Endodermal differentiation trajectories diverge with increasing all-trans retinoic acid exposure

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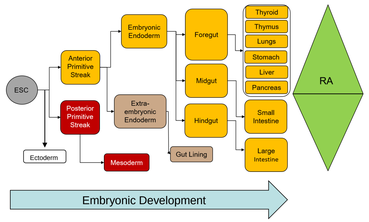
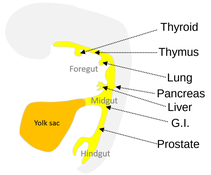
Background

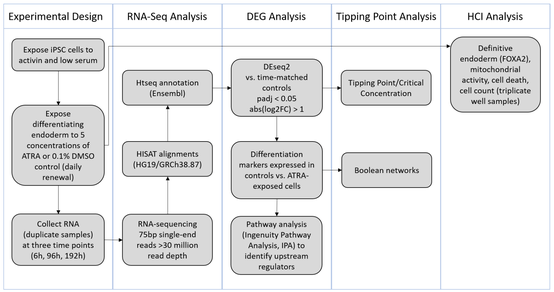
- Toxicological tipping points occur at chemical concentrations that overwhelm a cell's adaptive response [Shah et al. 2016].
- Human induced pluripotent stem cell (iPSC) derived endodermal differentiation (endogenesis) is an *in vitro* platform for probing the developmental impacts of a toxicological tipping point.
- Endogenesis is critical for primordial germ cells and organs including the stomach, intestine, colon, pancreas, liver, urinary bladder, prostate, trachea, lung, pharynx, thyroid, parathyroid glands, and visceral yolk sac.
- Retinoid signaling is critical for early development and directs morphogenesis, growth, and differentiation of the embryo including endogenesis via retinoic acid (RA) gradients.





Gut Tube

Study Overview

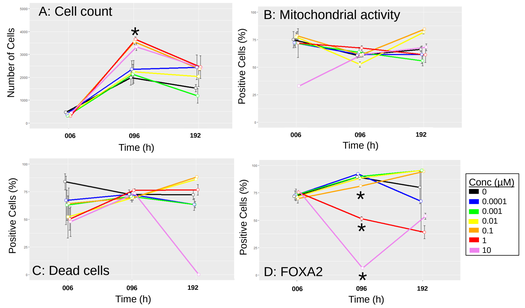


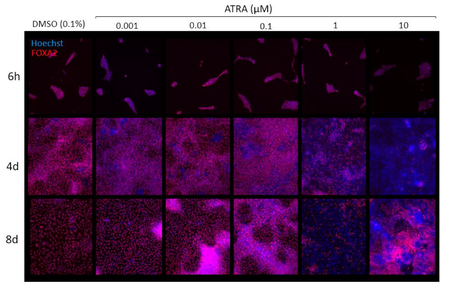
ATRA: all-trans retinoic acid; DEG: differentially expressed gene; HCI: high content imaging

Results

High Content Imaging (HCI): All-trans retinoic acid (ATRA) reduced FOXA2 protein expression in a concentration-dependent manner at 4 days post exposure.

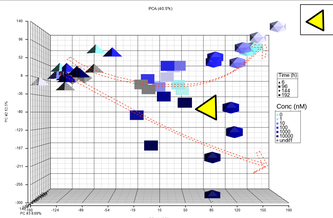
FOXA2 is an embryonic endoderm marker. Four-channel fluorescence imaging was used to measure cell counts (Hoechst), FOXA2 expression (α-FOXA2 antibody), mitochondrial activity (Mitotracker), and cell death (ImageIT-DEAD) over time. Significant changes in percentage of cells expressing the respective markers compared to DMSO controls were calculated by ANOVA with Tukey post-hoc test or Kruskal-Wallis with Dunn post-hoc test (n=3; padj < 0.05). Raw images were analyzed in CellProfiler v2.2.0.

