

Andreas Ian Lackner, Jürgen Pollheimer, Paulina Latos, Martin Knöfler and Sandra Haider*

Gene-network based analysis of human placental trophoblast subtypes identifies critical genes as potential targets of therapeutic drugs

<https://doi.org/10.1515/jib-2023-0011>

Received April 20, 2023; accepted November 7, 2023; published online December 22, 2023

Abstract: During early pregnancy, extravillous trophoblasts (EVTs) play a crucial role in modifying the maternal uterine environment. Failures in EVT lineage formation and differentiation can lead to pregnancy complications such as preeclampsia, fetal growth restriction, and pregnancy loss. Despite recent advances, our knowledge on molecular and external factors that control and affect EVT development remains incomplete. Using trophoblast organoid *in vitro* models, we recently discovered that coordinated manipulation of the transforming growth factor beta (TGF β) signaling is essential for EVT development. To further investigate gene networks involved in EVT function and development, we performed weighted gene co-expression network analysis (WGCNA) on our RNA-Seq data. We identified 10 modules with a median module membership of over 0.8 and sizes ranging from 1005 (M1) to 72 (M27) network genes associated with TGF β activation status or *in vitro* culturing, the latter being indicative for yet undiscovered factors that shape the EVT phenotypes. Lastly, we hypothesized that certain therapeutic drugs might unintentionally interfere with placentation by affecting EVT-specific gene expression. We used the STRING database to map correlations and the Drug-Gene Interaction database to identify drug targets. Our comprehensive dataset of drug-gene interactions provides insights into potential risks associated with certain drugs in early gestation.

Keywords: WGCNA; placental development; drug-gene interaction

1 Introduction

The human placenta is a unique transiently existing organ that acts as lungs, liver, gut, kidney, and endocrine glands for the developing fetus, supplying oxygen and nutrients, and eliminating waste products [1, 2]. During early gestation, epithelial villous cytotrophoblasts give rise to a layer of multinucleated hormone-producing syncytiotrophoblasts (STBs), and migratory extravillous trophoblasts (EVTs) (Figure 1A). EVT's invade the maternal decidua, the superficial uterine mucosal layer, transforming maternal arteries into wide-lumen, low-pressure vessels to ensure a smooth and adequate supply of maternal blood [3, 4]. Additionally, EVT's reshape the maternal immune system to ensure the acceptance of the fetal semi-allograft [5]. Failures in EVT differentiation are noticed in pregnancy complications such as preeclampsia, fetal growth restriction, and early pregnancy loss

*Corresponding author: Sandra Haider, Department of Obstetrics and Gynecology, Reproductive Biology Unit, Medical University of Vienna, Waehringer Guertel 18–20, 1090 Vienna, Austria, E-mail: sandra.haider@meduniwien.ac.at, <https://orcid.org/0000-0003-2409-1317>

Andreas Ian Lackner and Jürgen Pollheimer, Department of Obstetrics and Gynecology, Maternal-Fetal Immunology Group, Medical University of Vienna, Vienna, Austria

Paulina Latos, Center for Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria

Martin Knöfler, Department of Obstetrics and Gynecology, Reproductive Biology Unit, Medical University of Vienna, Vienna, Austria

 Open Access. © 2023 the author(s), published by De Gruyter.  This work is licensed under the Creative Commons Attribution 4.0 International License.

exposing the mother and the baby to risk for immediate life-threatening conditions during pregnancy and severe complications later in life [6–10]. EVT differentiation is initiated by the development of so-called villous cell columns that attach to the maternal uterus (decidua) (Figure 1A). Before EVT detach from the column to penetrate the decidua, these cells undergo various stages of differentiation to form a highly invasive phenotype.

In this context, transforming growth factor beta signaling (TGF β) was identified as one of the key regulators orchestrating EVT maturation [11, 12]. Our recent studies on trophoblast organoids (TO) demonstrated that EVTs lacking TGF β signaling develop into hyper-invasive, spindle-shaped cells, while TGF β -activated EVTs acquire a less-migratory, secretory phenotype, more closely mimicking the *in vivo* placental EVT (pEVT) phenotype. Sequential activation of TGF β signalling of TO-EVT is essential to reproduce correct EVT differentiation *in vitro* (Figure 1A) [13].

Transcriptional profiling is a powerful tool for identifying physiological and pathological tissue gene signatures and their regulatory mechanisms. While the DESeq2 workflow for analysis of bulk RNA sequencing data focuses on differentially expressed genes, the Weighted Gene Co-expression Network Analysis (WGCNA, version 1.71) algorithm identifies clusters of genes with similar expression patterns and reveals related biological functions [14]. To date, WGCNA has been employed for identifying gene co-expression networks in physiological processes such as lactation [14], in various cancers to determine therapeutic targets and biomarkers [15–18], and for investigating transcriptional regulators including micro and long non-coding RNAs [19, 20]. To gain more insights into the processes orchestrating EVT maturation, we employed WGCNA with our recent RNA-Seq data, where we compared isolated EVTs from first-trimester placental tissue (pEVTs) to EVTs derived from trophoblast organoids (TO-EVTs) from the same donors [13]. Our first aim was to decipher the complex gene networks involved in TGF β -dependent EVT maturation and identify any remaining differences between *in vivo* EVTs and *in vitro* TO-EVTs. Our second aim was to investigate external factors that could affect adequate EVT differentiation and placentation in early pregnancy. Only about 30 % of all human pregnancies result in a live birth. Besides known factors such as genetic aberration, infections, and immune rejection, about half of the patients will remain without a diagnosis [21]. A possible explanation might be external factors such as pharmaceutical drugs that unintentionally target critical EVT-specific genes in early weeks of gestation, thereby jeopardizing adequate placentation. We queried the STRING database to map gene interactions and the Drug-Gene Interaction database to identify potential drug targets. These findings provide a basis for high-throughput testing to identify possible cross-reactions of pharmaceuticals that could harm placentation in early pregnancy.

2 Materials and methods

2.1 Data collection

We performed bioinformatic analysis on RNA-Seq data sets ($n = 16$) from EVTs isolated either from first-trimester placental tissue (pEVTs) or derived from corresponding trophoblast organoids (TO), encompassing three distinct populations (Figure 1A and B) [13]: (1) *in vivo* placental extravillous trophoblasts (EVTs) comprising both TGF β -inactive and TGF β -active EVTs (pEVT^{on}, $n = 4$) due to a TGF β -activating *in vivo* environment, (2) *in vitro* trophoblast organoid (TO)-derived EVTs that were inhibited for TGF β signalling (TO-EVT^{off}, $n = 8$), and (3) *in vitro*-derived TO-derived EVTs, exposed to TGF β -activating conditions comprising both TGF β -inactive and TGF β -active TO-derived EVTs (TO-EVT^{on}, $n = 4$).

