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Validation of microRNAs regulated by vitamin D and lipid loading in immortalised hepatocytes

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MicroRNAs (miRNAs) play a critical role in the progression of non-alcoholic fatty liver disease (NAFLD)[(1)], but their differential regulation by dietary nutrients, such as vitamin D, is not yet fully understood. To investigate the role of vitamin D in NAFLD pathogenesis, these experiments aimed to measure miRNA expression in immortalised hepatocytes treated by vitamin D and/or fatty acids.

Immortalised hepatocytes, HepG2 cells, were cultured for 16 hours in the charcoal-stripped, serum-containing medium before 24-hour treatment with 100 nM calcitriol or fatty acid (500 M, 1:1 oleic acid and palmitic acid) or both. Vitamin D treatment was confirmed through CYP24A1 mRNA induction as a positive control. MiRNA samples from five biological experiments were collected by miRNeasy Tissue/Cells Advanced Mini Kit (QIAGEN) and synthesised to cDNA using TaqMan™ Advanced miRNA cDNA Synthesis Kit (Thermo Fisher).

Fourteen candidate dysregulated miRNAs, identified either from our miRNA sequencing experiment[(2)], or our comprehensive review about miRNAs dysregulated by vitamin D and NAFLD[(3)], were measured by qPCR using TaqMan™ Advanced miRNA Assay (Thermo Fisher), including let-7b-5p, miR-125b-1-3p, miR-17-3p, miR-34a-5p, miR-181a-2-3p, miR-27a-5p, miR-27a-3p, miR-27b-5p, miR-27b-3p, miR-125a-3p, miR-125b-2- 3p, miR-125b-5p, miR-155-5p and miR-155-3p. In addition, these were normalised to validated endogenous control miRNAs (miR-125a-5p, miR-222-3p and miR-455-3p). Data were analysed by one-way ANOVA with Dunnett's multiple comparisons test.

Surprisingly, only miR-125b-5p was found significantly down-regulated in both vitamin D (relative fold change (RFC): 0.83, $p = 0.02$) and cotreatment (RFC: 0.81, $p = 0.01$) relative to miR-455-3p. Multiple miRNAs (miR- 125b-1-3p, miR-155-3p and miR-181a-2-3p) remained undetected even after optimisation of cDNA synthesis. Other miRNAs showed a wide variance of RFC among 5 independent experiments and were not significantly dysregulated by vitamin D, fatty acid or cotreatment.

Our data identify miR-125b-5p as down-regulated by both vitamin D and lipid loading in immortalised hepatocytes. This is of interest as miR-125 has previously been found altered in serum from NAFLD patients and to be involved in liver fibrosis. Future functional and genetic experiments will further investigate the mechanism of miR-125-5p regulation by vitamin D.

In addition, inconsistent results were found between miRNA sequencing and qPCR measurements of candidate miRNAs. Variance is likely derived in part from the low levels of miRNA expression being measured and the sensitivity of RNA sequencing methodology. Therefore, a critical next step will be re-analysis of the miRNA sequencing raw data, focusing on adaptor trimming and analysis of unidentified sequences.

References

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