Nuclear factor programming improves stem-cell-derived hepatocyte phenotype

Cell Stem Cell
Previews

CellPress

Nuclear factor programming improves stem-cell-derived hepatocyte phenotype

Hassan Rashidi[1,2] and David C. Hay[3,*]
[1]UCL Great Ormond Street Institute of Child, London, UK
[2]UCL Institute for Liver and Digestive Health, Royal Free Hospital, London, UK
[3]Institute for Regeneration and Repair, Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK
*Correspondence: davehay@talktalk.net
https://doi.org/10.1016/j.stem.2022.04.009

In this issue of *Cell Stem Cell*, Ma et al. demonstrate that the activation of the nuclear receptor thyroid hormone receptor beta (NR1A2) improves the differentiation status of hepatocyte-like cells derived from human pluripotent stem cells.

To date, several protocols that generate human stem-cell derived hepatocyte-like cells (HLCs) have managed to successfully model human disease in the dish (Szkolnicka et al., 2014; Meseguer-Ripolles et al., 2021; Sinton et al., 2021). However, the generation of immature HLCs with transient phenotype is a major limitation, which has precluded their widespread commercial and clinical application (Rashidi et al., 2018). Additionally, these cultured cell populations still miss many physiological elements present *in vivo*, including biomechanical stimuli and interaction with other cell types (Rashidi et al., 2016; Szkolnicka and Hay, 2020). Epigenetic remodelling also plays important roles during lineage differentiation and somatic cell dedifferentiation. Therefore, understanding the epigenetic changes that regulate hepatocyte identity will be essential to efforts to create renewable somatic cell resources for *in vitro* and *in vivo* endeavors.

In this issue of *Cell Stem Cell*, Ma et al. show that the activation of the nuclear hormone receptor, thyroid hormone receptor beta (THRB), improves the differentiation status of HLCs derived from human pluripotent stem cells (PSCs). The authors developed a 3D differentiation system and profiled gene expression, chromatin accessibility, and enhancer landscapes of the differentiated cells and compared them to cryopreserved primary human hepatocytes (PHHs) to improve stem-cell-derived HLC phenotype in culture (Ma et al., 2022).

Principal component analysis confirmed that 3D HLCs were more similar to PHHs than to their 2D HLC counterparts, demonstrating reduced expression of a fetal marker, alpha-fetoprotein, and increased albumin expression. Other hepatocyte genes were also examined with glucose-6-phosphatase catalytic subunit 1, and expression of coagulation factor V and complement factor 5 was observed at similar levels between 3D HLCs and PHHs, levels which were superior to that in 2D HLCs. Enrichment of disease-associated non-coding single-nucleotide polymorphisms was detected in 3D PSC hepatocytes, suggesting that they could provide a better experimental system to study the mechanisms that underpin human disease. The authors also noted a strong increase in CYP3A4 expression (~80-fold) in 3D versus 2D culture. They put this into context, stating that 3D HLC levels only represented ~1% of the levels detected in PHHs, suggesting the presence of signaling defects in 3D HLCs when compared to PHHs.

Throughout their experimentation, the authors searched for key molecular features that underpinned the differences between HLCs and PHHs. By using assays for transposase-accessible chromatin sequencing and chromatin immunoprecipitation sequencing they found reduced enrichment of THRB motifs in accessible chromatin and active enhancers of 3D HLCs when compared to PHHs. However, THRB gene expression did not correlate with the reduced enrichment of THRB motifs, possibly due to differential THRB ligand availability in 2D and 3D HLCs. To test this, the authors targeted the proximal enhancer of CYP3A4, known to contain THRB-binding motif. Sustained exposure of the THRB ligand, thyroid hormone T3, was used to drive THRB heterodimer formation and nuclear translocation and resulted in improved CYP3A4 expression and function. These observations were further validated by using a genetically modified HepG2 cell line, RNAi, and an inducible CRISPR system in HLCs targeting the CYP3A4 proximal enhancer. Further transcriptomic analysis of liver metabolic genes demonstrated that drug (UGT1A1) and glycogen (GBE1) metabolism were also improved in T3-treated 3D HLCs (Figure 1).