2.2 Weighted gene co-expression network analyses (WGCNA)

We used our recent RNA-Seq data sets from pEVT^{on}, TO-EVT^{off}, and TO-EVT^{on} [13] to run WGCNA with parameters used in Mohr et al. [22]. After creating a DESeq2 (version 1.34.0) [23] object, we filtered out genes with very low counts (<10) in over 10 % of samples and performed variance stabilizing transformation, followed by batch correction using the limma package [24]. Next, we created an eSet object with feature data described in Figure 1 and applied additional filtering using two-Gaussian filtering. To improve the filtering process, we calculated a threshold using the package mixtools (version 2.0.0) [25]. We then removed outliers, defined by an absolute z -normalized inter-sample connectivity (zK) higher than 1.96, as recommended by Oldham et al. [26].

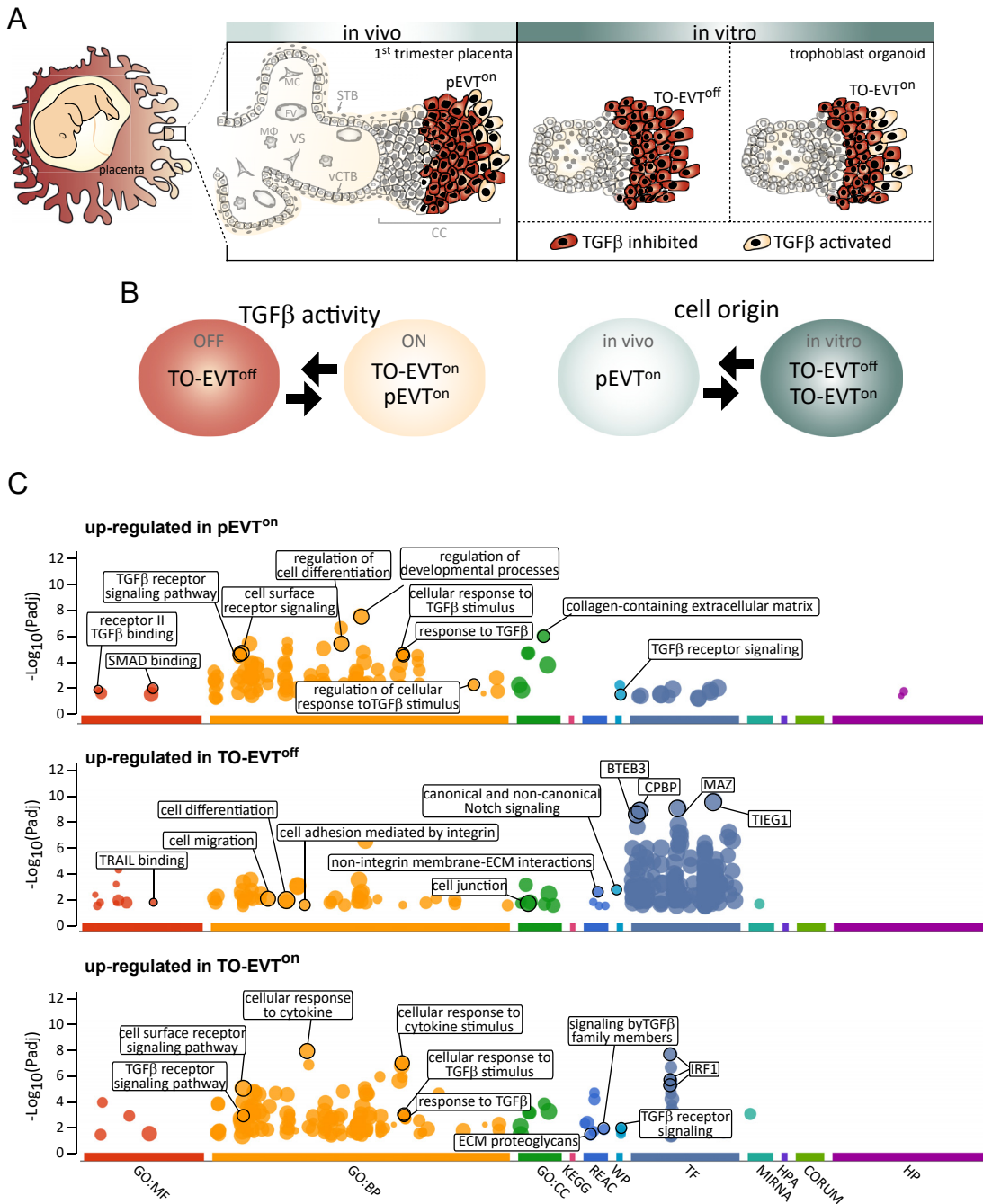


Figure 1: Maturation of in vitro-derived TO-EVT requires TGF β activation. (A) Illustration of placental EVT (pEVT) and trophoblast organoid EVT (TO-EVT), marked in red. Villous cytotrophoblasts (vCTB) proliferate to form a cell column (CC) and differentiate into pEVT^{on}. TO-EVT are differentiated under TGF β -inhibited (TO-EVT^{off}) and TGF β -activated (TO-EVT^{on}) conditions. Please note that only a subset of distally located EVTs display activated TGF β signaling (beige). (B) EVT populations for establishing TGF β - and cell origin-dependent WGCNA gene networks. (C) Manhattan plots depicting the g:Profiler results of enriched pathways in pEVT^{on}, TO-EVT^{off}, and TO-EVT^{on}. Circle size correlates with the size of the enriched pathway while the y-axis depicts the adjusted p -value as a negative decadic logarithm. Pathways of interest are encircled and named. VS, villous stroma; MC, mesenchymal cell; MØ, macrophage; vCTB, villous cytotrophoblasts; STB, syncytiotrophoblast; CC, cell column; pEVT, placental extravillous trophoblast; TO-EVT, trophoblast organoid-derived EVT; GO:MF, gene ontology: molecular function; GP:BP, gene ontology: biological process; GO:CC, gene ontology: cellular component; KEGG, Kyoto encyclopedia of genes and genomes; REAC, reactome; WP, WikiPathways; TF, transcription factor; MIRNA, MicroRNA; HPA, human protein atlas; CORUM, comprehensive resource of mammalian protein complexes; HP, human phenotype.

The WGCNA parameters included a “signed” network type, β : 12, deepsplit: 2, correlation: “bicor”, and pamStage: TRUE. Modules were calculated and genes with the absolute correlation between genes and module eigengenes below 0.8 were pruned until the network stabilized. We pruned genes with an absolute correlation between genes and module eigengenes below 0.8 until the network stabilized. To verify the association of calculated modules with phenotype information, we employed a mixed-effect model and calculated t -tests as described by Li et al. [27]. We calculated a normalized intramodular connectivity (k_{Within}), which is the module connectivity divided by the maximal connectivity as shown in the following formula, with α as a value for the adjacency in the co-expression network:

$$k_{\text{Within}} \cdot \text{norm}_i = \frac{\sum_{i \in \text{module}} \alpha_i}{\max \left(\sum_{i \in \text{module}} \alpha_i \right)}$$

We identified hub genes by selecting the top 10 % of genes based on their k_{Within} values for each module. We then used the STRING database (version 2.4.2) to map gene interactions and the Drug-Gene Interaction database to identify potential drug targets [28–31].

