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Defining trophoblast injury patterns in the transcriptomes of dysfunctional placentas

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Abstract

Trophoblast injury is central to clinically relevant placenta dysfunction. We hypothesized that the mRNA of primary human trophoblasts, exposed to distinct injuries *in vitro*, capture transcriptome patterns of placental biopsies obtained from common obstetrical syndromes. We deployed a CIBERSORTx deconvolution method to correlate trophoblastic RNAseq-based expression matrices with the transcriptome of omics-defined placental dysfunction patterns *in vivo*. We found distinct trophoblast injury patterns in placental biopsies from women with fetal growth restriction and a hypertensive disorder, or in biopsies clustered by their omics analysis. Our RNAseq data are useful for defining the contribution of trophoblast injuries to placental dysfunction syndromes.

Keywords

Placenta; trophoblast; apoptosis; ferroptosis; CIBERSORTx

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Declaration of Interest Statement

Statement: The authors certify that there are no financial interests that influence the results and findings obtained through this study. As stated on the title page, Yoel Sadovsky is a consultant to Bio-Rad Laboratories, Inc.

Introduction

Placental dysfunction is a leading cause of common obstetrical syndromes, such as preeclampsia (PE), fetal growth restriction (FGR), and preterm birth (PTB). Such dysfunction impacts villous trophoblasts, which govern placental gas and nutrient exchange, hormone production, immune and mechanical defense [1]. Whereas trophoblast hypoxia is typical early in pregnancy [2], fluctuating or persistent hypoxia later in pregnancy has been implicated in placental pathophysiology [2-4]. Further, inadequate trophoblast differentiation, apoptosis, and ferroptosis, are detectable in trophoblasts from placentas of women diagnosed with PE, FGR, or spontaneous PTB [5-9].

Cultured primary human trophoblasts (PHT cells) are commonly used for studying placental cell injury [10, 11]. Considering the heterogeneity of obstetrical syndromes, defining placental injury-related molecular patterns may provide insight into the underlying pathologies [12 , 13]. Moreover, our recent study implied that multiomics-based placental clusters (including transcriptomics, proteomics, and metabolomics) faithfully recapitulate histopathological changes [14]. We therefore hypothesized that mRNA from PHT cells, exposed to distinct injuries *in vitro*, would capture transcriptome patterns of placental biopsies obtained from common obstetrical syndromes.

Methods

All placentas used for PHT cell isolation in our studies were obtained from uncomplicated term pregnancies and deliveries at Magee-Womens Hospital in Pittsburgh, under an approved protocol (#19120076, University of Pittsburgh). The PHT cells were dispersed and cultured for three days as we previously detailed [15, 16]. The timeline of exposure to altered O_2 concentration or to ligands is shown in Fig. 1A. Exposure included Hams/Waymouth's medium (H/W 50/50, Thermo Fisher, Waltham, MA), staurosporine (100 nM, Cell Signaling, Danvers, MA), or RSL3 (200 nM, Selleck Chemicals, Houston, TX) [8, 13, 16-19]. RNA extraction, library preparation, and sequencing processes were performed as we described [13]. Our RNAseq analysis of placental biopsies was recently detailed [14]. RNA count data were transformed to approximate log scale normal distribution through variance-stabilizing transformation using DESeq2 (v1.36.0), conditioned on trophoblast injury [20, 21].

To correlate injury in PHT cells with placental tissue, we used CIBERSORTx [22] for bulk RNAseq deconvolution (Fig. S1). A signature matrix was constructed to represent unique gene expression patterns for each trophoblast injury. We performed differential expression analysis, and the first 194 differentially expressed genes (DEG) for each comparison, optimized on the basis of the most robust estimation of PHT injury signatures, were used to construct a signature matrix. The resulting matrix was used to estimate cell type proportions from the bulk placental RNAseq samples. We previously used a similarity network fusion (SNF) method to interrogate multiomics-based analysis of placental biopsies from different diseases [14].

