancy outcome in SLE (*20*). Down syndrome in trisomy 21 (T21) patients is also associated with serious birth defects (*1*). In vitro, VCT from T21 patients poorly fuse into ST (*1*). This may be due to the location of the IFN receptor on chromosome 21, rendering T21 cells hyper-responsive to IFN (*21*) and thus potentially expressing high amounts of IFITMs.

Like other restriction factors, *Ifitm* genes are polymorphic in humans and primates (*11, 12*). It will be worth determining whether placental pathologies of unknown etiology, such as certain preeclampsia or early spontaneous abortions, implicate IFITM proteins and variants. It is also tempting to suggest that blockade of IFITMs may represent a possible intervention strategy to prevent pregnancy complications linked to interferonopathies.

References

1. G. Pidoux *et al.*, Review: Human trophoblast fusion and differentiation: Lessons from trisomy 21 placenta. *Placenta* **33**, S81-S86 (2012).
2. L. J. Yockey, A. Iwasaki, Interferons and Proinflammatory Cytokines in Pregnancy and Fetal Development. *Immunity* **49**, 397-412 (2018).
3. P. A. Bolze, M. Mommert, F. Mallet, Contribution of Syncytins and Other Endogenous Retroviral Envelopes to Human Placenta Pathologies. *Prog Mol Biol Transl Sci* **145**, 111-162 (2017).
4. S. E. Ander, M. S. Diamond, C. B. Coyne, Immune responses at the maternal-fetal interface. *Sci Immunol* **4**, (2019).
5. J. J. Miner *et al.*, Zika Virus Infection during Pregnancy in Mice Causes Placental Damage and Fetal Demise. *Cell* **165**, 1081-1091 (2016).
6. J. E. Thaxton *et al.*, NKG2D blockade inhibits poly(I:C)-triggered fetal loss in wild type but not in IL-10-/- mice. *J Immunol* **190**, 3639-3647 (2013).
7. L. J. Yockey *et al.*, Type I interferons instigate fetal demise after Zika virus infection. *Science Immunology* **3**, eaao1680 (2018).
8. A. Dupressoir *et al.*, Syncytin-A knockout mice demonstrate the critical role in placentation of a fusogenic, endogenous retrovirus-derived, envelope gene. *Proceedings of the National Academy of Sciences* **106**, 12127-12132 (2009).
9. A. Dupressoir *et al.*, A pair of co-opted retroviral envelope syncytin genes is required for formation of the two-layered murine placental syncytiotrophoblast. *Proceedings of the National Academy of Sciences* **108**, E1164-E1173 (2011).
10. A. L. Brass *et al.*, The IFITM Proteins Mediate Cellular Resistance to Influenza A H1N1 Virus, West Nile Virus, and Dengue Virus. *Cell* **139**, 1243-1254 (2009).
11. G. Shi, O. Schwartz, A. A. Compton, More than meets the I: the diverse antiviral and cellular functions of interferon-induced transmembrane proteins. *Retrovirology* **14**, 53 (2017).
12. A. Zani, J. S. Yount, Antiviral Protection by IFITM3 In Vivo. *Current Clinical Microbiology Reports* **5**, 229-237 (2018).
13. Alex A. Compton *et al.*, IFITM Proteins Incorporated into HIV-1 Virions Impair Viral Fusion and Spread. *Cell Host & Microbe* **16**, 736-747 (2014).
14. U. C. Lange *et al.*, Normal Germ Line Establishment in Mice Carrying a Deletion of the Ifitm/Fragilis Gene Family Cluster. *Molecular and Cellular Biology* **28**, 4688-4696 (2008).
15. A. A. Compton *et al.*, Natural mutations in IFITM3 modulate post-translational regulation and toggle antiviral specificity. *EMBO reports* **17**, 1657-1671 (2016).
16. J. S. Yount, R. A. Karssemeijer, H. C. Hang, S-palmitoylation and ubiquitination differentially regulate interferon-induced transmembrane protein 3 (IFITM3)-mediated resistance to influenza virus. *J Biol Chem* **287**, 19631-19641 (2012).
17. K. Orendi, M. Gauster, G. Moser, H. Meiri, B. Huppertz, The choriocarcinoma cell line BeWo: syncytial fusion and expression of syncytium-specific proteins. *Reproduction* **140**, 759-766 (2010).
18. S. A. Degrelle, T. Fournier, Use of GATA3 and TWIST1 Immunofluorescence Staining to Assess In Vitro Syncytial Fusion Index. *Methods Mol Biol.* **1710**, 165-171 (2018).
19. C. B. Coyne, H. M. Lazear, Zika virus - reigniting the TORCH. *Nat Rev Microbiol* **14**, 707-715 (2016).
20. D. Andrade *et al.*, Interferon-alpha and angiogenic dysregulation in pregnant lupus patients who develop preeclampsia. *Arthritis Rheumatol* **67**, 977-987 (2015).
21. K. D. Sullivan *et al.*, Trisomy 21 consistently activates the interferon response. *Elife* **5**, Jul 29;5. pii: e16220. doi: 10.7554/eLife.16220 (2016)