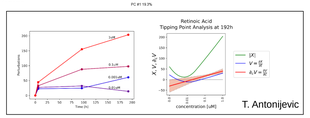




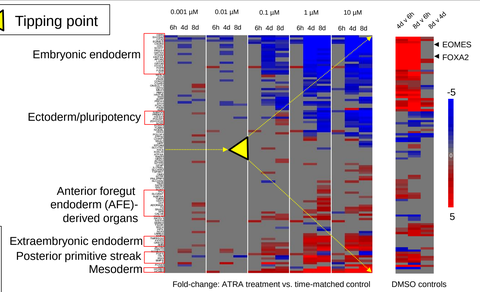
E. FOXA2 protein expression

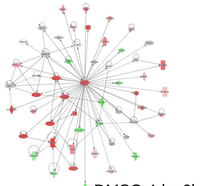
RNA-sequencing: Differentially expressed genes followed diverging trajectories at ATRA concentrations above and below a tipping point calculated at 0.0123 μM.



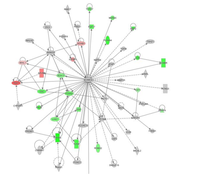


A tipping point defined by the persistence of effects at both of the 4d and 8d timepoints was identified using system trajectory and dynamics analysis of the differentially expressed genes (DEGs).





DMSO 4d v 6h

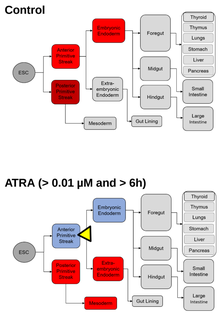


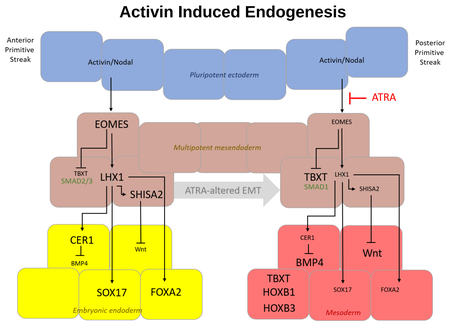
0.1 μM ATRA

Exogenous ATRA exposure shifted the endodermal differentiation trajectory of primitive streak from embryonic endoderm to mesoderm and extraembryonic endoderm. Pathway analysis identified eomesodermin (*EOMES*) as a top upstream regulator in both DMSO and ATRA samples above the tipping point at 4d.

Conclusions

ATRA exposure perturbs endogenous RA signaling to alter cell differentiation in the gastrula resulting in increased expression of primitive streak markers and a cell fate shift from embryonic endoderm to a mesoderm or extraembryonic endoderm phenotype. ATRA exposure disrupts Nodal activation of *EOMES* and downstream activation of *LHX1*, leading to increased expression of *BMP4* and *WNT* in favor of mesoderm.





Summary

- ATRA exposure above 0.01 μM significantly decreased FOXA2 protein expression at 4 days, indicating deviation from an embryonic endoderm differentiation trajectory.
- A tipping point of 0.0123 μM was identified based on ~10,000 differentially expressed genes (DEGs) at 8 days post-ATRA exposure. Increased expression of the mesendoderm marker, *TBXT (T)* at 4 days coincided with the tipping point and a cell fate trajectory shift.
- At ATRA concentrations above the tipping point, predominant cell fate shifted from embryonic endoderm to mesoderm or extraembryonic endoderm beginning at 4 days. This cell fate shift may be through a bias of epithelial mesenchymal transition (EMT) at the primitive streak stage of development in favor of mesoderm. Regional markers of the endodermal lineage also had altered expression patterns, consistent with expected effects of perturbed RA signaling on gut tube development [Wang et al. 2006].
- A set of 108 developmental marker genes is sufficient to screen for altered transcriptional profiles in human iPSC-derived endodermal cells to identify potential toxicants that shift developmental trajectories via perturbed RA signaling.

References

- Shah, et al. 2016. Using ToxCast™ Data to Reconstruct Dynamic Cell State Trajectories and Estimate Toxicological Points of Departure. *Environmental Health Perspectives*. Jul;124(7).
- Wang, et al. 2006. Retinoic Acid Regulates Morphogenesis and Patterning of Posterior Foregut Derivatives. *Developmental Biology*. Sep;297(2).
- Saili et al. *In prep.* Tipping point analysis of diverging endodermal differentiation trajectories following ATRA exposure.