In vitro embryotoxicity testing with human embryonic stem cells reveals markers for all-*trans*-retinoic acid

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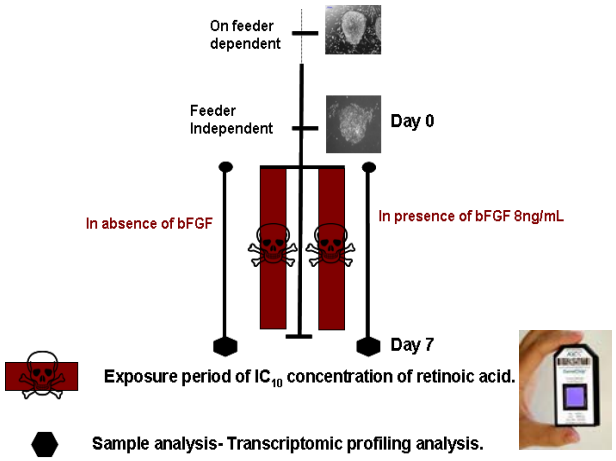
Abstract

Many characteristics of human embryonic stem cells (hESCs) including pluripotency are useful tools for studying the harmful effects of reference compounds during early development. The early embryogenesis is recapitulated by pluripotent embryonic stem cells when differentiated in vitro, which makes hESC an effective tool for developmental toxicology and embryotoxicology. All-trans-retinoic acid is known to be embryotoxic in laboratory animals and for humans when applied during critical stages of embryonic development. Characteristic malformation patterning of craniofacial structures and defects in cardiac development were observed in human offsprings. In rat and mice, All-trans-retinoic acid treatment resulted in craniofacial malformations and limb anomalies associated with embryo fetal alterations. In the following study, All-trans-retinoic acid (AtRA) was treated on undifferentiated hESC in the presence and absence of bFGF for a period of 7 days and a toxicogenomics profile was studied. The results revealed that mesoderm markers such as BMP2, COL1A1 and COL2A1, endoderm markers such as AFP, GATA4 and SERPINA1 were dysregulated. Ectodermal markers such as NEUROD1, PAX6 and TUBB3 were dysregulated in the presence or absence of bGFG. The results are further used to identify embryotoxic markers and also to test other compounds for embryotoxic potential.

Methods



Pathways in All *trans* Retinoic acid

Log Fold change ± 1 P ≤ 0.01	*AtRA With bFGF*	*AtRA Without bFGF*
Upregulated	*Pathways in Cancer*	*No significant pathways*
Downregulated	*No significant pathways*	*Melanogenesis PPAR signaling*

