Optimising Mesodermal Specification of hiPSCs with BMP4 and CHIR99021: Towards Enhanced Haematopoietic Stem Cell Production

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Introduction

- Haematopoeisis is the development of functional blood types which and occurs in **three waves**; primitive (firrst wave) and definitive haematopoeisis (second and third wave).[1,2]
- Development of blood occurs from the **hemogenic endothelium**, which is a subset of endothelial cells and of mesodermal derivation.
- With the current differentiation protocols generating low number of endothelial cells, means less haematopoietic progenitors, with **hiPSCs** model we can study the *in vitro* development of haematopoietic differentiation.

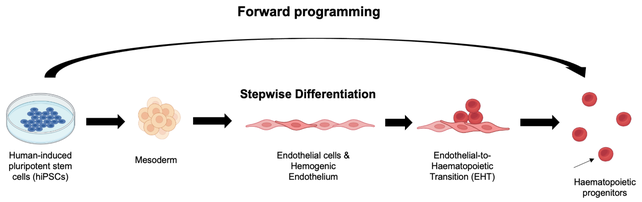


Figure 1. *Schematic of stepwise differentiation of haematopoeisis through mesoderm-endothelial origin using hiPSCs model. Adapted from Canu & Ruhrberg, 2021.*

Hypothesis & Aims

Increasing CHIR99021 concentration should optimise BMP and WNT signaling to induce mesodermal development and increase the hemogenic endothelial cell population.[3,4]
This project aims to improve the mesodermal commitment of hiPSCs fate in relation to ongoing protocols (Condition 1) by manipulating two pathways known to control mesodermal fate.

Methods

Mesodermal fate was induced by differentiating hiPSCs through Embryoid bodies (EBs) formation. EBs were kept under six different conditions of BMP4, CHIR99021 and bFGF for the 3-day process, and tested in four independent biological replicates against condition 1 as the control group (Figure 2).

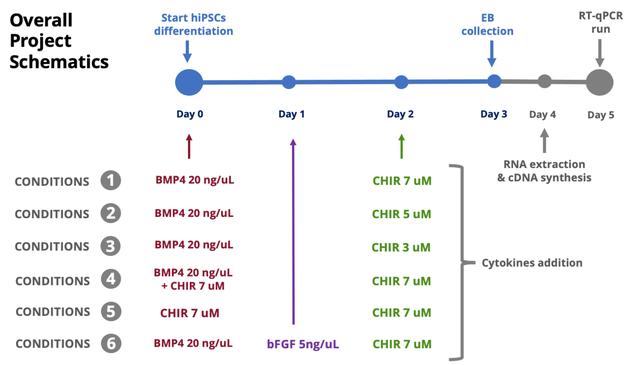
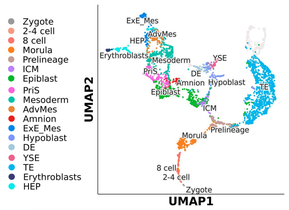


Figure 2. *Overall project schematic illustrate the differentiation process of hiPSCs into mesoderm cells (blue) and lab analytical techniques (grey). BMP4 was added at 20 ng/uL, bFGF was added at 5 ng/uL, and CHIR99021 was added between 3-7 uM depending on each condition.*

Marker Differentiation

For this project, we identified key embryonic marker genes as target genes for the gene expression analysis with RT-qPCR (Figure 3 and 4). Markers from extraembryonic mesoderm, intraembryonic mesoderm, endoderm, and amnion germ layers were selected. The primers were designed and validated for their efficiency before its use in hiPSCs differentiation (Figure 3).



