Spring Conference, 29–30 March 2021, Gut microbiome and health

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

Z. Zhang, R. Moon, H. Fuller, J.L. Thorne and J.B. Moore
School of Food Science and Nutrition, University of Leeds, Leeds, UK

Evidence for a role for vitamin D in metabolic-associated fatty liver disease (MAFLD) pathogenesis is conflicting[1]. MicroRNAs (miRNAs) play an essential role in both the progression of MAFLD[2], and in mediating the cellular response to vitamin D including the post-transcriptional regulation of the vitamin D receptor (VDR)[3]. However, the potential roles for vitamin D regulated miRNAs in the molecular pathogenesis of MAFLD remains unexplored. With an overall objective of investigating the role of vitamin D regulated miRNAs in MAFLD, the aims of these experiments were to: measure miRNA expression in immortalised hepatocytes (HepG2 cells) in response to vitamin D alone, or in combination with lipid loading; and to examine potential functional and mechanistic effects using bioinformatics.

Cells were cultured for 16hrs in charcoal-stripped serum-containing media prior to 24h treatment with either 100nM 1α,25-dihydroxyvitamin D3, or fatty acid (500μM, 1:1 oleic acid: palmitic acid), or both. miRNA samples from four biological experiments were collected using mirVANATM miRNA isolation reagents and synthesised to cDNA. TaqMan® array human miRNA A +B cards were used to measure miRNA expression in equally pooled cDNA samples. Data were examined by relative fold change, and also (in R version v4.0.3) by principal component analysis (PCA) and significance analysis of microarrays (SAM) approaches. Bioinformatic analyses included the identification of miRNA gene targets, geneset enrichment analysis (GSEA), and functional annotation and pathway analyses; utilizing the miRWALK (v3.0), DAVID (v6.8), and Reactome (v75) knowledgebases and their associated tools.

327 of 768 (42.6%) miRNAs were detected (below a threshold of Ct<32) across all four treatment groups. Of these, 7 miRNAs were identified as potential endogenous controls based on very low expression variance (<1%) across groups. A high percentage of detected miRNAs were found increased (fold change>1.33) and decreased (fold change <0.67) relative to vehicle treated cells (56.9%, 72.8% and 76.1% in vitamin D, fatty acid, and cotreated cells respectively). However, SAM analysis suggested across groups only 55 (16.8%) miRNAs were significantly upregulated, and none were detected as downregulated at delta = 1.266 and a median false discovery rate of 0.193. Utilising miRWALK, 459 unique gene targets were found for the 55 SAM identified miRNAs. GSEA identified the AMPK, Pi3K-Akt and FOXO signalling pathways among the most significantly ($P < 0.001$) regulated.

In summary, these experiments identify miRNAs regulated by vitamin D and lipid loading in immortalised hepatocytes. Bioinformatic results highlight signalling pathways with critical roles in insulin signalling known to be dysregulated in diabetes and MAFLD. Ongoing work is integrating these data with analyses from analogous experiments in immortalised hepatic stellate cells (LX-2). From this, a subset of candidate miRNAs will be followed up for independent verification of functional and mechanistic effects.

References

1. Zhang Z, Thorne JL & Moore JB (2019) *Curr Opin Clin Nutr Metab Care* **22**, 449–458.
2. Wang X, He Y, Mackowiak B *et al.* (2020) *Gut* **70**(4), 784–795.
3. Zenata O & Vrzal R (2017) *Oncotarget* **8**, 35390–35402.

Proceedings of the Nutrition Society