Global transcriptional profiling of time dependent multilineage differentiation of human embryonic stem cells and its implications for developmental toxicity assessment

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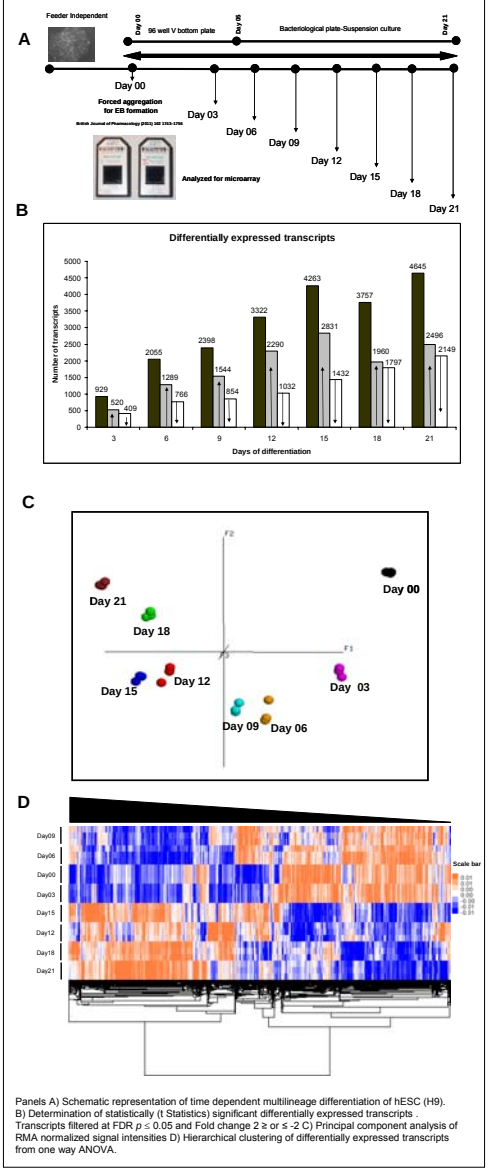
ABSTRACT

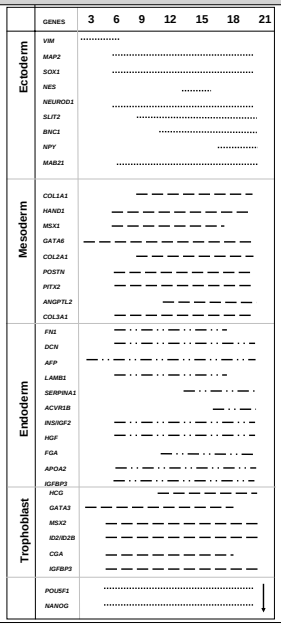
The better understanding of early human embryonic development has been hampered with the availability of samples and eventually addressed with *in vivo* embryonic development (ED). To study evolutionarily conserved, developmentally regulated pathways and genes for toxicity analysis, *in vivo* studies were extrapolated. As an initial step we have recapitulated the early human ED with multilineage differentiation of human embryonic stem cells (hESC) integrated with sensitive genomics approach in our prior publication. To further explore the human ED and its subsequent application in toxicogenomics we have assigned the time kinetic hESC ED assays. The global transcriptional profiling and the subsequent gene ontology analysis reveal that from differentiation day 3 the developmental biological processes were identified. Also, the gene signatures identified during the embryonic development were used for the assessment of early developmental toxicity. The known developmental toxicants and the negative compound were treated at sub lethal concentration during the embryoid body development for 14 days. The gene expression analysis for the toxicity markers were measured with RT-qPCR and reveal these positive compounds have distinctive effect on mRNA level for these markers (AFP, DCN, APOA2, IGFBP3, HAND1, POSTN, PITX2, COL3A1 and MSX1) but the negative compound penicillin doesn't show any significant effect.

Global transcriptomic profiling of time dependent multilineage differentiation of hESC

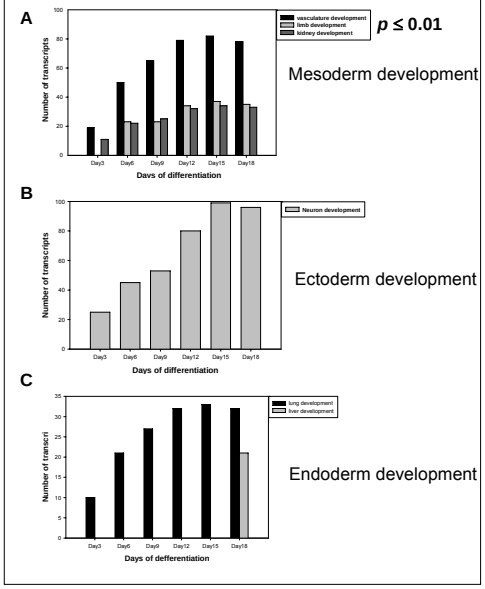


Panels A) Schematic representation of time dependent multilineage differentiation of hESC (H9). B) Determination of statistically (t Statistics) significant differentially expressed transcripts . Transcripts filtered at FDR $p \leq 0.05$ and Fold change 2 ≥ or ≤ -2 C) Principal component analysis of RMA normalized signal intensities D) Hierarchical clustering of differentially expressed transcripts from one way ANOVA.

