Effects of Inorganic Arsenic on the Epithelial-Mesenchymal Transition, Migration, and Invasion of Placental Cells

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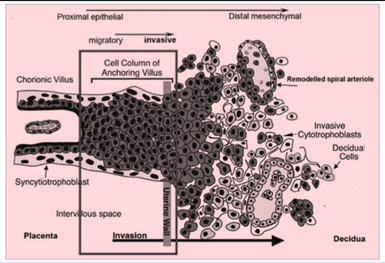
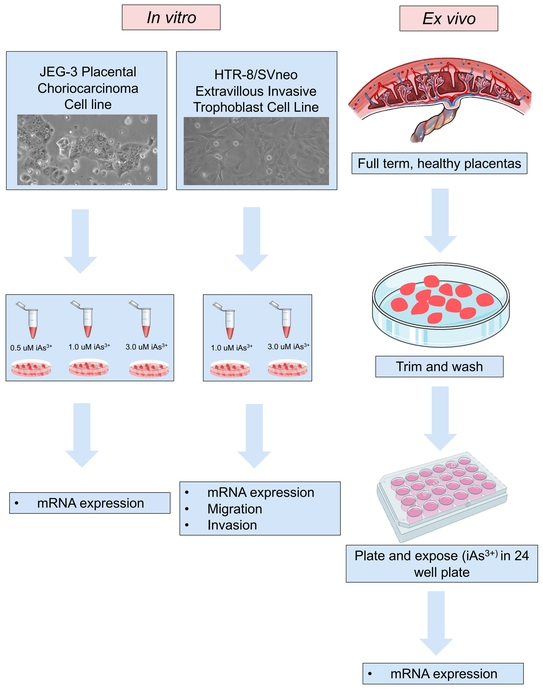
Background



(Laine et al., 2016)

The Process of Placentation
During placentation, a blood supply between the mother and fetus is established in the first trimester. Extravillous invasive trophoblasts (EVT) undergo an epithelial-mesenchymal transition (EMT) and invade the decidualized endometrium and remodel the arterial wall of the spiral arteries to allow blood flow. Shallow EVT invasion is characteristic of pre-eclampsia and fetal growth restriction, increased invasion is associated with placenta accreta, and uncontrolled invasion by EVT is associated with choriocarcinoma.

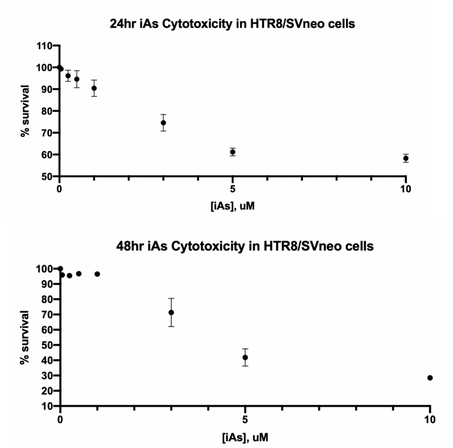
Experimental Design



Hypothesis and Objective

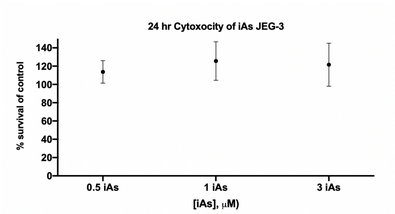
- **Hypothesis**: iAs has been shown to induce EMT and increase migration and invasion in cancer cell lines. Due to this, iAs may induce the EMT of placental trophoblasts and increase migration and invasion of EVTs.
- **Objective:** Measure changes in migration, invasion, and gene expression of EMT markers in placental cells after treatment with iAs.

Cytotoxicity: HTR-8/SVneo



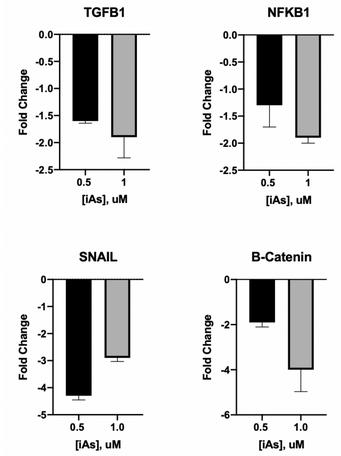
Resazurin Cytotoxicity Assays. Cytotoxicity of iAs in HTR-8/SVneo cells was assessed at 24 and 48 hours after treatment. 80% cell survival was used as the cutoff for cytotoxicity. Concentrations used were 0.05 uM, 0.25uM, 0.5 uM, 1.0uM, 3.0 uM, 5.0 uM, and 10 uM. Cytotoxic doses in this cell line were found to be those above 1.0 uM.