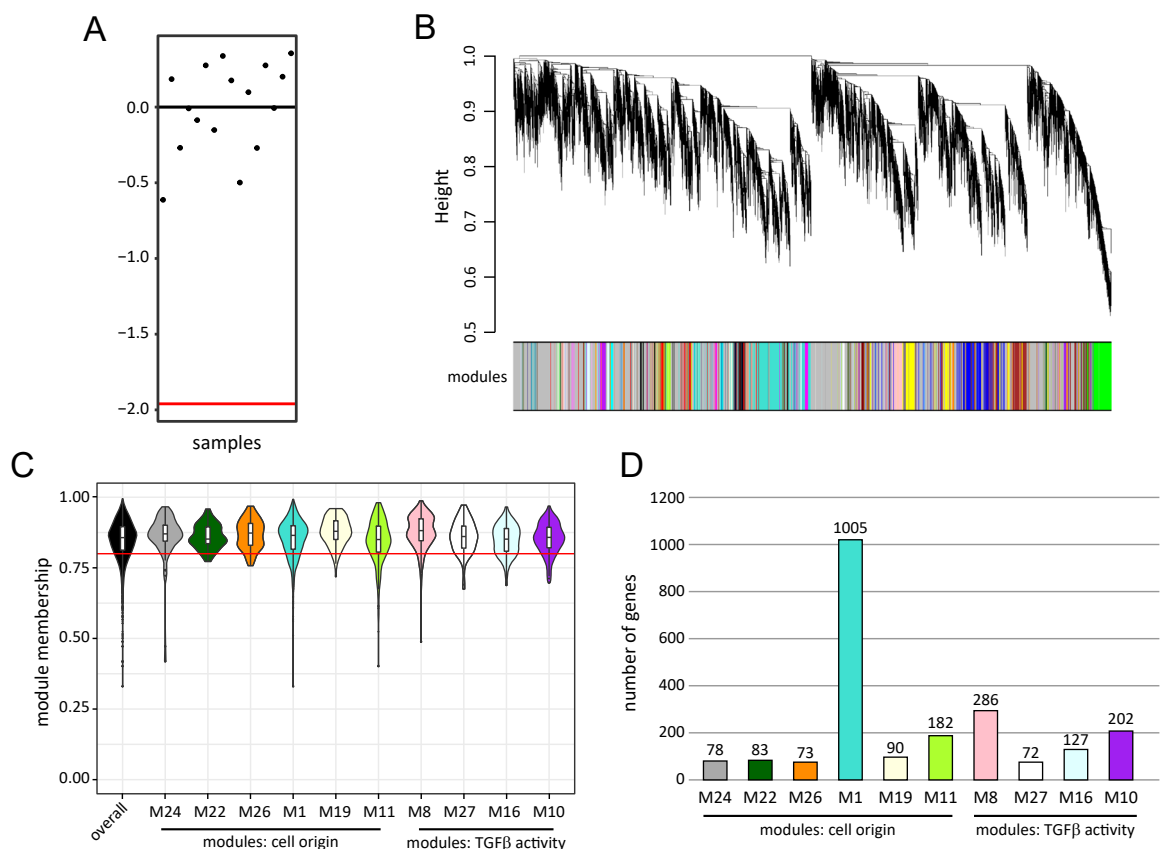


Figure 2: WGCNA identifies 10 modules in EVT populations. (A) z-normalized intersample connectivity (zKconnectivity). The red line denotes a zKconnectivity of -1.96 . (B) Dendrogram showing the euclidian distance between all detected modules. Each branch depicts a gene assigned to the respective modules. Grey areas represent genes not assigned to any module. (C) Violin plots depicting the membership score of selected modules: module membership for the entire network (overall), and for the cell origin modules M24, M22, M26, M1, M19, M11, and for TGF β modules M8, M27, M16, M10. The red line at 0.8 indicates the defined cut-off for module selection. (D) Bar graph displaying the number of genes within the modules.

3 Results and discussion

3.1 g:Profiler identified enriched biological pathways in EVT populations

To understand the differences between EVT subsets, we conducted g:Profiler analyses and created Manhattan plots to visualize functional enrichment across multiple categories, such as molecular function, biological process, cellular compartment, and human phenotype ontology (Figure 1C). To this end, we studied two stages of EVT differentiation in TO-EVTs, including TGF β -inhibited (TO-EVT^{off}) and TGF β -activated (TO-EVT^{on}), and compared those with isolated, primary pEVTs (pEVT^{on}). We found that the TO-EVT^{off} subset showed enrichment of “canonical and non-canonical Notch signaling”. During EVT formation, trophoblasts undergo certain steps of differentiation orchestrated by various signaling pathways. Among these active NOTCH1 signaling was demonstrated as a prerequisite for initiating and stabilizing EVT formation [32]. Hence, the up-regulation of Notch signaling in TO-EVT^{off} might indicate an accumulation of an immature EVT phenotype. Additionally, functional enrichment analysis revealed potentially increased migratory properties in TO-EVT^{off}, with pathways related to cell migration and adhesion being upregulated. These results align with our previous findings that inhibition of TGF β prompts an invasive, less mature EVT phenotype [13]. When comparing pEVT^{on} and TO-EVT^{on}, we observed a significant overlap in upregulated pathways related to TGF β signalling, confirming that pEVT^{on} exhibit a TGF β signature and that TO-EVT require TGF β activation to resemble *in vivo* pEVT^{on}.

3.2 Weighted gene co-expression network analysis (WGCNA) of EVT RNA-Seq data

To explore the relationships between different gene sets (modules), we used WGCNA to analyse the RNA-Seq data of pEVT^{on}, TO-EVT^{off}, and TO-EVT^{on}. We also looked for significant differences between female and male samples. First, we checked that all samples had a zKconnectivity above -1.96 (Figure 2A). We then excluded genes with low expression levels across all samples, resulting in a co-expression network of 7743 genes. Using an iterative approach by Mohr et al. [26], we identified 50 modules (M) containing between 1005 (M1) and 33 (M50) genes (Figure 2B). All modules and assigned genes are listed in the Supplementary Table 1. We calculated associations between pEVT^{on} and TO-EVT^{on} from female and male donors, and the module eigengenes (Supplementary Figure 1). Surprisingly, in this study, no modules exhibited statistically significant variations between samples differentiated by fetal sex. However, potential differences in module expression relative to fetal sex may be characterized by a small effect size. Taken this into account, the sample size of the current investigation may be too small to detect such nuanced alterations. This underscores the necessity for subsequent investigations with augmented sample sizes enhancing statistical power to elucidate a potential influence of fetal sex on the modules examined herein. Hence, we did not investigate sex-specific gene expression networks further. However, we identified 10 modules (M1, M8, M10, M11, M16, M19, M22, M24, M26, and M27) that were associated with *TGF β activity* and *cell origin*, and we selected these for further analysis (Figure 2C and D). These modules had a median module membership of above 0.8, indicating that the gene assignments were stable.

