Modeling endodermal differentiation trajectories following all-trans retinoic acid exposure

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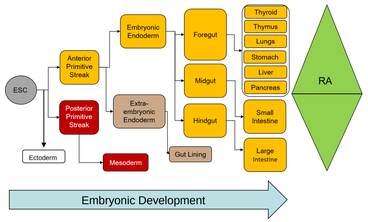
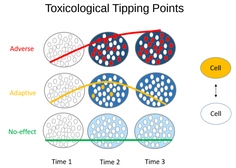
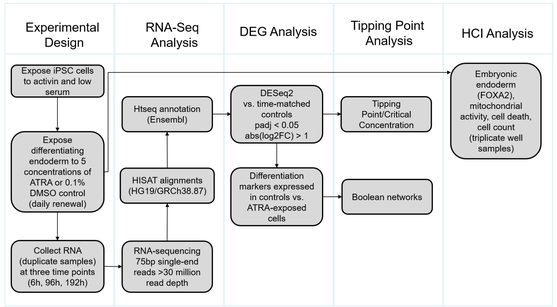
Background

- 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.





Study Overview

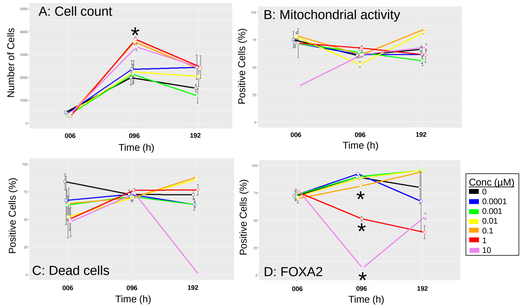


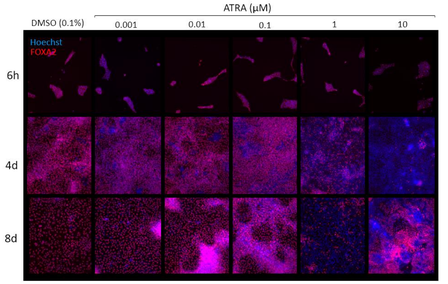
ATRA: all-trans retinoic acid; DEG: differentially expressed gene; HCI: high content imaging

Results

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

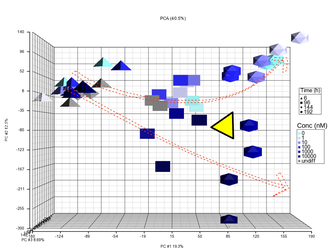
FOXA2 is an embryonic endoderm marker. Four-channel fluorescence imaging was used to measure cell counts (Hoechst), FOXA2 expression (α-FOXA2 antibody), mitochondrial activity (Mitotracker), and cell death (ImageIT-DEAD) over time. Significant changes in percentage of cells expressing the respective 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.

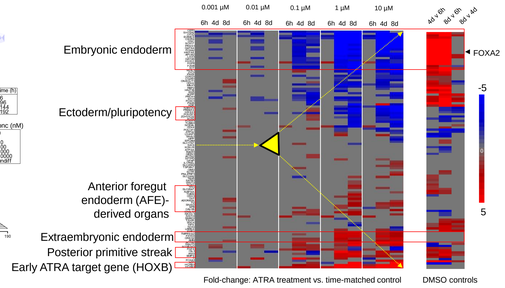




E. FOXA2 protein expression

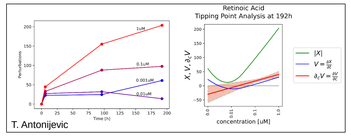
RNA-sequencing: Differentially expressed genes followed diverging trajectories at ATRA concentrations above and below a tipping point calculated at 0.0123 μM.





Tipping point

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



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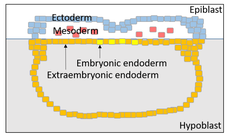
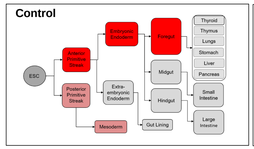
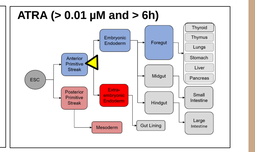
Exogenous ATRA exposure shifted the endodermal differentiation trajectory of anterior primitive streak from embryonic endoderm to extraembryonic endoderm. Extraembryonic endoderm markers were not differentially expressed over time in controls, but increased at ATRA concentrations above the tipping point.

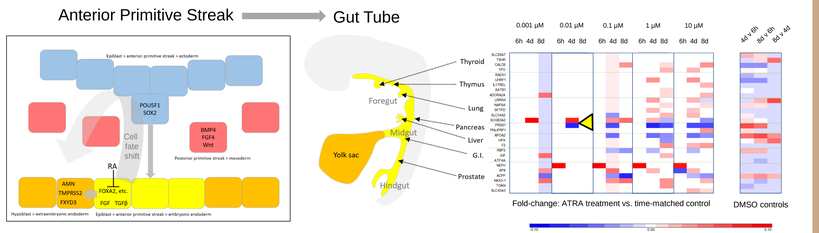
Conclusions

ATRA exposure perturbs endogenous RA signaling to alter cell differentiation in the gastrula resulting in enhanced genesis of multipotent ectodermal cells and a cell fate shift away from embryonic endoderm to an extraembryonic phenotype. This shift is expected to perturb subsequent gut tube formation.









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 key pluripotency genes and ectodermal markers (i.e., NANOG, SOX2, POU5F1) at 8 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 extraembryonic endoderm beginning at 4 days. This cell fate shift may be through enhanced genesis of multipotent ectodermal cells at an anterior primitive streak stage of development. Expression of gut tube derived organ markers was also altered, consistent with expected effects of perturbed RA signaling on gut tube development [Wang et al. 2006].
- A set of ~100 developmental marker genes is sufficient to screen for altered transcriptional profiles in 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).