Statistics and data availability

Differential expression analysis was performed with the lmerSeq package [23], using a linear mixed model. False discovery rate (FDR) was controlled using the Benjamini-Hochberg method. Differential expression with respect to SNF clusters was analyzed with the DESeq2 package [20], with FDR control as above. The significance of associations of trophoblast injury signatures, estimated by CIBERSORTx with clinical diagnoses or SNF clusters was assessed using the Kruskal-Wallis test and Dunn's *post hoc* test. The family-wise error rate was controlled using the Holm-Bonferroni method. Our RNAseq data from PHT cells have been deposited in the NCBI Sequence Read Archive, Bio Project ID PRJNA995610.

Results and Discussion

To identify injury patterns in PHT cells, we cultured them in six conditions, including standard conditions in 21% O_2 and 8% O_2 as controls, 0% O_2, H/W medium for hindering differentiation, staurosporine for induction of apoptosis, and RSL3 for ferroptosis [8, 17, 18, 24, 25]. Key DEG comparisons are shown in Table S1 and in Fig. S2.

We constructed a signature matrix to capture unique gene expression patterns that characterize each PHT injury (Table S2 and Fig. S1). Applying CIBERSORTx to the mRNA data from the six clinically defined placental biopsy (n=271) groups, the 21% O_2 signature accounted for 55-60% of the transcripts across all disease paradigms (Fig. 1B and Table S3). The ferroptosis signature was particularly prominent in the FGR+HDP group. In contrast, placentas from pregnancies with isolated FGR correlated with apoptotic signature, consistent with previous results of higher apoptotic signaling in FGR placentas [26].

Our previous investigation harnessed SNF to reclassify the six clinically defined placental groups into four multiomics-defined clusters (Fig. 1A). The SNF clusters better correlated with the histopathology and were more distinctive of placental injuries [14]. We therefore compared the PHT injury signatures to placental biopsies that were analyzed using the unsupervised, multiomics-based SNF method (Fig. 2 and Table S4) and performed Bayesian model selection to compare the association of gene expression signatures in the six PHT culture conditions with clinical diagnoses vs. with SNF clusters. The Bayesian Information Criterion score was used to evaluate the posterior likelihood for each multiple linear regression model under a uniform prior. PHT injury signatures were better explained by the SNF clusters than by the clinical diagnoses (Bayes factor: 1.07×10^{31}). Notably, the differentiation-impaired (H/W) signature was highest in cluster II and lowest in Cluster III, effectively differentiating these from other clusters. Ferroptosis was prominent in cluster III, and 0% O_2 mainly differentiated clusters I and IV from clusters II and III.

Our study was based on PHT cells, cultured under various conditions. Naturally, the *in vitro* settings may not fully capture the placental environment *in vivo*. Moreover, while single-cell RNAseq could have been used to define cell injury in the placental biopsies, we relied on integrated multiomics data, which remain challenging at the single cell level. Nevertheless, our work represents a stride toward a comprehensive understanding

of placental pathophysiology, providing insights into the contribution of PHT injuries to placental syndromes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- We compared RNAseq matrices in cultured trophoblasts and in placental samples.
- CIBERSORTx deconvolution method correlated *in vitro* and *in vivo* injury patterns.
- Correlations were strongest in preeclampsia and fetal growth restriction clusters.