3.2.1 TGF β -dependent eigengene modules

We identified four gene modules (M8, M10, M16, and M27) associated with TGF β activity, consisting of 286, 202, 127, and 72 network genes, respectively (Figure 3, Supplementary Figure 2). Modules M8 and M27 showed increased eigengene expression in response to TGF β signalling, while M10 and M16 exhibited reduced eigengene expression. Notably, we did not find significant differences between pEVT^{on} and TO-EVT^{on}. Within the module network genes of M8 and M27, we identified several well-known TGF β signalling-associated genes expressed in EVT [13] including SKI, SKIL, NUAK1, FN1, HPGD, ESAM, KRT7, LITAF, ID1, and CLIC4 [33–42]. In contrast, the network

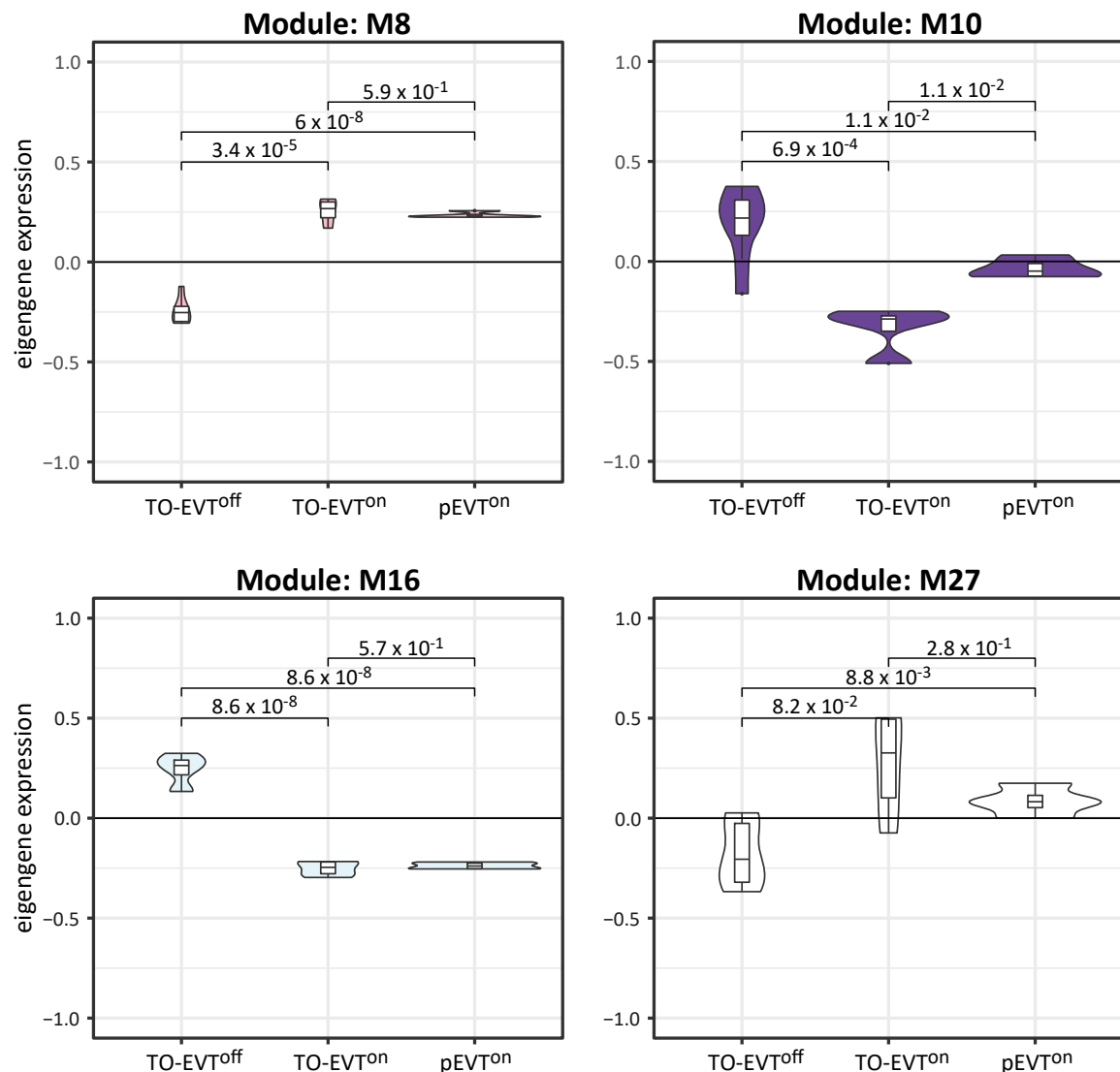


Figure 3: Eigengene plots of TGF β -associated modules. Plots are colored according to the module color. P -values between pEVT^{on}, TO-EVT^{off}, and TO-EVT^{on} are depicted in the plots.

genes of M10 and M16 were enriched in genes associated with an immature EVT phenotype, including PEG10, FZD6, FREM1, CDK2, CDH5, ITGA6, ITGB4, and COMT [32, 43–47]. These findings reasserted that *in vitro* activation of the TGF β pathway promotes a pEVT^{on}-like gene signature.

3.2.2 Cell origin-dependent eigengene modules

Although activation of TGF β signalling led to a significant convergence of TO-EVT^{on} genotypes to their *in vivo* counterparts, our WGCNA analyses revealed six modules that showed expressional differences between *in vivo* pEVTs and *in vitro*-derived TO-EVTs (Figure 4, Supplementary Figure 3). In these modules no significant differences were found between TO-EVT^{off} and TO-EVT^{on}. We observed downregulated eigengene expressions in M1 (1005 network genes), M11 (182 network genes), and M19 (90 network genes) in pEVT^{on}, while M22 (83 network genes), M24 (78 network genes), and M26 (73 network genes) showed increased eigengene expressions in pEVT^{on}. These results suggest that the current TO-EVT culture conditions do not fully replicate pEVT^{on}