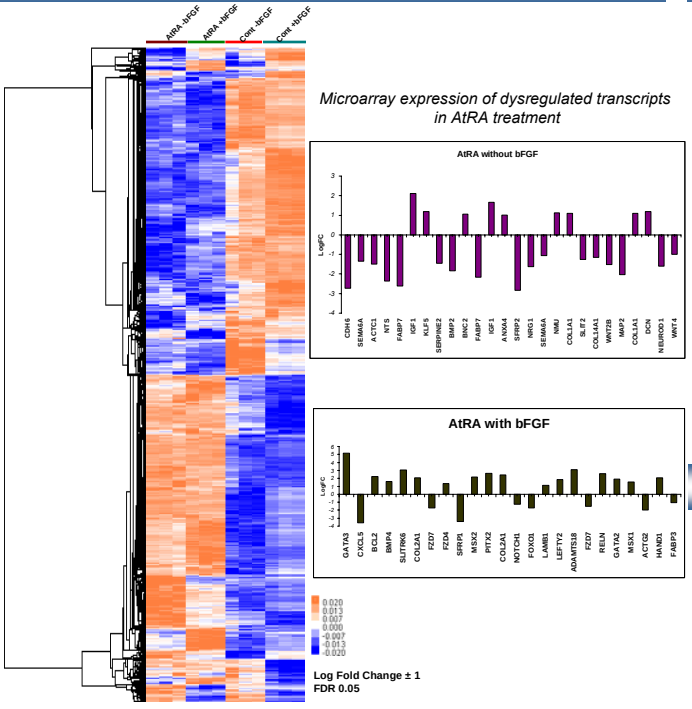
Common pathways

Upregulated	*Axon Guidance*
Downregulated	*WNT signaling pathway*

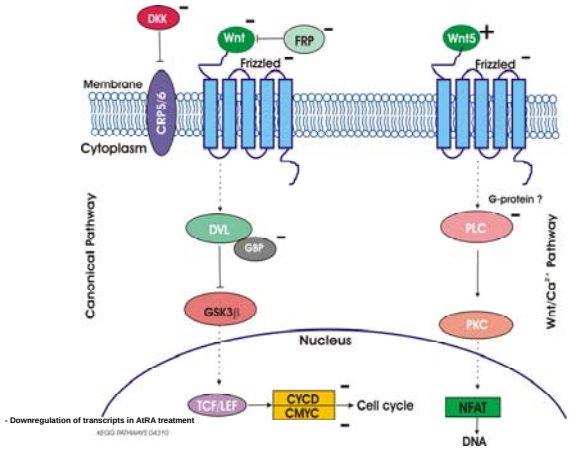
Gene Ontology for All *trans* Retinoic acid

	AtRA with bFGF			AtRA without bFGF		
	Biological process	gene count	p Value	Biological process	gene count	p Value
upregulated	GO:0060173~limb development	16	1.54E-08	GO:0044057~regulation of system process	9	6.04E-04
	GO:0048736~appendage development	16	1.54E-08	GO:0042391~regulation of membrane potential	6	0.001384517
	GO:0035108~limb morphogenesis	15	6.87E-08	GO:0008284~positive regulation of cell proliferation	9	0.003926025
	GO:0048598~embryonic morphogenesis	25	2.35E-07	GO:0051240~positive regulation of multicellular organismal process	7	0.00384147
	GO:0001501~skeletal system development	25	4.69E-07	GO:0048878~chemical homeostasis	10	0.004060185
	GO:0035295~tube development	19	4.16E-06	GO:0042445~hormone metabolic process	5	0.004072579
	GO:0030182~neuron differentiation	25	9.87E-05	GO:0051960~regulation of nervous system development	6	0.006495326
	GO:0007411~axon guidance	11	1.99E-04	GO:0006873~cellular ion homeostasis	8	0.008008513
downregulated	GO:0001944~vasculature development	16	1.99E-05	GO:0000902~cell morphogenesis	19	8.09E-08
	GO:0001568~blood vessel development	14	2.34E-04	GO:0060284~regulation of cell development	14	4.50E-07
	GO:0002040~sprouting angiogenesis	4	3.54E-04	GO:0060485~mesenchyme development	8	1.55E-06
	GO:0043405~regulation of MAP kinase activity	9	0.002465	GO:0060485~mesenchyme development	8	1.55E-06
	GO:0043549~regulation of kinase activity	15	0.002633	GO:0050767~regulation of neurogenesis	12	2.33E-06
	GO:0048514~blood vessel morphogenesis	11	0.002821	GO:0001569~patterning of blood vessels	5	7.50E-05
	GO:0000165~MAPKKK cascade	10	0.00368	GO:0060348~bone development	8	4.35E-04

Global cluster for All *trans* Retinoic acid



WNT signaling in All *trans* Retinoic acid



Summary

1. *All trans retinoic acid tends to dysregulated the pluripotent state of hESC irrespective of exogenous bFGF.*
2. *Wnt signaling elicits multiple functions in stem cells, regulating not only promotion of proliferation, but also lineage selection. Any dysregulation in WNT signaling in response to embryo-toxicants may alter crucial developmental process.*
3. *AtRA was found to cause alteration WNT signaling, which can be further exploited for other embryo-toxicant testing*
4. *Although how signaling pathways interact with canonical WNT signaling remains to be elucidated, we can consider WNT as an important signaling pathway in embryotoxicity studies.*