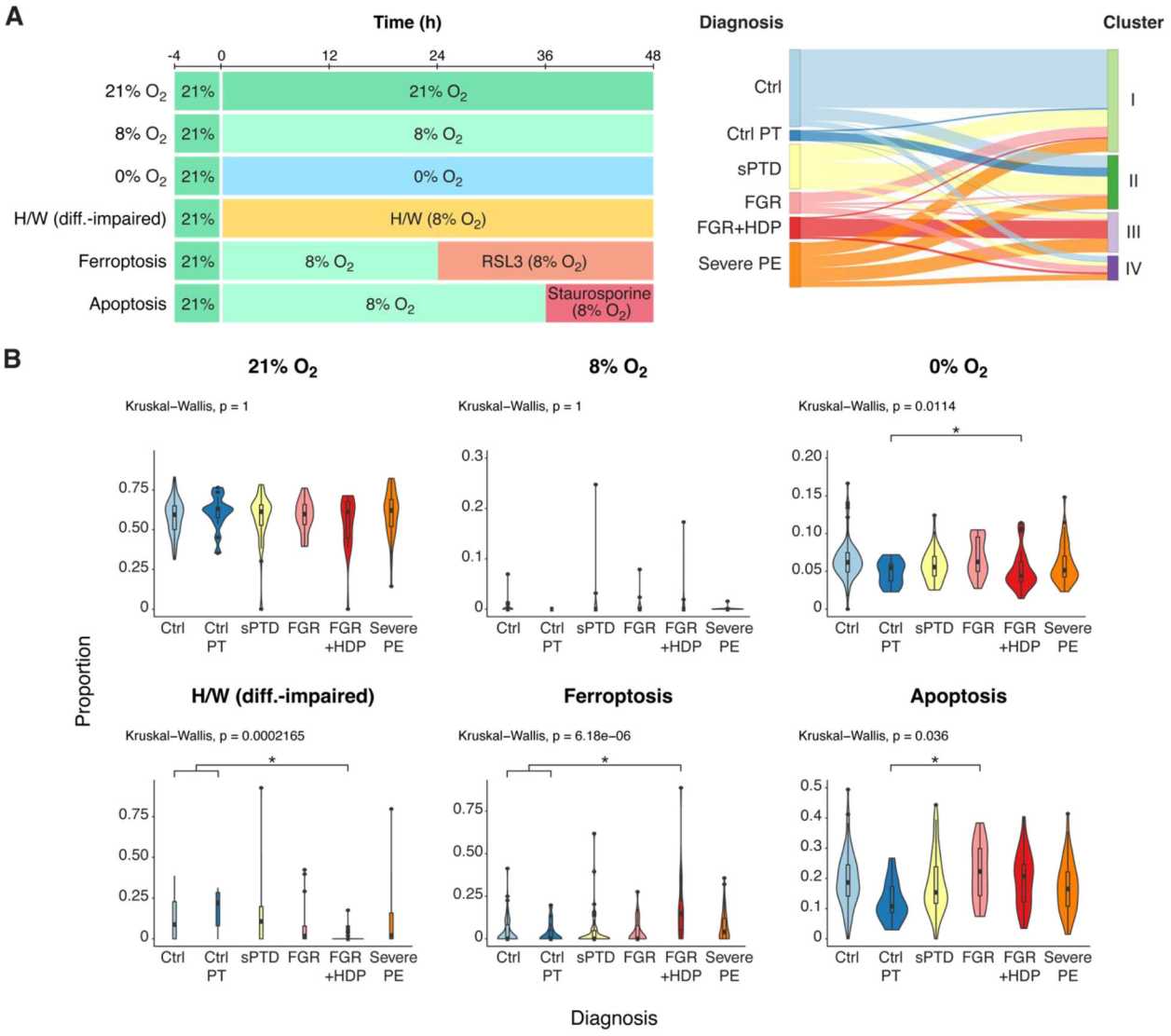

Figure 1. The effect of culture conditions on the representation of PHT cell transcripts among relevant clinical conditions .

(A) An overview of the datasets and experiments used in this study. Left panel: an illustration of the experimental design for PHT cell exposure. PHT cells, isolated from placentas of five uncomplicated term pregnancies, were initially cultured in 20% O_2 for 4 h and subsequently exposed to varying oxygen conditions—8% O_2, 20% O_2, hypoxia at near 0% O_2, or to H/W (differentiation-impaired) medium—for 48 h. Cells were exposed to RSL3 induction of ferroptosis (24 h) or staurosporine induction of apoptosis (12 h). At 48 h the cells were harvested for total RNA sequencing. Right panel: a Sankey plot depicting the allocation of placental biopsies from six clinical conditions into four molecular-based clusters (Barak et al, ref. 14), created on the basis of transcriptomic, proteomic, and metabolomic analyses, with Similarity Network Fusion analysis applied to identify clusters (Ctrl, Control; Control-PT, control preterm; sPTD, spontaneous preterm delivery; FGR, fetal growth restriction; FGR+HDP, fetal growth restriction with hypertensive disorder of pregnancy; PE, preeclampsia. (B) A violin plot depicting the effect of culture conditions