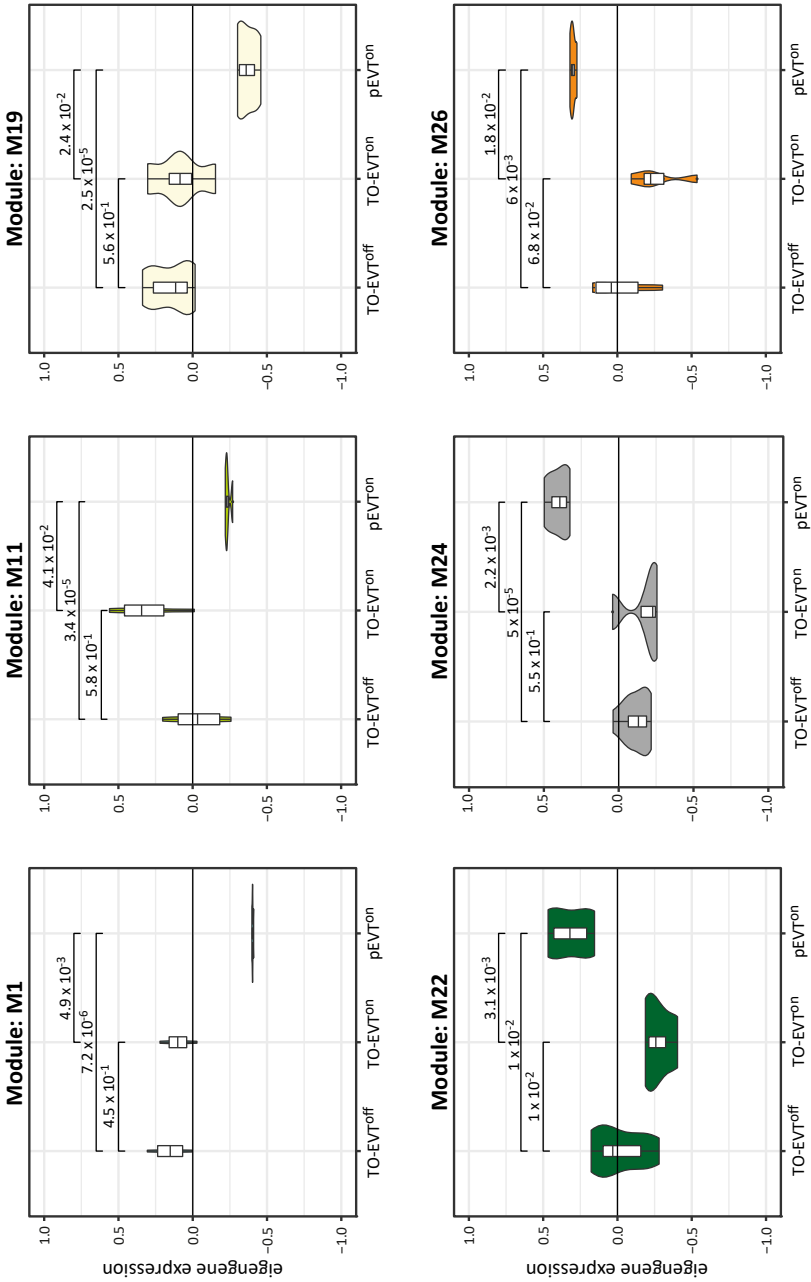


Figure 4: Eigengene plots of cell origin-associated modules. Plots are colored according to the module color. P -values between pEVT^{on}, TO-EVT^{off}, and TO-EVT^{on} are depicted in the plots.

maturation, and our module-based data might provide insights for identifying the complete spectrum of factors that shape pEVT^{on} genotypes in future studies.

3.3 Module network genes are targeted by pharmaceutical drugs

Our final aim was to investigate the susceptibility of EVT-specific genes to pharmaceutical drugs. We analysed the network genes of all modules (M1 – M50) using the Drug-Gene Interaction and STRING database to identify genes that can be targeted by drugs (Supplementary Table 2). Our findings revealed that numerous EVT-related genes can be affected by various drugs, such as inhibitors, modulators, agonists, inducers, antibodies, antagonists, suppressors, antisense oligonucleotides, or interacting molecules. The complexity of potential interactions between module network genes and drugs is illustrated in Figure 5 (M1, M11, M19, M22, M24, and M26) and Supplementary Figure 4 (M8, M10, M16, and M27). We further identified important drug targets from EVT network genes across the detected modules, including ITGB1 (M4, Supplementary Table 2), and HMGCR (M1, Figure 5), which have been associated with important EVT functions.

Over 800 distinct drugs have been identified to potentially interact with nuclear factor erythroid 2-like BZIP transcription factor 2 (*NFE2L2*, M1, Figure 5 and Supplementary Table 2). In human placentas, NRF2 (*NFE2L2*) has been detected in extravillous trophoblasts, with increased protein levels observed in endovascular and interstitial trophoblasts from placentas affected by fetal growth restriction and preeclampsia [48]. NRF2 plays

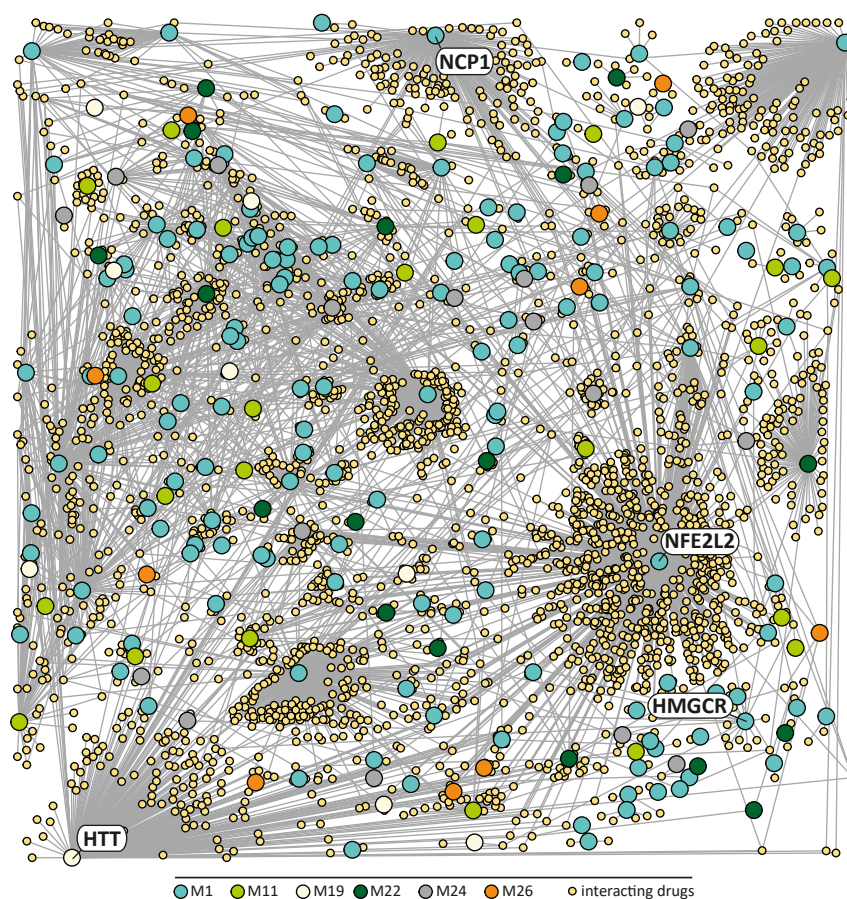


Figure 5: Drug-gene interaction plot for cell origin-associated modules. Network plot depicting module network genes and interacting drugs. Module network genes are colored according to the module color. *Niemann-Pick-C1* (NCP1), *nuclear factor erythroid 2 Like BZIP transcription factor 2* (NFE2L2), *3-Hydroxy-3-Methylglutrylo-CoA Red* (HMGCR), and *huntingtin* (HTT) are depicted in the interaction plot.