Selected germ layer markers expression during the embryonic development- Microarray results



Gene ontology analysis for selected biological process during the embryonic development

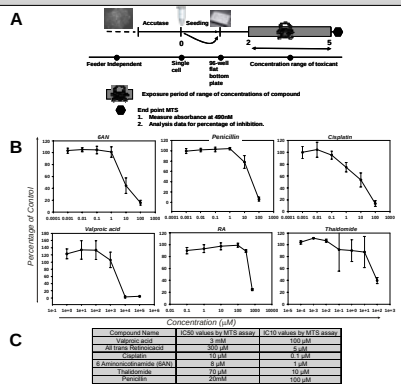


Pathways assessment for the differentially expressed Transcripts in embryoid body development

$p \leq 0.01$

Days of differentiation	KEGG Pathways
Day 03-00	hsa05218:Prostate cancer hsa04310:Wnt signaling pathway
Day 06-00	hsa05216:Melanoma hsa05214:Glioma hsa05412:Arrhythmogenic right ventricular cardiomyopathy (ARVC)
Day 09-00	hsa04512:ECM-receptor interaction hsa05218:Melanoma hsa05215:Prostate cancer hsa05214:Glioma
Day 12-00	hsa04512:ECM-receptor interaction hsa04510:Melanogenesis hsa05217:Basal cell carcinoma hsa05215:Prostate cancer hsa05216:Melanoma hsa05217:Basal cell carcinoma hsa04530:Tight junction
Day 15-00	hsa05216:Prostate cancer hsa05218:Melanoma hsa04512:ECM-receptor interaction
Day 18-00	hsa04510:Focal adhesion hsa05200:Pathways in cancer hsa04115:p53 signaling pathway hsa04360:Axon guidance hsa05211:Renal cell carcinoma hsa05210:Colorectal cancer hsa04512:ECM-receptor interaction

Determination of cytotoxicity concentration for toxicological evaluation



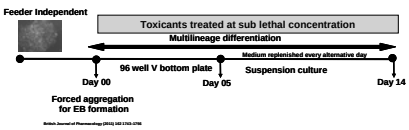
C

Compound Name	IC50 values by MTS assay	IC10 values by MTS assay
Valproic acid	3 mM	100 µM
All trans Retinoicacid	300 µM	5 µM
Cisplatin	10 µM	0.1 µM
6 Aminonicotinamide (6AN)	8 µM	1 µM
Thalidomide	70 µM	10 µM
Penicillin	20mM	100 µM

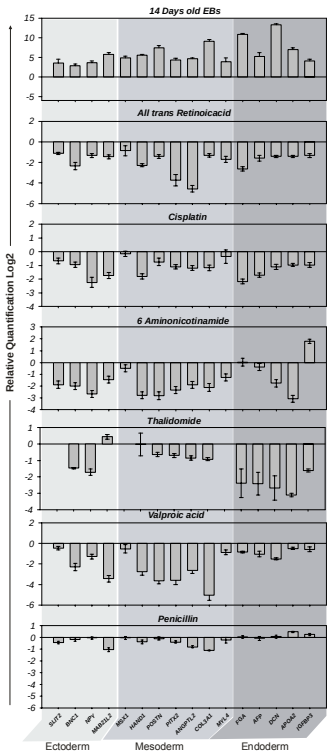
Panels A) Schematic representation of cell (H9) viability (MTS) assay. B) Cell viability assay for positive and negative compounds C) Summary of lethal and sub lethal concentrations for the toxicants and negative compound

Multilineage differentiation of hESC for developmental toxicity prediction

A Differentiation scheme and drug treatment



B Germ layer markers (RT-qPCR) assessment during multilineage differentiation in presence and absence of toxicants



C Interference of germ layer markers during the multilineage differentiation

Compounds tested	Number of markers repressed ≤ Log2 Fold 1
6 Aminonicotinamide	12
Cisplatin	9
Valproic acid	9
Thalidomide	8
All trans retinoic acid	15
Penicillin	1