Human Induced Pluripotent Stem Cell (hiPSC)-Derived Osteoblasts for *In Vitro* Assessment of Skeletal Malformations

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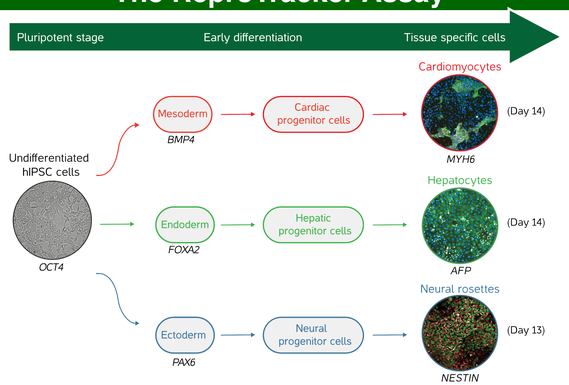
Introduction

- Human developmental toxicants can negatively impact embryonic development. Several drugs, such as warfarin, have been reported to cause bone malformations, including nasal hypoplasia and short limbs.
- To help evaluate developmental toxicity, we developed ReproTracker, a human induced pluripotent stem cell (hiPSCs)-based biomarker assay that predicts the teratogenicity of substances in vitro. This assay utilizes three different lineage-specific cells: hepatocytes, cardiomyocytes, and neural rosettes.
- In this study, we introduced osteoblasts to the ReproTracker platform, specifically aimed at capturing the direct effects of teratogenic agents on bone development.

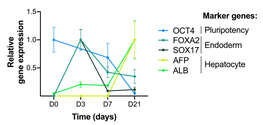
The ReproTracker Assay



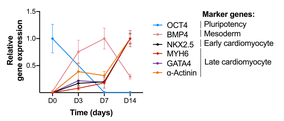
Differentiation of hiPSCs towards specialized cell types within the ReproTracker assay. Pluripotent stem cells are directed to differentiate towards mesoderm, endoderm, and ectoderm, after which they are further matured into cardiomyocytes, hepatocytes and neural rosettes.

Biomarker analysis

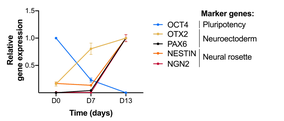
Hepatocyte differentiation



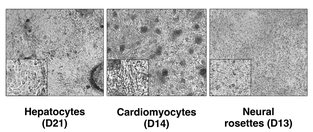
Cardiomyocyte differentiation



Neural rosette differentiation

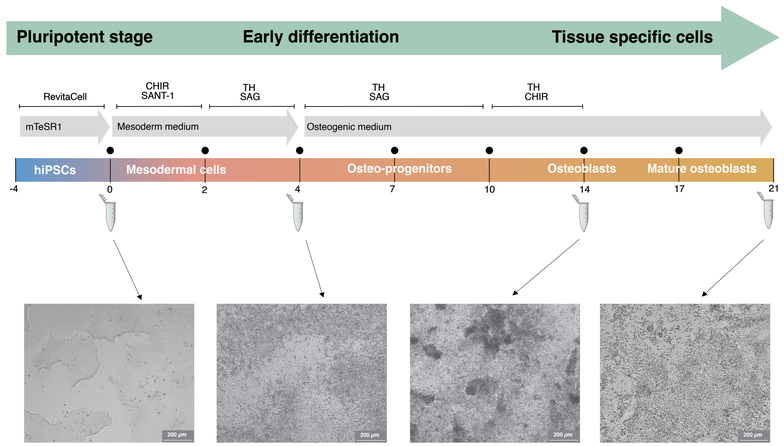


Morphology profiling

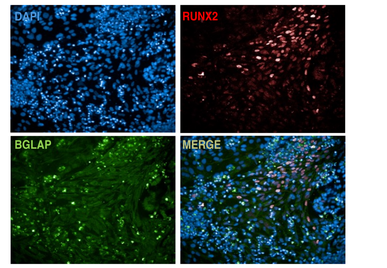


Biomarker expression analysis and cellular morphology/functionality. Successful differentiation of the hiPSCs is determined by quantitative assessment of the expression of various selected biomarker genes. Proper stem cell differentiation is evaluated by morphological profiling and assessment of time-dependent expression patterns of cell-specific biomarkers. In ReproTracker, a decrease in expression of the biomarker genes and morphological disruption of the differentiated cells following compound exposure indicates teratogenicity.

