The trichloroethylene metabolite *S*-(1,2-dichlorovinyl)-L-cysteine stimulates metabolomic changes in human placental trophoblast BeWo cells undergoing syncytialization

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INTRODUCTION

- In placenta, cytotrophoblasts fuse to form multinucleated syncytiotrophoblasts in a process known as **syncytialization**.[1]
- Syncytiotrophoblasts cover the placenta villi and are in contact with maternal blood to provide the maternal-fetal interface.[1]
- The human placental trophoblast cell line, **BeWo**, is widely used as an *in vitro* model of syncytialization.[1]
- **Forskolin**, an adenylate cyclase activator that increases intracellular cAMP, stimulates BeWo cells to syncytialize.[1]
- Trichloroethylene (TCE) exposure has been associated with adverse pregnancy outcomes in women, including decreased fetal weight and small for gestational age.[2-4]
- The TCE metabolite *S*-(1,2-dichlorovinyl)-L-cysteine (DCVC) stimulates reactive oxygen species generation, pro-inflammatory response, apoptosis, and energy utilization in placental cells.[5-6]
- In the differentiating slime mold model of cellular multinucleation, amino acid supply decreases and carbohydrates accumulate, highlighting the importance of energy utilization during cellular multinucleation.[7-8]
- Preeclampsia is a hypertensive disorder of pregnancy characterized by changes in energy utilization and oxidative stress.[9-10]

OBJECTIVE

We examined DCVC-stimulated effects on metabolites in BeWo cells during the process of syncytialization. The targeted metabolomics platform used at the University of Michigan (UM) Metabolomics Core for this study was the tricarboxylic acid (TCA) plus platform.

METHODS

Experimental Design

Seed BeWo cells at 800,000 cells/dish

24 hour pretreatment

Treat cells per following:

	Treatment group			
Chemical	1	2	3	4
Forskolin (μM)	0	100	100	100
DCVC (μM)	0	0	10	20

Syncytialization

DCVC effect during syncytialization

48 hour treatment

Remove media, wash cells, and freeze cells at -80 °C

*The vehicle control (treatment group 1) received 0.1% v/v DMSO

Targeted metabolomics performed on cell samples at the UM Metabolomics Core

Tricarboxylic acid (TCA) Plus Assay

Analyzed for 54 metabolites by electrospray ionization (ESI) on a liquid chromatography quadrupole time-of-flight (LC-QTOF) mass spectrometer.

Data processing: LOESS analysis and normalization to protein (pmol/μg protein), or LOESS analysis (relative response).

RESULTS

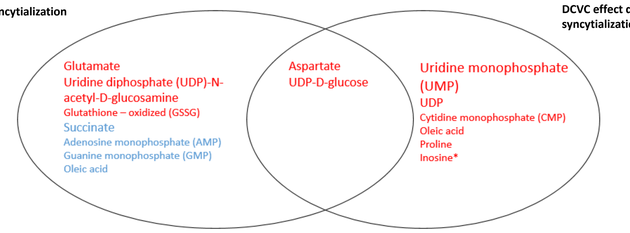

Figure 1: Comparison of metabolites altered by syncytialization alone and DCVC during syncytialization. Metabolites highlighted in blue or red indicate metabolites increased or decreased, respectively. The metabolites in smallest, moderate, and largest font sizes correspond to significant differences: p<0.05, p<0.01, and p<0.001, respectively. A student's unpaired two-tailed t-test was performed for the syncytialization-specific analysis whereas a one-way ANOVA was performed for the DCVC effect analysis. All processed data were generalized log-transformed prior to statistical analysis. N=5 experiments.