Ma et al. (2022) then confirmed the interaction of THRB with heterogeneous nuclear ribonucleoproteins, transcription initiating factor proteins, and proteins from the ATP-dependent chromatin remodeling complex polybromo-associated Brg/Brahma associated factors (pBAF). RNAi-mediated reduction of a key component of the pBAF remodeling complex, polybromo-1 (PBRM1), decreased CYP3A4 expression and function. These findings suggest that THRB regulation of chromatin accessibility is mediated in part via the pBAF chromatin remodeling complex. The authors went on to explore cell expansion and cryopreservation of HLCs. They designed a medium formulation, incorporating factors important in liver regeneration, to expand HLCs *in vitro* and study their safety profile. Single-cell preparations of 2D and 3D HLC types were transplanted into the spleen of immunocompromised mice (Figure 1). HLCs from 3D spheres successfully engrafted into host livers and remained stable for 6 months, producing human albumin. Conversely, transplantation of 2D HLCs resulted in tumor formation after several months, which is consistent with previous studies (Payne et al., 2011).

Overall, the work by Ma et al. (2022) is important for the field. The ability to

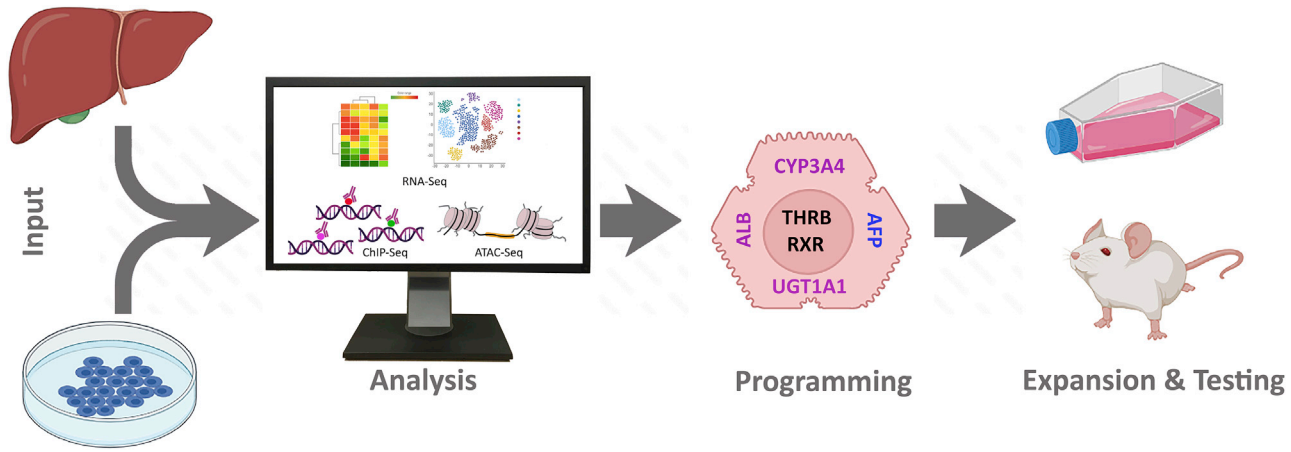

Figure 1. Schematic of the study performed by Ma et al. to identify key molecular features that underpin the differences in stem-cell-derived HLCs and PHHs

Thyroid hormone T3 was used to drive THRB nuclear translocation and improve HLC phenotype. Figure created with Biorender.com.

program key pathways by using a simple cell culture additive, such as T3, is not only enabling for liver research, but could also be applied to other organ systems in the future. The THRB interactome analysis done by Ma et al. (2022) provides essential mechanistic information in this context. If it were possible to perform those experiments in fresh or cryopreserved primary hepatocytes in the future, other key processes regulating the hepatocyte genome could be identified and targeted. It will also be important to evaluate T3-primed HLC drug induction and drug metabolizing capacity and compare this to freshly isolated PHHs.

The authors also demonstrate the importance of working with 3D cells to better capture the human tissue structure and functional relationships, which is consistent with previous studies (Takebe et al., 2013; Rashidi et al., 2018; Lucendo-Villarin et al., 2020). Going forward, it would be interesting to evaluate how improved HLCs interact with other key cell types of the liver, such as endothelial, stellate, and Kuppfer cells and whether these interactions would augment tissue performance and stability *in vitro* and *in vivo*. The authors also studied cell expansion and cryopreservation, and it is encouraging to see that this was achievable by using research-grade materials. The challenge will be to manufacture their product in a more defined manner for technology expansion, automation, and large-scale pre-clinical testing to assess the safety, supportive value, and stability of T3-programmed 3D HLCs in different liver disease and regeneration models.

