Organoid cultures boost human liver cell expansion

Organoid cultures boost human liver cell expansion

Stuart J Forbes MD, PhD.

MRC Centre for Regenerative Medicine, University of Edinburgh, and Scottish Liver Transplant Unit, Royal Infirmary of Edinburgh, NHS Lothian, Edinburgh, UK.

Email: stuart.forbes@ed.ac.uk

Tel: + 00 44 131 651 9515

Abbreviations:

α1-antitrypsin, A1AT; cyclic adenosine monophosphate, cAMP; carbon tetrachloride, CCl4; epithelial cell adhesion molecule, EpCAM; embryonic stem cell, ESC; induced pluripotent stem cell, iPSC; Lgr5, leucine-rich repeat containing G protein-coupled receptor 5; transforming growth factor beta, TGFbeta;

Long-term culture of genome-stable bipotent stem cells from adult human liver. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, Ellis E, van Wenum M, Fuchs SA, de Ligt J, van de Wetering M, Sasaki N, Boers SJ, Kemperman H, de Jonge J, Ijzermans JN, Nieuwenhuis EE, Hoekstra R, Strom S, Vries RR, van der Laan LJ, Cuppen E, Clevers H. Cell. 2015 Jan 15;160(1-2):299-312.

A method of cell culture termed organoid culture has been developed to expand tissue stem cells from various organs. In the recent Cell article, Huch, Gehart et al. applied organoid culture to cells derived from human liver, including from standard biopsies, to expand liver epithelial cells.[1] Cells grown as organoids could be massively expanded, remained genetically and phenotypically stable and could be differentiated into hepatocyte like cells. Cells expanded from patients with genetic liver diseases expressed the disease phenotype following differentiation. Organoid expansion was only successful using EpCAM positive biliary ductular cells rather than mature hepatocytes.

Primary human hepatocytes are used therapeutically for transplantation due to their synthetic and metabolic capacity. Hepatocytes also have potential for therapeutic use in “bioartificial livers” and are important cells to the pharma sector for drug testing. However, fresh human hepatocytes have been challenging to work with as they do not expand significantly ex-vivo in conventional monolayer culture and rapidly de-differentiate. This has fuelled the search for a culture-expandable human hepatocyte, either by devising culture techniques to allow primary hepatocytes to expand in vitro, or by identifying a clonogenic liver stem cell that could be expanded significantly in vitro without undergoing genetic mutations, yet retaining the ability to differentiate into hepatocytes when supplied with appropriate differentiation signals. In the absence of the former there has been increasing focus on the development of hepatocyte-like cells from human pluripotent stem cells such as embryonic stem cell

(ESC) or induced pluripotent stem cell (iPSC) derived hepatocytes.[2,3] However recent studies have raised concerns regarding the phenotypic maturity of these cells,[4] another lingering concern is their phenotypic and genetic stability and, consequently, their safety in vivo. This publication is therefore timely.

Leading up to this work has been an impressive series of observations in the field of adult stem cells. Lgr5 is a cell marker that has been used to great effect to identify adult cells with stem cell like properties, initially in mouse gut but later in a variety of tissues. An important aspect that developed out of this work was the use of the organoid culture technique whereby stem cells could be expanded under the influence of Wnt signalling, whilst keeping some of their 3 dimensional cell-cell interactions.[4] This was first developed in the growth of gut organoids but was later applied to mouse liver where liver organoids could be grown from Lgr5+ epithelial cells isolated from adult mouse liver, and following expansion the cells could be differentiated into hepatocytes.[6]

Huch, Gehart et al. report in the journal Cell that the organoid cell culture technique was successfully utilised to expand primary untransformed epithelial cells from adult human liver (see figure).[1] This seminal study was able to show massive expansion of the epithelial cells in organoids. Of note the authors identified several key factors that enables the expansion of the organoids including the need for Wnt signalling, the addition of cAMP agonists and the inhibition of TGFbeta signalling.

Critically, for disease modelling and possible therapeutic use, the expanding epithelial cells were genetically stable and few base pair substitutions in protein coding regions were identified during the expansion phase of the organoids. Given the high degree of clonogenicity of the organoids, significant cell numbers can be expanded from small numbers of starting cells and this includes standard liver biopsies- opening the door for many diagnostic research and potentially therapeutic possibilities. Given that the expanded cells retained their ability to be differentiated into mature hepatocytes, one can envisage possible clinical cell therapy using the organoid expansion technique. Using “differentiation conditions” the expanding progenitor cells were differentiated into hepatocytes that had synthetic and drug metabolising capacity in vitro. When 1–2 × 10^6 human liver organoid cells from 4 donors were injected intrasplenically into 5 immunodeficient mice with liver injury they engrafted the liver at low levels in 2 of the 5 recipients. At 120 days post-transplant, donor cells were mostly seen as single cells or doublets, but occasionally as small cells clusters. These mice had very low, but detectable levels of human albumin in their blood. The sub-therapeutic albumin levels are likely due to the very low engraftment levels and their future use in models with a selective advantage for the transplanted cells would be interesting.