Pathway Analysis

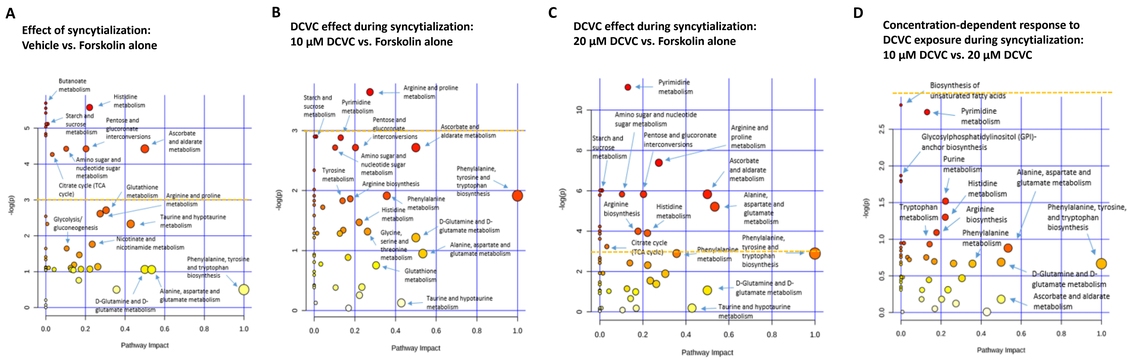

Figure 2: Pathway analysis to identify metabolic pathways most affected by syncytialization and DCVC during syncytialization. Comparisons for A-D are described above each respective graph. Pathway analysis (specific for *Homo Sapiens*) was performed by Metaboanalyst 4.0 on the data (generalized log-transformed). The circle color corresponds to significance of the pathway (white to red in order of increasing significance) whereas circle size corresponds to pathway impact, which is calculated as the matched metabolites as a cumulative percentage contributing to total pathway importance. The gold dashed line depicts –log(p)=3, which equals p=0.05 under the $\log_e$ (or ln) scale used in this analysis.

Purine metabolism pathway

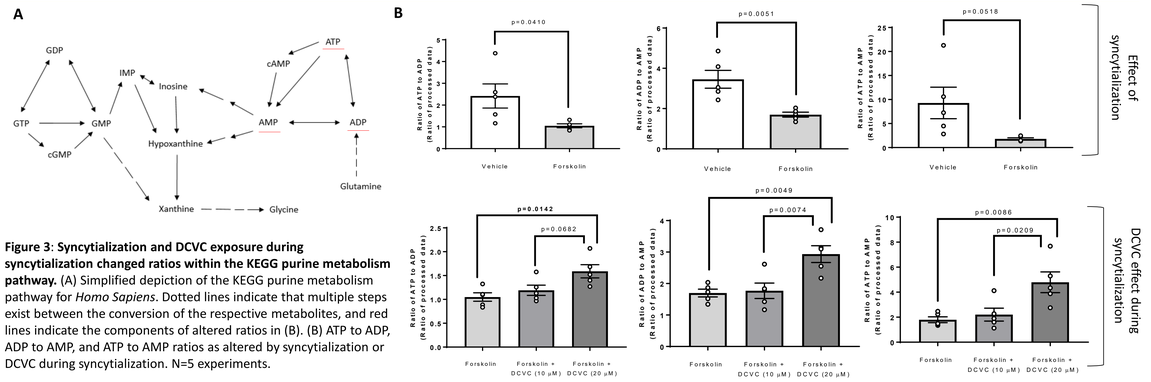

Figure 3: Syncytialization and DCVC exposure during syncytialization changed ratios within the KEGG purine metabolism pathway. (A) Simplified depiction of the KEGG purine metabolism pathway for *Homo Sapiens*. Dotted lines indicate that multiple steps exist between the conversion of the respective metabolites, and red lines indicate the components of altered ratios in (B). (B) ATP to ADP, ADP to AMP, and ATP to AMP ratios as altered by syncytialization or DCVC during syncytialization. N=5 experiments.

DISCUSSION

- Forskolin induction of syncytialization produced numerous changes in energy metabolism.
- DCVC exposure during forskolin-stimulated syncytialization modified energy metabolism distinct from changes associated with syncytialization.
- Changes in critical metabolites and pathways are relevant to adverse pregnancy outcomes.

Metabolite or Pathway Altered	Syncytialization effect	DCVC effect during syncytialization	Biological Significance	Relevance to adverse pregnancy outcomes (previous studies)
Oleic acid	Increased	Decreased	Nonessential monounsaturated fatty acid	Negatively correlated to birthweight and gestational age; predicts preterm delivery.[11]
Aspartate	Decreased	Decreased	Nonessential acidic amino acid	Decreased in early-onset preeclampsia.[9] Normally preferentially locates in the placenta.[12]
Histidine metabolism	P<0.01	P<0.05	Metabolism of histidine involving glutamate and aspartate as breakdown products	Decreased excretion of histidine in toxic pregnancies (e.g., pregnancy characterized by preeclampsia or hyperemesis gravidarum).[13]

- Forskolin-stimulated syncytialization increased AMP at the expense of ADP and ATP.
- DCVC exposure during syncytialization increased ATP at the expense of ADP and AMP compared to syncytialized cells not exposed to DCVC (forskolin alone treatment).

CONCLUSIONS

- Syncytialization in BeWo cells is accompanied by changes in energy metabolism, similar to observations in other systems (e.g., slime mold).
- We are the first to show DCVC to decrease energy supply metabolites during syncytialization in placental cells.
- Future research is warranted to fully understand the initiators and mechanisms between DCVC exposure and adverse pregnancy outcomes.

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