Transcriptome profiling to identify ATRA-responsive genes in human iPSC-derived endoderm for high-throughput point of departure analysis

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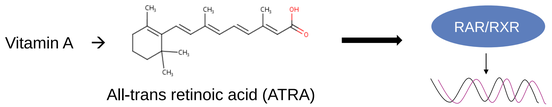
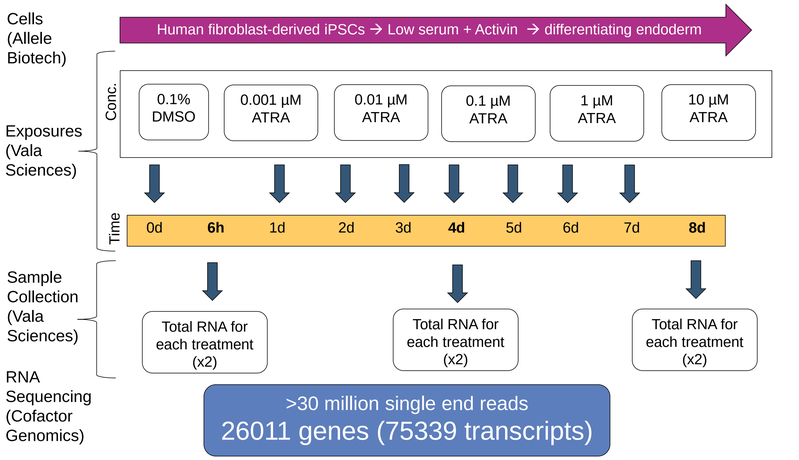
Background

OBJECTIVE: Identify biomarkers for high content analysis of tipping points in a developmental context

- Toxicological tipping points occur at chemical concentrations that overwhelm a cell's adaptive response [Shah et al. 2016]
- Human iPSC-derived endodermal differentiation (endogenesis) is an *in vitro* platform for probing the developmental impacts of a toxicological tipping point
- Endogenesis is critical for organs such as the stomach, intestine, colon, pancreas, liver, urinary bladder, trachea, lung, pharynx, thyroid, and parathyroid glands, visceral yolk sac
- Retinoid signaling is critical for early development and directs morphogenesis, growth, and differentiation of the embryo including endogenesis via retinoic acid gradients
- Epigenetic changes mediate retinoid-induced stem cell differentiation [Gudas. 2013]



Methods

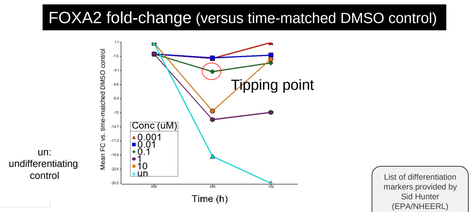


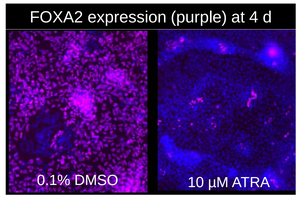
Data Analysis (US EPA)

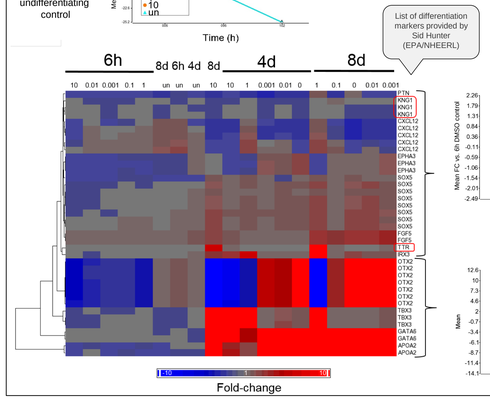
Log2(RPKM+1) values were used for differential gene expression analysis (ANOVA) and filtered (p < 0.00001; -2 > fold-change >2)

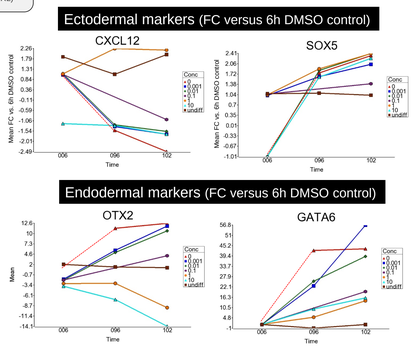
Results (pathway analysis)

ATRA reduced FOXA2 expression and other endoderm-specific biomarkers in a concentration-dependent manner at 4 d

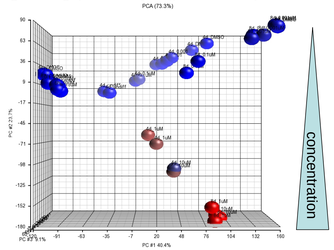


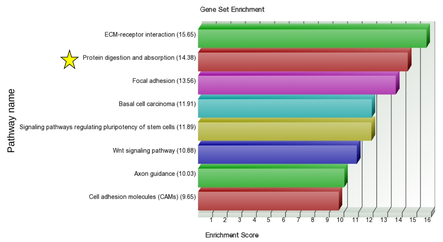






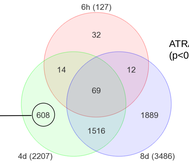
KEGG pathway analysis of differentially expressed genes (6033) returned 8 highly enriched signaling pathways (p<0.0001)



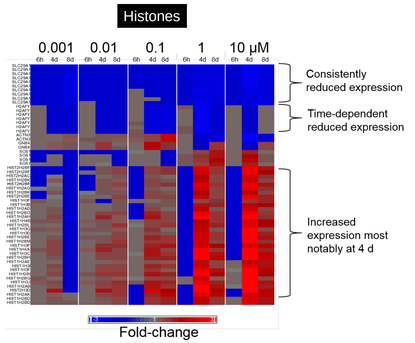


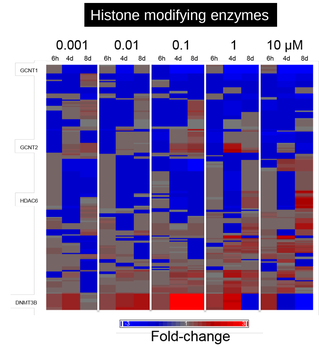
Results (epigenetics)

Enriched pathways uniquely associated with 4 d ATRA exposure were driven by differential expression of 29 histone-encoding genes



KEGG Pathway Analysis: two highly enriched pathways (p < 0.0001)





Summary and Conclusions

- Endodermal differentiation based on FOXA2 expression occurred between 6 h and 4 d
- ATRA first suppressed differentiation at 0.1 µM (by 4 d) indicated by multiple pathways such as protein digestion and absorption that characterize the trajectories for tipping point analysis
- ATRA increased the expression of 29 histone encoding genes by 4 d, suggesting DNA remodeling is a target process for tipping point analysis
- Endogenesis may be a suitable ToxCast platform for molecular-level characterization of toxicological tipping points during cell differentiation

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)
- Gudas. 2013. Retinoids induce stem cell differentiation via epigenetic changes. *Semin Cell Dev Biol*. Dec; 24(0)
- *The views expressed in this poster are those of the authors and may not reflect U.S. EPA policy*