Characterization of human stem cell- derived hepatocytes

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Abstract

Chronic liver diseases represent a major worldwide health concern. Liver transplantation is the only effective cure for these severe and chronic diseases, but due to the lack of human donors, this treatment is only available to a limited number of patients. An alternative treatment is the cell-based transplantation of hepatocytes or hepatocyte-like cells (HLCs) derived from human stem cells.

Our aims are to produce a physiologically relevant human liver model from human stem cells, and to scale-up model production for utilisation in disease modelling and drug testing.

The Wnt/β-catenin signalling pathway is involved in hepatoblast proliferation and differentiation during liver development and regeneration. Here we show that the inhibition of GSK3β stabilizes β-catenin during human Embryonic Stem Cell (hESC) -derived hepatoblast differentiation, resulting in increased β-catenin levels and the early appearance of a truncated form of β-catenin. In addition, HLC morphology was improved, as well as the upregulation of HNF4α and albumin expression.

Although hESCs are able to differentiate into almost any cell type including hepatocytes, their genetic instability in culture limits their utilization in regenerative medicine and cell- based transplantation therapy. Adult liver stem/hepatic progenitor cells (HPCs) on the other hand are genetically stable when cultured in vitro and can differentiate into both hepatocytes and biliary epithelial cells following severe liver injury.

We show that EpCAM+ HPCs isolated from human liver tissue have the capacity to proliferate in extended 3D culture (organoids) whilst maintaining progenitor characteristics. Importantly, they can also differentiate into hepatocyte-like. cells, resulting in the loss of hepatic progenitor cell markers, and an increase in the expression of differentiated hepatocyte-specific genes.

However, differentiated hepatic organoids showed low albumin expression compared to primary human hepatocytes, suggesting that these may have arisen from a limited population of cells or that the organoid-derived hepatocytes were not fully mature.

Compared to EpCAM+ sorted cells alone, fluorescent cell sorting using a combination of markers (EpCAM, CD133 and CD24) produced a more homogeneous cell population that differentiated more effectively into hepatocytes.

To improve the differentiation characteristics of our HLCs, acellular liver scaffolds were produced by the decellularization of rat liver using detergents. These scaffolds directed the cultured HPCs and non-parenchymal cells to their natural organellar localization and improved albumin expression in differentiated HPCs, thereby providing a viable model for drug testing and bio-engineered liver transplantation.

Generating functional hepatocytes from HPCs could provide an unlimited supply of hepatocyte-like cells, reducing animal use in drug screening, the testing of new chemical entities and the modelling human liver diseases.