Amnion	Primer Efficiency	Extraembryonic Mesoderm	Primer Efficiency
GABRP	1.99	NPY	1.96
ISL1	1.84	MESP1	2.08
TFAP2A	1.98	KDR / FLK1	2.03
DLX5	1.97	TBXT	1.89
Intraembryonic Mesoderm	**Primer Efficiency**	**Endoderm**	**Primer Efficiency**
PARM1	1.93	GATA4	1.98

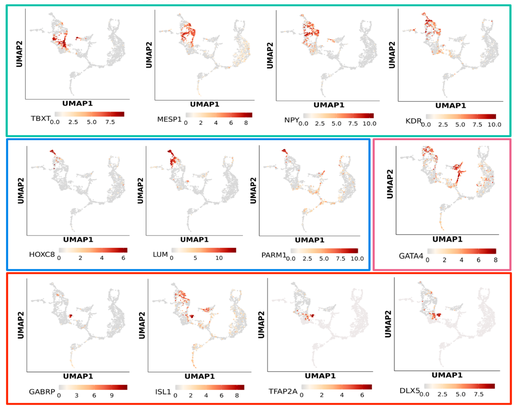


Figure 3. *Selection of germline markers separated by group. Top: UMAP reannotation on subset cell information, and primer efficiency table for qPCR analysis. Bottom: UMAP of selected markers, scale bar below shows qualitative annotation for no expression (gray) and high expression (red) of the gene of interest.*

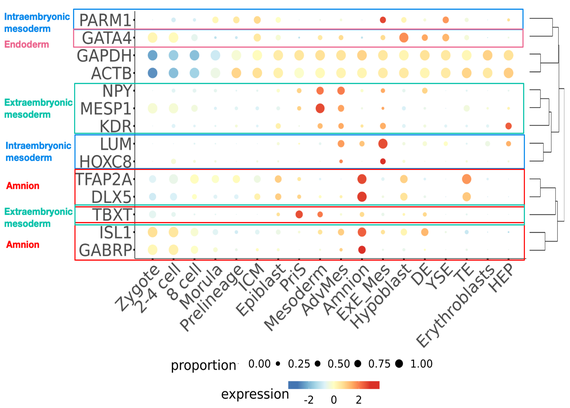


Figure 4. *Dotplot showing gene expression profile of all the selected gene markers used in the experiment and separated by embryonic cell information. Created with human embryonic reference tool(v1.1.1.17) by Petropoulous Lanner Labs.*

Microscopic Morphology of Embryoid Bodies

Under microscopic view, morphology of differentiated embryoid bodies show slight variation in size and aggregation under different culture (Figure 5). Condition 3 seems to have the lowest aggregation of EBs. Condition 5 had the largest EBs amongst all conditions, while Condition 6 exhibited the best aggregation and roundness of EB colonies. Batch 3 was excluded due to low cell viability and RNA yield.

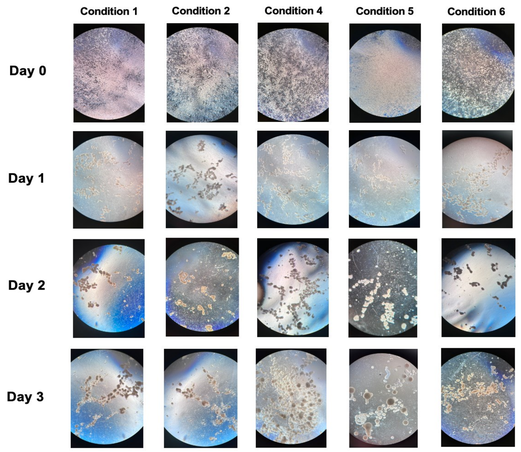


Figure 5. *Differentiation of hiPSCs into embryoid bodies (EBs) from condition 1,2,4,5,6 between the three days of differentiation.*

Mesodermal Differentiation in Response to BMP and WNT Manipulation

Condition 3 was totally excluded due to low RNA yield across all replicates, indicated poor EB quality. Mesoderm markers showed the highest expression in Condition 4, suggesting a potential synergy between BMP4 and CHIR99021. Contrary to expectations, extraembryonic mesoderm markers were undetected mainly due to inadequate primers, hence requiring further testing. Amnion markers were expressed except for Condition 5, only *GABRP* showed opposite results. *GATA4*, the sole endoderm marker exceeded detection limits and requires more markers to assess endodermal fate.