Cytotoxicity: JEG-3



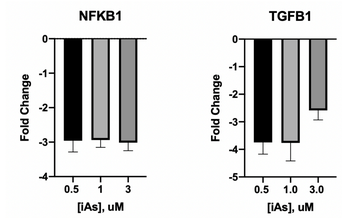
Resazurin Cytotoxicity Assays. Cytotoxicity of iAs in JEG-3 cells was assessed at 24 hours after treatment. 80% cell survival was used as the cutoff for cytotoxicity. Survival is reported relative to the control. Concentrations used were 0.5 uM, 1.0 uM and 3.0 uM. All doses tested were found to be non-cytotoxic.

Gene Expression: HTR-8/SVneo



RT-qPCR. Fold changes of differentially expressed genes after treatment with iAs in HTR-8/SVneo cells ($p<0.05$). TGFB1 inhibits trophoblast invasion when upregulated but induces EMT(Lash et al., 2005; Xu et al., 2009). NFKB1 is elevated in preeclamptic placentas, which characteristically have reduced invasion of trophoblast cells but induces EMT (Vaughan et al., 2012; Huber et al., 2004). SNAIL is a classic marker of EMT and downregulates E-cadherin and activates B-catenin. (Laine et al., 2016)

Gene Expression: JEG-3



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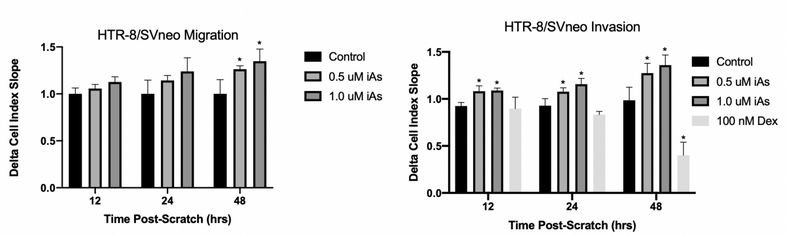
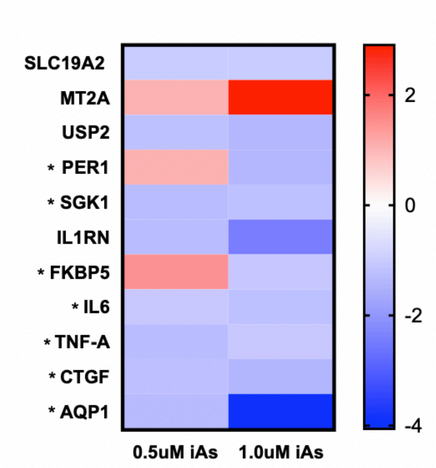
Migration & Invasion



ACEA Cell Invasion and Migration Assay. Migration and invasion of HTR-8/SVneo cells in response to iAs treatment. iAs increases migration in a time and dose dependent manner, with statistical significance 48 hours after treatment. iAs increases invasion to a greater extent than migration in a time and dose dependent manner, with statistical significance 12, 24, and 48 hours after treatment. Dex decreased invasion at 24 and 48 hours after treatment Findings are similar to studies of inorganic arsenic and glucorticoid treatment on the invasion and migration of cancer cell lines.

Gene Expression: *Ex Vivo*



Fluidigm. Fold changes in gene expression of 13 statistically significant genes ($p < 0.05$) in placental explants treated with 0.5 uM and 3uM of iAs. These were corrected for sex and FDR. * marks genes that have been associated with EMT in studies of other cell types. Increased expression of PER1 is associated with inhibition of EMT (Han et al., 2017). Increased expression of SGK1, FKBP5, TL6, TNF, CTGF, and AQP1 is associated with induction of EMT (Liu et al., 2018; Rotoli et al., 2016; Zhou et al., 2017; Li et al., 2012; Sonnylal et al., 2013; Yun et al., 2016).

Conclusions

- iAs increases the migration and invasion of HTR-8/SVneo cells in a time and dose-dependent manner
- iAs-induced increased migration and invasion may be mediated through the glucorticoid receptor (GR) pathway
- Placental gene expression of EMT-associated genes *in vitro* and *ex vivo* is consistent with iAs inhibition of EMT in other cell types

Future Directions

- Further evaluation of EMT gene expression signature in response to iAs treatment using high throughput technologies
- Validation of the GR pathway as a potential mediator in iAs effects using co-exposures of iAs and glucocorticoids
- Migration and invasion modeling *in vivo* in response to iAs treatment
- Better characterization of the regulation of EMT in the placenta

Acknowledgments

- Fry Lab Members: Lisa Smeester, Hadley Hartwell, Jackie Bangma