An important question is what would be the best mode of therapeutic application? This could potentially range from ex vivo use in a bioartificial liver to direct cell therapy. For cell therapy, in addition to significantly increasing cell engraftment, it would be important to ascertain whether the organoid derived hepatocytes form functional connections with other liver cells. Retention of homeostatic qualities is also vital, i.e. do the cells divide appropriately following injury but remain quiescent at other times. Furthermore are the cells phenotypically stable following injury in vivo?

Obviously for autologous therapy of genetic liver diseases of the hepatocyte some form of gene correction ex-vivo would be required. This has been previously tested using iPSCs derived hepatocytes derived from patients with alpha-1-antitrypsin (A1AT) deficiency.[7]

Another positive finding is that the differentiated hepatocytes recapitulated the disease phenotypes which may facilitate "disease in the dish" modelling and drug testing. The authors grew organoids from liver biopsies of patients with A1AT deficiency and upon differentiation the hepatocytes exhibited the disease phenotype with an accumulation of the mutant A1AT in hepatocytes and lack of functional A1AT secretion. The disease modelling was not limited to hepatocytes. When a single biopsy from one Alagille syndrome patient was tested, the authors could grow organoids but when a biliary differentiation protocol was applied the differentiating cells failed to form luminal structures and the cells underwent apoptosis. Alagille syndrome is caused by mutations in the Notch-signaling pathway leading to biliary atresia and it would be interesting to know whether such a phenotype could be corrected in vitro. Whilst this was only a single sample, it does indicate the potential of organoids for disease modelling of biliary diseases.

Only EpCAM positive ductal cells could serve as expendable cells in the organoids growth system whereas mature EpCAM negative hepatocytes could not be expanded. This is in interesting direct contrast to recent in vivo mouse lineage tracing reports, which could not identify ductal cells or hepatic progenitor cells with hepatocyte regeneration potential, and suggested all hepatocyte regeneration occurred through hepatocyte self-duplication.[8] Whilst the studies are entirely different and cannot be directly compared, it does raise the question as to why hepatocytes self-replicate so readily in vivo and ductular cells do not robustly regenerate hepatocytes in published mouse models. This may be due to several **theoretical** reasons: (1) Mouse hepatocytes and ductular cells may be different in their proliferation and differentiation potential to human cells, (2) In vitro organoid expansion conditions are not recapitulated in vivo and although useful are "artificial" and do not inform the in vivo situation in mouse and human liver, or (3) The published mouse models of liver injury and regeneration do not accurately model the severe liver injury seen in human disease where there is often significant hepatocyte senescence and a major ductular response. Answering these questions will help us understand the regenerative potential of liver's different cellular compartments, why regeneration fails in disease and how best to remedy this.