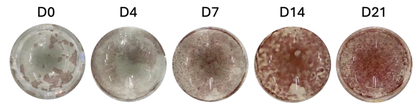
Osteoblast Differentiation



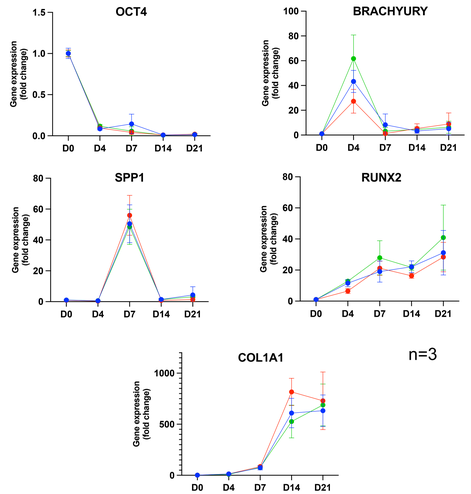
Schematic representation of hiPSCs differentiation towards osteoblast-like cells with representative brightfield images at the different time points.



Immunostaining of the cultures at different developmental stages showing the expression of the relevant biomarkers. Cultures are stained for RUNX2 (red), BGLAP (green) and counterstained with DAPI (blue) at D21



Alizarin Red Staining at different stages of the differentiation. Red staining indicates the presence of calcium depositions in the cultures

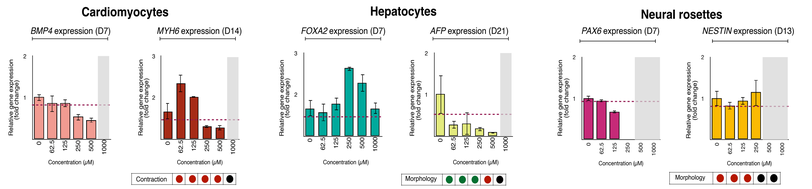


Gene expression of key bone developmental biomarkers at the different developmental stages. Biomarkers included represent pluripotency (*OCT4*), mesoderm (*BRACHYURY*) and osteoblasts (*RUNX2, SPP1, COL1A1*). Data represent mean ± standard deviation from 3 biological replicates as depicted by three different colors

Results

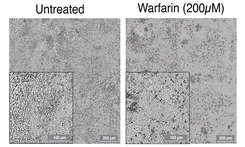
Warfarin

ReproTracker results from the trilineage differentiation

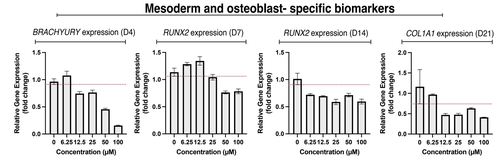


Exposure of differentiating cells to non cytotoxic concentrations of warfarin selectively decreased expression levels of different key biomarkers starting at concentrations of 62.25 µM

Morphology assessment



Biomarker expression analysis



Teratogen in human, ReproTracker and rat

- ReproTracker LOAEL ~6.25µM
- Rat study LOAEL ~13 µM (based on 0.5 mg/kg)
- Human Cmax ~18-20 µM (therapeutic dose)

Non-teratogen in rabbit

- Rabbit study LOAEL n.d.

Conclusions

- Integration of osteogenesis in the ReproTracker assay expands the biological coverage of developmental process recapitulated by the assay.
- Bone-specific biomarkers as an additional end point allow for the evaluation of teratogenic agents in the ReproTracker Assay.
- The assay allows for the detection of skeletal developmental toxicants.

References

- DOI: 10.1016/j.etap.2015.12.006
- DOI:10.1097/00007691-198703000-00001
- DOI: 10.1080/15569520701860999