Supplementary References

22. J. Lu *et al.*, The IFITM proteins inhibit HIV-1 infection. *J Virol* **85**, 2126-2137 (2011).
23. S. Blaise, A. Ruggieri, M. Dewannieux, F.-L. Cosset, T. Heidmann, Identification of an Envelope Protein from the FRD Family of Human Endogenous Retroviruses (HERV-FRD) Conferring Infectivity and Functional Conservation among Simians. *Journal of Virology* **78**, 1050-1054 (2004).
24. J.-L. Blond *et al.*, An Envelope Glycoprotein of the Human Endogenous Retrovirus HERV-W Is Expressed in the Human Placenta and Fuses Cells Expressing the Type D Mammalian Retrovirus Receptor. *Journal of Virology* **74**, 3321-3329 (2000).
25. A. Dupressoir *et al.*, Syncytin-A and syncytin-B, two fusogenic placenta-specific murine envelope genes of retroviral origin conserved in Muridae. *PNAS* **102**, 725-730 (2005).
26. H. Clabault, L. Laurent, J. T. Sanderson, C. Vaillancourt, Isolation and purification of villous cytotrophoblast cells from term human placenta. *Methods in Molecular Biology* **1710**, 219-231 (2018).
27. M. S. Longtine, A. Barton, B. Chen, D. M. Nelson, Live-cell imaging shows apoptosis initiates locally and propagates as a wave throughout syncytiotrophoblasts in primary cultures of human placental villous trophoblasts. *Placenta* **33**, 971-976 (2012).
28. T. Cokelaer, D. Desvillechabrol, R. Legendre, M. Cardon, 'Sequana': a Set of Snakemake NGS pipelines. *The Journal of Open Source Software* **2**, 352 (2017).
29. S. Andrews, FastQC: a quality control tool for high throughput sequence data. (2010).
30. M. Martin, Cutadapt removes adapter sequences from high-throughput sequencing reads kenkyuhi hojokin gan rinsho kenkyu jigyo. *EMBnet.journal* **17**, pp. 10-12 (2013).
31. A. Dobin *et al.*, STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21 (2013).
32. D. R. Zerbino *et al.*, Ensembl 2018. *Nucleic Acids Research* **46**, D754-D761 (2018).
33. Y. Liao, G. K. Smyth, W. Shi, FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923-930 (2014).
34. R Core Team. *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.*
35. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **15**, 1-21 (2014).
36. K. Strimmer, fdrtool: a versatile R package for estimating local and tail area-based false discovery rates. *Bioinformatics* **24**, 1461–1462 (2008).
37. Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological)* **57**, 289-300 (1995).

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Author contribution: JB, DAD, TH, XM, TF, ML and OS: designed experimental strategy. JB, QN, DAD, FP, MA and KR: Designed and performed experiments with cell lines. SAD and TF designed and performed experiments with primary trophoblasts. JB, TC, CM, QN, OD and XM designed and performed mouse experiments. EP and KHH performed the bioinformatic analysis. AD and TH provided vital materials and expert advice. JB and OS wrote the manuscript. All authors agreed on the final version.

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Supplementary Materials

Materials and Methods

Fig S1 – S9

References (22 – 37)

Movie S1

Figure Legends

Figure 1. IFITMs inhibit Syncytin-mediated cell fusion. (A) Left: 293T-GFP1-10 and -GFP11 cells were co-cultured at a 1:1 ratio and co-transfected with Syncytin-1 and IFITM or control plasmids. Cell fusion was quantified by measuring the GFP+ area at 18h. Middle: Representative image. White lines display the GFP area. Right: Quantification of GFP areas. Results are mean±sd from 4 independent experiments. Bar: 200 µm. **(B)** Left: BeWo β-Gal-α and -ω were transduced with IFITM or control vectors. Cells were co-cultured at a 1:1 ratio and fusion was induced by Forskolin for 48h. Right: Fusion index (β-Gal activity) measured with a colorimetric (CPRG) assay. Results are mean±sd from 4 independent experiments. **(C)** Left: WT or IfitmDel MEFs were co-transfected with GFP and Syncytin-A plasmids. Syncytia were quantified after 24h. Right: Quantification of syncytia (cells with >3 nuclei) per well. Results are mean±sd from 3-6 independent experiments. Statistical analysis: One-Way ANOVA, ns: non-significant, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Figure 2. IFN-β inhibits fusion in primary human villous cytotrophoblasts (VCTs). (A) Quantification of fusion. Mononucleated VCTs express the nuclear GATA3 protein, downregulated after fusion. After 48h, nuclei are visualised by DAPI and plasma membranes with E-Cadherin. Representative images are shown. Syncytia, defined as large cells containing multiple GATA3-negative nuclei, have been delimited in white. Bar: 50 µm. **(B)** Fusion index was quantified and calculated as (100 − % (number of GATA3+ nuclei/total number of DAPI nuclei) **(C)** hCG levels in culture supernatants at 72h. Results are mean±sd from 8 independent experiments. Statistical analysis: One-Way ANOVA, $****p < 0.0001$.

Figure 3. IFITMs are key mediators of IFN induced fetal demise in mice. (A) Gestating dams were injected intra-peritoneally with Poly-IC or PBS at E7.5. Number, size and resorption of the

embryos were assessed at E9.5. Number of litters: WT PBS: 3, WT Poly-IC: 6, Ifnar-/- PBS: 3, Ifnar-/- Poly-IC: 3, IfitmDel PBS: 3, IfitmDel Poly-IC: 7. **(B)** Percentage of resorption for the indicated conditions. Numbers in brackets indicate the number of resorptions/total number of feto-placental units. Statistical analysis: Mann-Whitney, ns: non-significant, ****p < 0.0001. **(C)** Representative images of E9.5 embryos. Bar: 500 µm. **(D)** WT and IfitmDel placentas (E9.5) stained for E-cadherin (red), and Hoechst (blue). White arrows indicate ST. Representative images from 3 independent experiments are shown. Bar: 50 µm.