a crucial role in the VEGF-NRF2-mediated oxidative stress response by regulating the expression of protective genes, such as heme oxygenase-1 and glutathione peroxidase [49]. As early placentation primarily occurs under hypoxic conditions, defence against reactive oxygen species becomes essential from the 9th/10th week onwards, when maternal blood begins to flow into the intervillous space [50]. In summary, drug-induced alterations in NRF2 levels may pose an unidentified risk for the basal defence mechanisms against oxidative stress during early placentation.

During pregnancy, Niemann-Pick-C1 (*NPC1*, M1, Figure 5, Supplementary Table 2) participates in the uptake of cholesterol, a vital nutrient necessary for fetal growth [51]. Although current knowledge regarding NPC1 function in placental physiology is limited, sequencing data reveal high RNA expression levels of *NPC1* in trophoblast subtypes, suggesting a role in the transport of cholesterol to the foetus during human pregnancy [13, 52]. Consequently, impaired NPC1 expression could negatively impact fetal nutrition throughout gestation.

Nearly 200 drugs potentially target huntingtin (*HTT*, M19, Figure 5, Supplementary Table 2), a gene originally identified in the context of Huntington's disease and involved in various cellular processes such as organelle transport, spindle pole assembly during mitosis, and protein transport [53]. Gene expression profiles reveal high expression of *HTT* in trophoblast subtypes, particularly in extravillous trophoblasts [13, 52]. While the functions of *HTT* in human trophoblasts have not yet been demonstrated, mice lacking huntingtin exhibited impaired trophoblast giant cell differentiation [54]. These findings suggest that drug-induced alterations in *HTT* expression could disrupt trophoblast differentiation, which is essential for optimal maternal adaptations during pregnancy.

Integrin beta 1 (*ITGB1*, M4, Supplementary Table 2) is expressed at high levels in endovascular extravillous trophoblasts (EVTs) deeply embedded within maternal decidual tissues [55, 56]. This increased expression is believed to be necessary for the trophoblasts to withstand and migrate against maternal blood flow [56]. Our Drug-Gene Interaction database query identified eight pharmaceuticals that have the potential to target *ITGB1*, including antagonists (e.g., Finategrast, Volociximab, Intetumumab), inhibitors (e.g., Abituzumab, Natalizumab), and antibodies (e.g., Etaracizumab). Finategrast, for example, is a small-molecule antagonist of the integrin $\alpha 4 \beta 1$, which is an integrin dimer composed of *ITGA4* and *ITGB1* and is used to reduce trafficking of lymphocytes into the central nervous system for the treatment of multiple sclerosis [57]. Through its antagonistic effect on *ITGB1*, it could interfere with the establishment of the maternal-fetal interface and compromise fetal growth.

One interesting pharmacological target that has come to our attention is the interleukin-1 receptor type 1 (*IL1R1*; M8, Supplementary Figure 4, Supplementary Table 2). Evidence suggests that *IL1R1* is upregulated during trophoblast differentiation and exhibits high expression levels in extravillous trophoblasts (EVTs). Our prior laboratory experiments have demonstrated that trophoblast motility increases upon stimulation with one of its ligands, IL-1 β . Additionally, we observed that IL-1 β stimulation induces the expression of urokinase plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1), and PAI-2 in trophoblast cells. These findings suggest that the *IL1R1* signalling pathway and the subsequent activation of the plasminogen system may be crucial for ensuring proper decidual invasion [58]. Anakinra, a recombinant form of IL-1Ra, directly binds to and blocks *IL1R1*. This compound is utilized to treat pro-inflammatory states in various diseases, including familial Mediterranean fever, rheumatoid arthritis, and cryopyrin-associated periodic syndromes. However, data regarding the use of anakinra during pregnancy are limited. Consequently, the European Medicines Agency recommends avoiding its use in pregnant patients and patients with childbearing potential who are not using contraception. In conclusion, potential effects Anakinra on EVT invasion may have significant implications for the establishment of the maternal-fetal interface [59].

We have identified 3-Hydroxy-3-Methylglutaryl-CoA Reductase (*HMGCR*, M1, Figure 5, Supplementary Table 2) as a target for 19 different drugs, primarily statins, which inhibit cholesterol synthesis. Only recently EVTs were shown to exhibit increased levels of free and esterified cholesterol, the precursors of steroid hormones such as progesterone [60]. Placental progesterone expression is considered a key factor supporting pregnancy maintenance in the first weeks of gestation [61]. Hence, reduced progesterone expression caused

unintentionally by statins may pose a severe risk for successful pregnancy outcomes. Our findings align with a recent meta-analysis, which found higher rates of spontaneous abortions in patients exposed to statins [62].

4 Conclusions

In this study, we conducted a comprehensive bioinformatic analysis based on gene expression profiles from *in vivo* EVT and *in vitro* TO-derived EVT from first-trimester placental tissue. Using WGCNA, we identified expected TGF β -dependent gene networks confirming our previous studies where we demonstrated the importance of the signalling pathway in EVT maturation [13]. However, we also detected large gene networks specific to the cells' origin still pointing out differences between *in vivo* and *in vitro*-derived EVTs despite TGF β activation. These findings indicate that while activating TGF β signalling in TO-EVT largely resembles pEVT gene expression profiles, further studies are required to better mimic *in vivo* EVT phenotypes. Surprisingly, we found that fetal sex did not result in sex-specific gene modules. Our drug-gene interaction analysis identified several EVT-specific genes as potential drug targets, including *ITGB1*, *RXRA*, and *HMGCR*. Compromised placentation provoked by drug side effects might be one explanation for idiopathic early pregnancy loss. Consequently, there is an urgent need for data collection and further investigation of unintended pharmaceutical effects counteracting EVT development and differentiation. We propose using TO-EVT cultures as a highly reliable, animal-free model for testing pharmaceutical effects on EVT formation and function to protect and support early pregnancy.

Acknowledgment: The authors would like to thank Thomas Mohr of the Institute of Cancer Research, Medical University of Vienna, Austria, for supplying his original scripts and helping with the analysis.