References

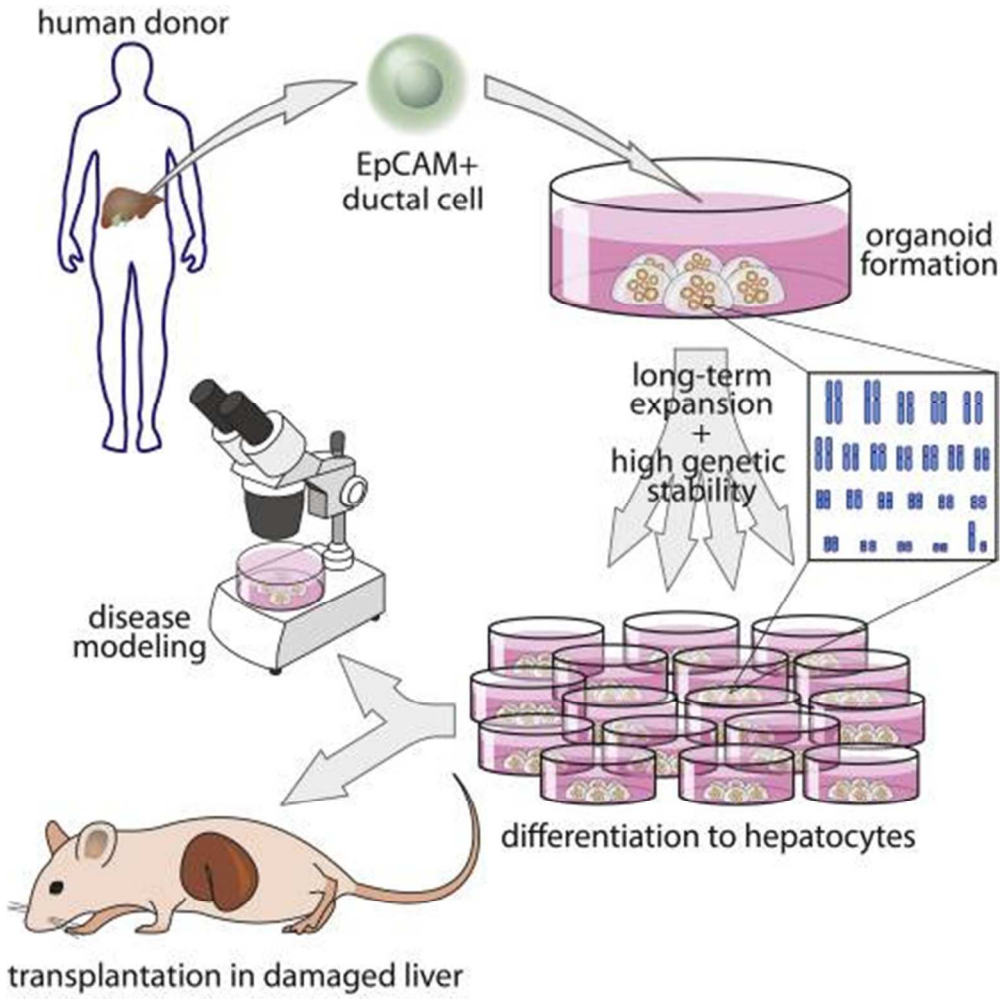
1. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver.. Cell. 2015 Jan 15;160(1-2):299-312.
2. Hay DC, Fletcher J, Payne C, Terrace JD, Gallagher RC, Snoeys J, et al. Highly efficient differentiation of hESCs to functional hepatic endoderm requires ActivinA and Wnt3a signaling. Proc Natl Acad Sci U S A. 2008 Aug 26;105(34):12301-6

3. Sullivan GJ, Hay DC, Park IH, Fletcher J, Hannoun Z, Payne CM, Dalgetty D, et al. Generation of functional human hepatic endoderm from human induced pluripotent stem cells. Hepatology. 2010 Jan;51(1):329-35.
4. Baxter M, Withey S, Harrison S, Segeritz CP, Zhang F, Atkinson-Dell R, et al. Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. J Hepatol. 2015 Mar;62(3):581-9
5. T. Sato, R.G. Vries, H.J. Snippert, M. van de Wetering, N. Barker, D.E. Stange, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature, 459 (2009), pp. 262–265 M.
6. Huch, C. Dorrell, S.F. Boj, J.H. van Es, V.S. Li, M. van de Wetering, et al. In vitro expansion of single Lgr5+ liver stem cells induced by Wnt-driven regeneration. Nature, 494 (2013), pp. 247–250
7. Yusa K, Rashid ST, Strick-Marchand H, Varela I, Liu PQ, Paschon DE, et al. Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Nature. 2011 Oct 12;478(7369):391-4.
8. K. Yanger, D. Knigin, Y. Zong, L. Maggs, G. Gu, H. Akiyama, et al. Adult hepatocytes are generated by self-duplication rather than stem cell differentiation. Cell Stem Cell, 15 (2014), pp. 340–349

Figure legend

Human liver cell expansion using the organoid culture system.

Liver tissue was perfused and epithelial cells were separated into ductal cells and hepatocytes before plating in organoid culture medium. EpCAM+ ductal cells were readily and massively expandable as organoids, whereas EpCAM- hepatocytes could not be expanded. The EpCAM+ ductal cells maintained a high degree of genetic stability during the expansion, were bipotential and could be differentiated into cells with a hepatocyte or biliary cell phenotype and function. Organoids grown from the livers of patients with genetic biliary diseases expressed the disease phenotype upon differentiation. Cells grown as organoids could be transplanted into immunodeficient mice but had a low engraftment level in the Retrorsine/CCl4 model used and consequently produced low levels of human serum albumin. (reprinted under Creative Commons license)



131x131mm (96 x 96 DPI)