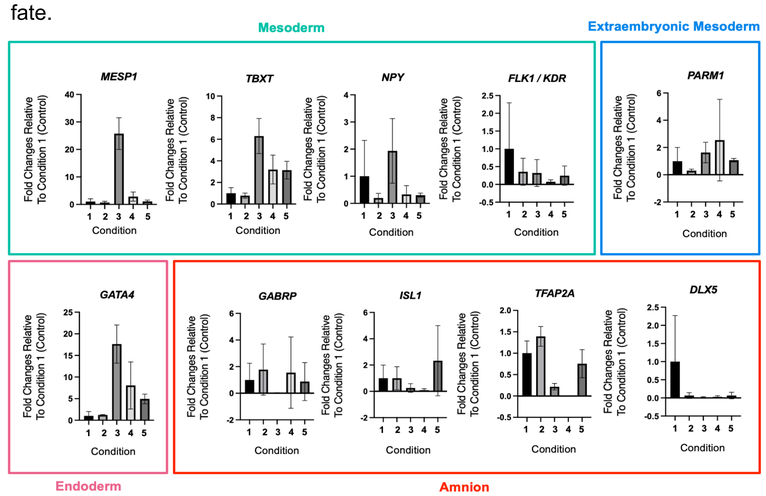


Figure 6*. (A) RT-qPCR analysis include fold changes relative to condition 1 (BMP4 (d0) + CHIR 7uM (d2) as the control group, Condition 2 (BMP4 (d0) + CHIR 5uM (d2), Condition 4 (BMP4 + CHIRr 7uM (d0), Condition 5 (CHIR 7uM (d0)), and Condition 6 (BMP4 (d0) + bFGF (d1) + CHIR 7uM (d2). GraphPad.*

Conclusion

We aimed to investigate and increase mesodermal expression, which our findings suggested that through condition 4, from the beginning of differentiation with concurrent stimulation of BMP4 and CHIR99021 in mesodermal induction, led to higher mesodermal expression and inhibiting amnion differentiation.

Future Perspective

- Use of additional markers for extraembryonic mesoderm and endoderm markers to validate findings.
- Study of FGF signalling pathway in the induction of mesoderm with BMP4, bFGF, and CHIR99021 application from day 0. Extend differentiation to eight days with VEGF, and analyse CD34+ cell development for enhanced accuracy in cell fate determination.[3]
- Using fluorescent endoderm SOX17 reporter line to allow visualisation of rapid change in gene expression and real-time tracking during hiPSCs cell differentiation for insight on haematopoietic development.

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References & Citations

1. Dzierzak, E., Bigas, A. Blood Development: Hematopoietic Stem Cell Dependence and Independence. *Cell Stem Cell*. **22** (5), pp.639-651 (2018). ISSN 1934-5909. https://doi.org/10.1016/j.stem.2018.04.015.
2. Canu, G., Ruhrberg, C. First blood: the endothelial origins of hematopoietic progenitors. *Angiogenesis*, **24**, 199–211 (2021). https://doi.org/10.1007/s10456-021-09783-9
3. Antonella Fidanza, Patrick S. Stumpf, Prakash Ramachandran, Sara Tamagno, Ann Babtie, Martha Lopez-Yrigoyen, A. Helen Taylor, Jennifer Easterbrook, Beth E. P. Henderson, Richard Axton, Neil C. Henderson, Alexander Medvinsky, Katrin Ottersbach, Nicola Romanò, Lesley M. Forrester; Single-cell analyses and machine learning define hematopoietic progenitor and HSC-like cells derived from human PSCs. ***Blood***, **136** (25), pp. 2893–2904 (2020). doi: https://doi.org/10.1182/blood.2020006229
4. Sturgeon, C., Ditadi, A., Awong, G. *et al.* Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells. *Nat Biotechnol*, **32**, pp.554–561 (2014). https://doi.org/10.1038/nbt.2915
5. Petropoulos, Sophie, and Fredrik Lanner. “Multi-Data Integration Including 6 Embryonic Reference ScRNA-Seq Data - Default.” *Clintec.ki.se*, 2024, petropoulos-lanner-labs.clintec.ki.se/shinys/app/ShinyEmbryoRef. Accessed 20 Aug. 2024.