Research ethics: Analyses were performed from placental tissue-derived data collected elsewhere (doi: 10.1073/pnas.2120667119). Utilization and analyses were approved by the Medical University of Vienna ethics board (no. 084/2009).

Author contributions: A.I.L. performed the analyses. J.P., P.L., M.K., and S.H. designed the study. S.H. performed the figures. A.I.L. and S.H. wrote the manuscript.

Competing interests: The authors state no conflict of interest.

Research funding: Austrian Science Funds: P-34588 and P-36159, both assigned to S.H.

Data availability: The raw data can be obtained on request from the corresponding author.

References

1. Burton GJ, Fowden AL. The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci* 2015;370:20140066.
2. Staud F, Karahoda R. Trophoblast: the central unit of fetal growth, protection and programming. *Int J Biochem Cell Biol* 2018;105:35–40.
3. Huppertz B. Traditional and new routes of trophoblast invasion and their implications for pregnancy diseases. *IJMS* 2019;21:289.
4. Harris LK. Review: trophoblast-vascular cell interactions in early pregnancy: how to remodel a vessel. *Placenta* 2010;31:S93–8.
5. Vondra S, Höbner AL, Lackner AI, Raffetseder J, Mihalic ZN, Vogel A, et al. The human placenta shapes the phenotype of decidual macrophages. *Cell Rep* 2023;42:111977.
6. Khong TY, Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *BJOG An Int J Obstet Gynaecol* 1986;93:1049–59.
7. Romero R, Kusanovic JP, Chaiworapongsa T, Hassan SS. Placental bed disorders in preterm labor, preterm PROM, spontaneous abortion and abruptio placentae. *Best Pract Res Clin Obstet Gynaecol* 2011;25:313–27.
8. Hustin J, Jauniaux E, Schaaps JP. Histological study of the materno-embryonic interface in spontaneous abortion. *Placenta* 1990;11:477–86.
9. Benschop L, Duvekot JJ, van Lennep JER. Future risk of cardiovascular disease risk factors and events in women after a hypertensive disorder of pregnancy. *Heart* 2019;105:1273–8.
10. Cheng SB, Sharma S. Preeclampsia and health risks later in life: an immunological link. *Semin Immunopathol* 2016;38:699–708.
11. Haider S, Kunihs V, Fiala C, Pollheimer J, Knöfler M. Expression pattern and phosphorylation status of smad2/3 in different subtypes of human first trimester trophoblast. *Placenta* 2017;57:17–25.

12. Li Y, Yan J, Chang HM, Chen ZJ, Leung PCK. Roles of TGF- β superfamily proteins in extravillous trophoblast invasion. *Trends Endocrinol Metabol* 2021;32:170–89.
13. Haider S, Lackner AI, Dietrich B, Kunihs V, Haslinger P, Meinhardt G, et al. Transforming growth factor- β signaling governs the differentiation program of extravillous trophoblasts in the developing human placenta. *Proc Natl Acad Sci USA* 2022;119:e2120667119.
14. Farhadian M, Rafat SA, Panahi B, Mayack C. Weighted gene co-expression network analysis identifies modules and functionally enriched pathways in the lactation process. *Sci Rep* 2021;11:2367.
15. Bian Y, Huang J, Zeng Z, Yao H, Tu J, Wang B, et al. Construction of survival-related co-expression modules and identification of potential prognostic biomarkers of osteosarcoma using WGCNA. *Ann Transl Med* 2022;10:296.
16. Di Y, Chen D, Yu W, Yan L. Bladder cancer stage-associated hub genes revealed by WGCNA co-expression network analysis. *Hereditas* 2019;156:7.
17. Xu L, Xiao T, Xu L, Yao W. Identification of therapeutic targets and prognostic biomarkers in cholangiocarcinoma via WGCNA. *Front Oncol* 2022;12:977992.
18. Yin X, Wang P, Yang T, Li G, Teng X, Huang W, et al. Identification of key modules and genes associated with breast cancer prognosis using WGCNA and ceRNA network analysis. *Aging* 2021;13:2519–38.
19. Wani N, Barh D, Raza K. Modular network inference between miRNA–mRNA expression profiles using weighted co-expression network analysis. *J Integr Bioinform* 2021;18:20210029.
20. Wang Q, Roy B, Dwivedi Y. Co-expression network modeling identifies key long non-coding RNA and mRNA modules in altering molecular phenotype to develop stress-induced depression in rats. *Transl Psychiatry* 2019;9:125.
21. Macklon NS. Conception to ongoing pregnancy: the “black box” of early pregnancy loss. *Hum Reprod Update* 2002;8:333–43.
22. Mohr T, Katz S, Paulitschke V, Aizarani N, Tolios A. Systematic analysis of the transcriptome profiles and co-expression networks of tumour endothelial cells identifies several tumour-associated modules and potential therapeutic targets in hepatocellular carcinoma. *Cancers* 2021;13:1768.
23. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* 2014;15:550.
24. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res* 2015;43:e47–7.
25. Benaglia T, Chauveau D, Hunter DR, Young D. Mixtools: an R package for analyzing finite mixture models. *J Stat Software* 2009;32:1–29.
26. Oldham MC, Langfelder P, Horvath S. Network methods for describing sample relationships in genomic datasets: application to huntington’s disease. *BMC Syst Biol* 2012;6:63.
27. Li J, Zhou D, Qiu W, Shi Y, Yang JJ, Chen S, et al. Application of weighted gene co-expression network analysis for data from paired design. *Sci Rep* 2018;8:622.
28. Freshour SL, Kiwala S, Cotto KC, Coffman AC, McMichael JF, Song JJ, et al. Integration of the drug–gene interaction database (DGIdb 4.0) with open crowdsourcing efforts. *Nucleic Acids Res* 2021;49:D1144–51.
29. Cotto KC, Wagner AH, Feng YY, Kiwala S, Coffman AC, Spies G, et al. DGIdb 3.0: a redesign and expansion of the drug–gene interaction database. *Nucleic Acids Res* 2018;46:D1068–73.
30. Griffith M, Griffith OL, Coffman AC, Weible JV, McMichael JF, Spies NC, et al. DGIdb: mining the druggable genome. *Nat Methods* 2013;10:1209–10.
31. Wagner AH, Coffman AC, Ainscough BJ, Spies NC, Skidmore ZL, Campbell KM, et al. DGIdb 2.0: mining clinically relevant drug–gene interactions. *Nucleic Acids Res* 2016;44:D1036–44.
32. Haider S, Meinhardt G, Saleh L, Fiala C, Pollheimer J, Knöfler M. Notch1 controls development of the extravillous trophoblast lineage in the human placenta. *Proc Natl Acad Sci USA* 2016;113:E7710–9.
33. Tecalco-Cruz AC, Ríos-López DG, Vázquez-Victorio G, Rosales-Alvarez RE, Macías-Silva M. Transcriptional cofactors Ski and SnoN are major regulators of the TGF- β /Smad signaling pathway in health and disease. *Signal Transduct Targeted Ther* 2018;3:15.
34. Tecalco-Cruz AC, Sosa-Garrocho M, Vázquez-Victorio G, Ortiz-García L, Domínguez-Hüttlinger E, Macías-Silva M. Transforming growth factor- β /SMAD target gene SKIL is negatively regulated by the transcriptional cofactor complex SNON-SMAD4. *J Biol Chem* 2012;287:26764–76.
35. Zhang T, He X, Caldwell L, Goru SK, Severino LU, Tolosa MF, et al. NIAK1 promotes organ fibrosis via YAP and TGF- β /SMAD signaling. *Sci Transl Med* 2022;14:ea4028.
36. Igotz RA, Massagué J. Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. *J Biol Chem* 1986;261:4337–45.
37. Yan M, Rerko RM, Platzer P, Dawson D, Willis J, Tong M, et al. 15-Hydroxyprostaglandin dehydrogenase, a COX-2 oncogene antagonist, is a TGF- β -induced suppressor of human gastrointestinal cancers. *Proc Natl Acad Sci USA* 2004;101:17468–73.
38. Shingai Y, Yokota T, Okuzaki D, Sudo T, Ishibashi T, Doi Y, et al. Autonomous TGF β signaling induces phenotypic variation in human acute myeloid leukemia. *Stem Cell* 2021;39:723–36.
39. Fowler EW, Venrooy EV, Witt RL, Jia X. TGF β R inhibition represses TGF- β 1 initiated keratin-7 expression in human salivary gland progenitor cells. *Bioengineering* 2021;1–37. <https://doi.org/10.1101/2021.10.08.463706>.