on the representation of PHT cell transcripts among relevant clinical conditions. The x-axis defines the clinical diagnosis, and the y-axis delineates the proportion of transcripts defining each condition. P-values were calculated by the nonparametric Kruskal-Wallis test, with FDR controlling for multiple comparisons using the Benjamini-Hochberg method. For significant variables the Dunn's post hoc test was performed. Ctrl, control; Control-PT, control preterm; sPTD, spontaneous preterm delivery; FGR, fetal growth restriction; FGR+HDP, fetal growth restriction with hypertensive disorder of pregnancy; PE, preeclampsia.

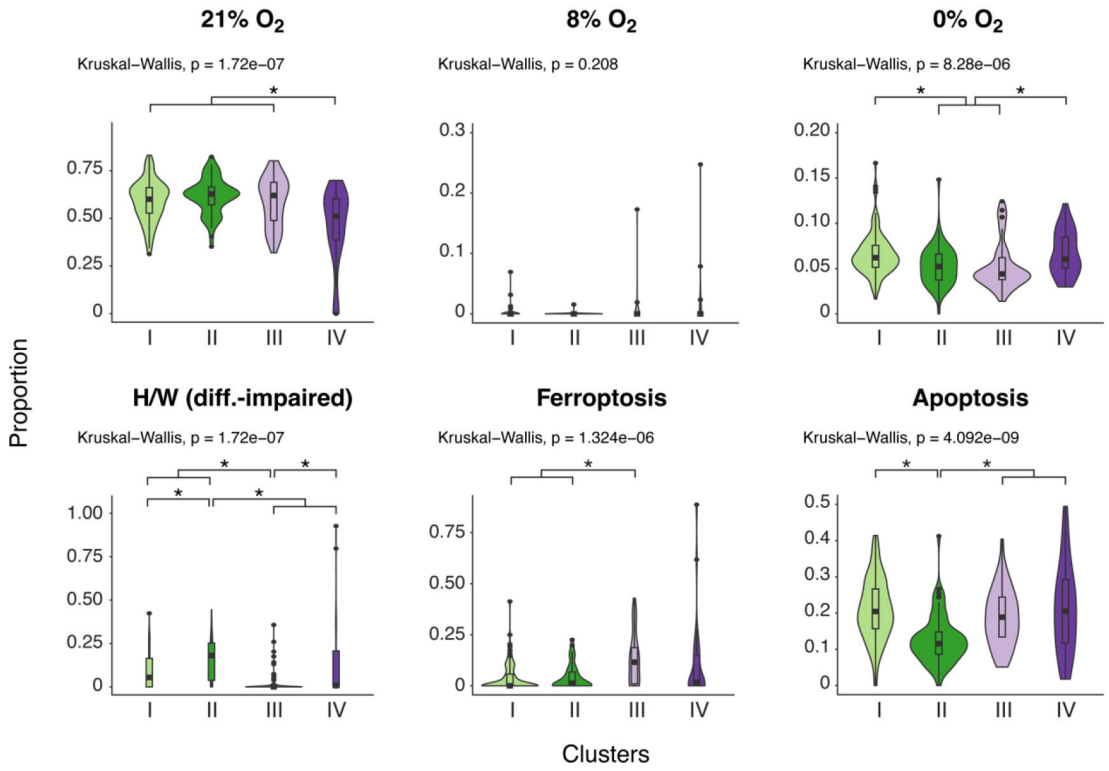

Figure 2. A violin plot depicting the effect of culture conditions on the representation of PHT cell transcript SNF clusters.

The x-axis defines the SNF clusters, and the y-axis delineates the proportion of transcripts defining each cluster. P-values were calculated by the nonparametric Kruskal-Wallis test, with FDR controlling for multiple comparisons using the Benjamini-Hochberg method. For significant variables, Dunn's post hoc test was performed.