Epigenetics and liver fibrosis

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is a range of conditions covering a spectrum of pathological changes in the liver from steatosis through steatohepatitis to fibrosis and cirrhosis then ultimately hepatocellular carcinoma.[1] NAFLD is caused by accumulation of fats in the liver and is normally seen in overweight and obese people.[1] There is no treatment available for NAFLD besides managing diet, cholesterol level and obesity.

However, as the understanding of the pathogenesis of NAFLD has been significantly advanced over the years, recent studies suggest that epigenetic regulations of genes involved in generation of DNA methylation and histone modifications marks exists. These in turn regulate transdifferentiation of quiescent hepatic stellate cells into matrix-secreting myofibroblasts that lead to initiation of fibrogenesis and subsequent fibrosis in the liver.

- Epigenetics is defined as "study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself" that are hereditable via cell division.

Aim

To identify the changes in expression of enzymes that regulate DNA methylation and histone modifications in liver exposed to fat.

Methods

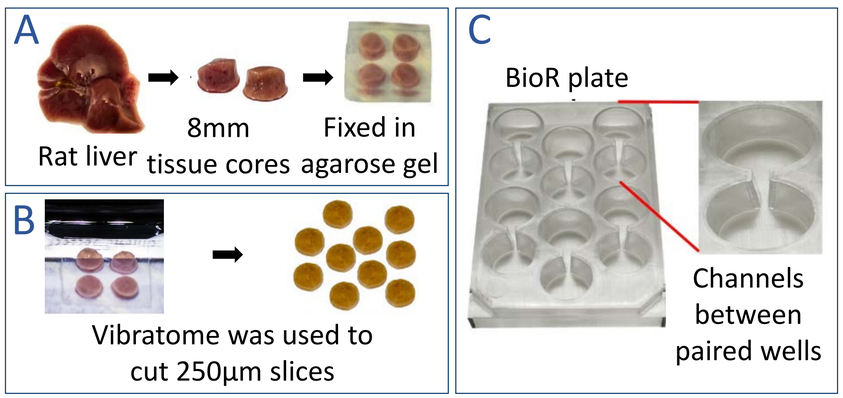

Figure 1 Preparation of PCLS

Cell cultures were previously used to study liver fibrosis but they lacked cell-cell interactions as the cells are grown on plastic. Thus, precision-cut liver slices (PCLS) were used in this study as the cell numbers and composition remain intact. The PCLS were cultured using a novel bioreactor culture system to keep the tissue alive up to 6 days.[3] PCLS were obtained using the steps shown in Figure 1 (A,B) and were cultured in Williams Medium E (W4128, Sigma-Aldrich) in 12-well BioR plates as shown in Figure 1 (C) and rocked on a bioreactor platform. A channel is present between every 2 wells to allow bidirectional flow of media to oxygenate the PCLS and prevent accumulation of toxic products and metabolites.

PCLS samples were prepared by the following methods:

- Control : cultured for 96 hours
- Lipid-treated: cultured for 24 hours then a mixture of fatty acids and bovine serum albumin solution in a 10 to 1 ratio was added to the samples every 24 hours to mimic fat accumulation in the liver in NAFLD patients

RNA was isolated and cDNA was synthesised from each sample. Quantitative PCR was performed to determine the changes in mRNA expression of enzymes that regulate DNA methylation and histone modifications in each sample.

Results and Discussion

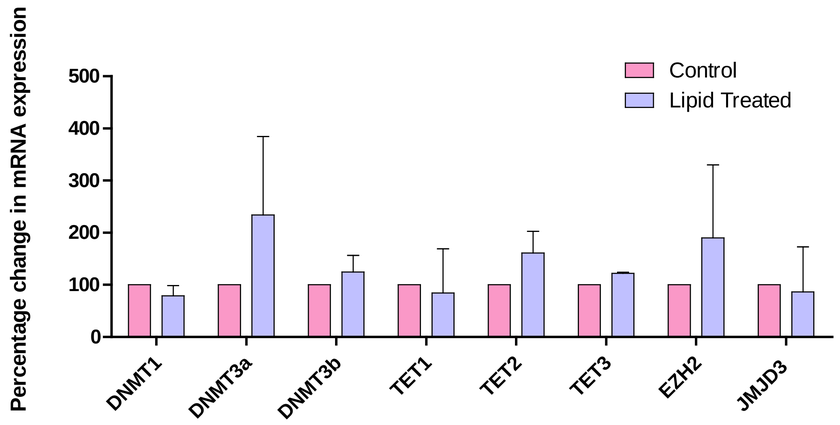

Figure 2 Epigenetic marker levels

Results presented in Figure 2 showed that DNMT3a, DNMT3b, TET2, TET3 and EZH2 were upregulated in lipid-treated PCLS whereas DNMT1, TET1 and JMJD3 were unchanged when compared to control PCLS.

Control sample had been normalised to 100 so that a better comparison between control and lipid-treated PCLS can be made. GAPDH gene is measured as a control as it is a housekeeping gene that can be found in all cells.

DNMT3a and 3b are involved in de novo methylation of DNA whereas DNMT1 is involved in maintaining DNA methylation pattern in cells.[5] TET genes play a key role in activation of gene transcription by carrying out DNA hydroxymethylation.[5] EZH2 acts as a lysine methyltransferase which transfer methyl groups to lysine 27 of histone H3 thus causing transcriptional repression of target gene; whereas JMJD3 reverses the methylation of lysine residues on histones.[5]

Conclusion

The data demonstrate that expression of epigenetic modulators alter in response to accumulation of fats in the liver.

Further studies need to be done to have a better understanding of the roles and specific actions of these genes during fibrosis in order to develop drugs that are able to reverse the expression of the epigenetic regulatory markers shown in Figure 2 in order to prevent and treat liver fibrosis.

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