Title: Nuclear factor programming improves stem-cell-derived hepatocyte phenotype

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In this issue of Cell Stem Cell, Ma et al., 2022, 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 (Ma, 2022).

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, reliance on undefined animal products and generation of immature HLCs with transient phenotype are the major limitations, which has precluded their widespread commercial and clinical applications (Rashidi et al., 2018). In addition, these cultured cell populations still miss many physiological elements present in vivo including biomechanical stimuli and interaction with other organs (Rashidi et al., 2016, Szkolnicka, 2020). Understanding the epigenetic changes that take place during liver cell specification and that regulate hepatocyte identity will be essential to our ability to create renewable somatic cell resources for in vitro and in vivo endeavors.

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 phenotypes in culture (Ma, 2022).

Principal component analysis confirmed that 3D HLCs were more similar to PHHs than their 2D HLC counterparts, demonstrating reduced expression of a fetal marker, alpha-fetoprotein (AFP), and increased albumin expression. Other hepatocyte genes were also examined with Glucose-6-Phosphatase Catalytic Subunit 1 (G6PC), coagulation factor V (F5), Complement factor 5 (C5) expression observed at similar levels between 3D HLCs and PHHs, which was superior to 2D HLCs. Enrichment of disease-associated non-coding single nucleotide polymorphisms (SNPs) were 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. Using ATAC-seq and ChIP-seq 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 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 (hnRNPs), transcription initiating factor proteins (TAFs) 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 BAF 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 immune compromised 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 program key pathways using a simple cell culture additive, such as T3, is not only enabling for the liver field, 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 may be identified and targeted. It will also be important to evaluate HLC drug inducibility and metabolizing capacity and compare this to 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 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.

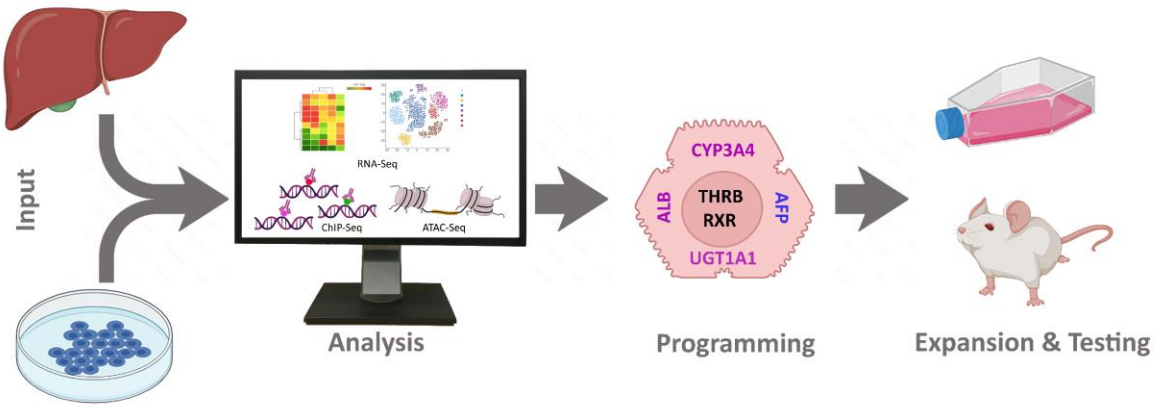

Figure 1: Schematic of the study performed by Ma et al., 2022, to identify key molecular features that underpin the differences in stem cell derived hepatocyte-like cells (HLCs) and PHHs. Thyroid hormone T3 was used to drive THRB nuclear translocation and improve HLC phenotype. Figure created with Biorender.com.

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