40. Meyer C, Liu Y, Kaul A, Peipe I, Dooley S. Caveolin-1 abrogates TGF- β mediated hepatocyte apoptosis. *Cell Death Dis* 2013;4:e466–6.
41. Liang YY, Brunicardi FC, Lin X. Smad3 mediates immediate early induction of id1 by TGF- β . *Cell Res* 2009;19:140–8.
42. Shukla A, Malik M, Cataisson C, Ho Y, Friesen T, Suh KS, et al. TGF- β signalling is regulated by schnurri-2-dependent nuclear translocation of CLIC4 and consequent stabilization of phospho-smad2 and 3. *Nat Cell Biol* 2009;11:777–84.
43. Apps R, Sharkey A, Gardner L, Male V, Trotter M, Miller N, et al. Genome-wide expression profile of first trimester villous and extravillous human trophoblast cells. *Placenta* 2011;32:33–43.
44. Dietrich B, Haider S, Meinhardt G, Pollheimer J, Knöfler M. WNT and NOTCH signaling in human trophoblast development and differentiation. *Cell Mol Life Sci* 2022;79:292.
45. Olvera M, Harris S, Amezcua CA, McCourty A, Rezak S, Koo C, et al. Immunohistochemical expression of cell cycle proteins E2F-1, Cdk-2, Cyclin E, P27kip1, and Ki-67 in normal placenta and gestational trophoblastic disease. *Mod Pathol* 2001;14:1036–42.
46. Aplin JD. Expression of integrin A β 4 in human trophoblast and its loss from extravillous cells. *Placenta* 1993;14:203–15.
47. Kanasaki K, Palmsten K, Sugimoto H, Ahmad S, Hamano Y, Xie L, et al. Deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol is associated with pre-eclampsia. *Nature* 2008;453:1117–21.
48. Kweider N, Huppertz B, Wruck CJ, Beckmann R, Rath W, Pufe T, et al. A role for Nrf2 in redox signalling of the invasive extravillous trophoblast in severe early onset IUGR associated with preeclampsia. *PLoS One* 2012;7:e47055.
49. Lee JM, Johnson JA. An important role of nrf2-ARE pathway in the cellular defense mechanism. *BMB Rep* 2004;37:139–43.
50. Burton GJ, Cindrova-Davies T, Yung HW, Jauniaux E. Hypoxia and reproductive health: oxygen and development of the human placenta. *Reproduction* 2021;161:F53–65.
51. Kallol S, Albrecht C. Materno-fetal cholesterol transport during pregnancy. *Biochem Soc Trans* 2020;48:775–86.
52. Vento-Tormo R, Efremova M, Botting RA, Turco MY, Vento-Tormo M, Meyer KB, et al. Single-cell reconstruction of the early maternal–fetal interface in humans. *Nature* 2018;563:347–53.
53. Saudou F, Humbert S. The biology of huntingtin. *Neuron* 2016;89:910–26.
54. Seong IS, Woda JM, Song JJ, Lloret A, Abeyrathne PD, Woo CJ, et al. Huntingtin facilitates polycomb repressive complex 2. *Hum Mol Genet* 2010;19:573–83.
55. Moser G, Drewlo S, Huppertz B, Armant DR. Trophoblast retrieval and isolation from the cervix: origins of cervical trophoblasts and their potential value for risk assessment of ongoing pregnancies. *Hum Reprod Update* 2018;24:484–96.
56. Thirkill TL, Hendren SR, Soghomonians A, Mariano NF, Barakat AI, Douglas GC. Regulation of trophoblast beta1-integrin expression by contact with endothelial cells. *Cell Commun Signal* 2004;2:4.
57. Miller DH, Weber T, Grove R, Wardell C, Horrigan J, Graff O, et al. Fingertag for relapsing remitting multiple sclerosis: a phase 2, randomised, double-blind, placebo-controlled trial. *Lancet Neurol* 2012;11:131–9.
58. Prutsch N, Fock V, Haslinger P, Haider S, Fiala C, Pollheimer J, et al. The role of interleukin-1 β in human trophoblast motility. *Placenta* 2012;33:696–703.
59. Kineret. EPAR — product information; 2018. Available from: https://www.ema.europa.eu/en/documents/product-information/kineret-epar-product-information_en.pdf.
60. Vondra S, Kunihs V, Eberhart T, Eigner K, Bauer R, Haslinger P, et al. Metabolism of cholesterol and progesterone is differentially regulated in primary trophoblastic subtypes and might be disturbed in recurrent miscarriages. *Journal of Lipid Research* 2019;60:1922–34.
61. Ewington LJ, Tewary S, Brosens JJ. New insights into the mechanisms underlying recurrent pregnancy loss: RPL revisited. *J Obstet Gynaecol Res* 2019;45:258–65.
62. Vahedian-Azimi A, Bianconi V, Makvandi S, Banach M, Mohammadi SM, Pirro M, et al. A systematic review and meta-analysis on the effects of statins on pregnancy outcomes. *Atherosclerosis* 2021;336:1–11.

Supplementary Material: This article contains supplementary material (<https://doi.org/10.1515/jib-2023-0011>).