In conclusion, Ma et al. (2022) offer interesting mechanistic insights into the regulation of hepatocyte gene expression and epigenetic status and provide the field with a more sophisticated HLC for basic and applied research.

DECLARATION OF INTERESTS

D.H. is a founder, director, and shareholder in Stimuliver ApS and Stemnovate Limited.

REFERENCES

Lucendo-Villarin, B., Meseguer-Ripolles, J., Drew, J., Fischer, L., Ma, E., Flint, O., Simpson, K.J., Machesky, L.M., Mountford, J.C., and Hay, D.C. (2020). Development of a cost-effective automated platform to produce human liver spheroids for basic and applied research. Biofabrication *13*, 015009. https://doi.org/10.1088/1758-5090/abbdb2.

Ma, H., De Zwaan, E., Guo, Y.E., Cejas, P., Thiru, P., Van de Bunt, M., Jeppesen, J.F., Syamala, S., Daall'Agnese, A., Abraham, B.J., et al. (2022). The nuclear receptor THRB facilitates differentiation of human PSCs into more mature hepatocytes. Cell Stem Cell *29*, 795–809.

Meseguer-Ripolles, J., Lucendo-Villarin, B., Tucker, C., Ferreira-Gonzalez, S., Homer, N., Wang, Y., Starkey Lewis, P.J., M Toledo, E., Mellado-Gomez, E., Simpson, J., et al. (2021). Dimethyl fumarate reduces hepatocyte senescence following paracetamol exposure. Iscience *24*, 102552. https://doi.org/10.1016/j.isci.2021.102552.

Payne, C.M., Samuel, K., Pryde, A., King, J., Brownstein, D., Schrader, J., Medine, C.N., Forbes, S.J., Iredale, J.P., Newsome, P.N., and Hay, D.C. (2011). Persistence of functional hepatocyte-like cells in immune-compromised mice. Liver Int. *31*, 254–262. https://doi.org/10.1111/j.1478-3231.2010.02414.x.

Rashidi, H., Alhaque, S., Szkolnicka, D., Flint, O., and Hay, D.C. (2016). Fluid shear stress modulation of hepatocyte-like cell function. Arch. Toxicol. *90*, 1757–1761. https://doi.org/10.1007/s00204-016-1689-8.

Rashidi, H., Luu, N.T., Alwahsh, S.M., Ginai, M., Alhaque, S., Dong, H., Tomaz, R.A., Vernay, B., Vigneswara, V., Hallett, J.M., et al. (2018). 3D human liver tissue from pluripotent stem cells displays stable phenotype in vitro and supports compromised liver function in vivo. Arch. Toxicol. *92*, 3117–3129. https://doi.org/10.1007/s00204-018-2280-2.

Sinton, M.C., Meseguer-Ripolles, J., Lucendo-Villarin, B., Wernig-Zorc, S., Thomson, J.P., Carter, R.N., Lyall, M.J., Walker, P.D., Thakker, A., Meehan, R.R., et al. (2021). A human pluripotent stem cell model for the analysis of metabolic dysfunction in hepatic steatosis. Iscience *24*, 101931. https://doi.org/10.1016/j.isci.2020.101931.

Szkolnicka, D., Farnworth, S.L., Lucendo-Villarin, B., Storck, C., Zhou, W.L., Iredale, J.P., Flint, O., and Hay, D.C. (2014). Accurate Prediction of drug-Induced liver Injury using stem cell-derived populations. Stem Cells Translational Med. *3*, 141–148. https://doi.org/10.5966/sctm.2013-0146.

Szkolnicka, D., and Hay, D.C. (2020). Liver stem cells. In Principles of Tissue Engineering, Fifth Edition, R. Lanza, J.P. Vacanti, and A. Atala, eds. (Academic Press), pp. 723–736.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., Zhang, R.R., Ueno, Y., Zheng, Y.W., Koike, N., et al. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. Nature *499*, 481–484. https://doi.org/10.1038/nature12271.