The PNPLA3 I148M variant enhances the disease phenotype of hepatic stellate cells in an in vitro model of non-alcoholic fatty liver disease

Tomasz Kostrzewski1, Paloma Maraver1, Ana Levi2, Kareene Smith1, Krista Rombouts1 and David Hughes1

1. CN Bio Innovations Limited, BioPark Hertfordshire, Welwyn Garden City, UK, AL7 3AX.
2. University College London, Institute for Liver & Digestive Health, Royal Free Hospital, London, UK

Correspondence: david.hughes@cn-bio.com

RESULTS

The single nucleotide polymorphism 148M (in the gene encoding patatin-like phospholipase domain-containing-3, PNPLA3) is strongly associated with adverse outcomes for patients with NAFLD. PNPLA3 I148M has been associated with increased hepatic inflammation, steatosis and enhanced development and severity of liver fibrosis. The mutation has been shown to directly affect hepatocytes and become hyper-proliferative by interfering with lipase activities causing intracellular lipid droplets to accumulate (2). PNPLA3 I148M has been shown to affect the foragenic phenotype of hepatic stellate cells (HScs) (3). HScs are the key players in extra cellular matrix deposition in the liver and primary 148M HScs have been found to produce more inflammatory mediators and have a higher lipid droplet content (4).

However, the effects of this mutation in HSc has only been explored using isolated HSc. To demonstrate the utility of this model we have analysed how the 148M mutation in the PNPLA3 gene are associated with disease progression and have been recently shown to directly influence HSc disease phenotype.

MATERIALS & METHODS

PNPLA3 I148M variant in hepatic stellate cells promote the NASH phenotype in the co-culture microcultures

PNPLA3 I148M variant in hepatic stellate cells promote the NASH phenotype in the co-culture microcultures. The triple-culture model was run with matched WT controls to account for wild type control. Gene expression profiles were compared with disease associated genes and increased levels of fat loading. These findings confirm the direct effect of the PNPLA3 I148M mutation on the disease phenotype generated by HScs.

Figure 4 – Obeticholic acid reduces NASH phenotype in co-culture microcultures

Figure 5 – PNPLA3 I148M variant in hepatic stellate cells promote the NASH phenotype in the co-culture microcultures

Figure 6 – The cytokine profile in the 3D in vitro NASH model is altered by fat loading, LPS dosing and the PNPLA3 I148M variant in hepatic stellate cells

Figure 7 – Physiobank is a perfused cell culture system, with open well plates, design for the culture of primary human cells. A) Physiobank™ LC-12 plate along with 0.06 x 106 Hepatocytes and 0.06 x 106 HScs. Cultures were run for 14 days under lean, fat and fat + LPS conditions. B) IL-6 production in the culture medium. C) Fat loading as measured by Oil Red O staining. Data are mean ± SD, n = 3. * = P < 0.05.

REFERENCES

1. Wiberg C, Petersen IV, Maes M, Gengyo-Andia MC, Ochsenrau KH, Ribo A, Ghiotto P, Villanueva A, Schwarz S, Witters LM, et al. Hepatic stellate cells promote the NASH phenotype in the co-culture microcultures. The triple-culture model was run with matched WT controls to account for wild type control. Gene expression profiles were compared with disease associated genes and increased levels of fat loading. These findings confirm the direct effect of the PNPLA3 I148M mutation on the disease phenotype generated by HScs.

This work further demonstrates that the 3D in vitro NASH model is ideally suited to exploring the molecular mechanisms that underlie the development of human NASH and is a useful tool for analysing the efficacy of novel anti-NASH therapeutics against a range of different target pathways.

CONCLUSION

Using the 3D perfused culture platform PhysioMimix™ we have generated fully human models of NAFLD and NASH. We have used these to create a fully human in vitro NASH model that recapitulates key features of human NASH. We have demonstrated the utility of these models by studying the effect of the PNPLA3 I148M mutation on disease phenotype and gene expression profiles in primary human hepatic stellate cells.

Table 1 – PNPLA3 I148M variant in hepatic stellate cells alters gene expression profile of the NASH in vitro model

Table 2 – Costimulatory molecules are expressed in the co-culture microcultures

CONCLUSION

Using the 3D perfused culture platform PhysioMimix™ we have generated fully human models of NAFLD and NASH. We have used these to create a fully human in vitro NASH model that recapitulates key features of human NASH. We have demonstrated the utility of these models by studying the effect of the PNPLA3 I148M mutation on disease phenotype and gene expression profiles in primary human hepatic stellate cells.

Table 1 – PNPLA3 I148M variant in hepatic stellate cells alters gene expression profile of the NASH in vitro model

Table 2 – Costimulatory molecules are expressed in the co-culture microcultures

REFERENCES

1. Wiberg C, Petersen IV, Maes M, Gengyo-Andia MC, Ochsenrau KH, Ribo A, Ghiotto P, Villanueva A, Schwarz S, Witters LM, et al. Hepatic stellate cells promote the NASH phenotype in the co-culture microcultures. The triple-culture model was run with matched WT controls to account for wild type control. Gene expression profiles were compared with disease associated genes and increased levels of fat loading. These findings confirm the direct effect of the PNPLA3 I148M mutation on the disease phenotype generated by HScs.

This work further demonstrates that the 3D in vitro NASH model is ideally suited to exploring the molecular mechanisms that underlie the development of human NASH and is a useful tool for analysing the efficacy of novel anti-NASH therapeutics against a range of different target pathways.