Transcriptome landscape of HepG2 cells with deleted late genes of cholesterol synthesis: focus on RORC and the circadian clock

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Cholesterol synthesis is a metabolic pathway with many steps and still open mysteries. Evidence suggests that cholesterol intermediates between zymosterol and desmosterol may serve as ligands of nuclear receptors from ROR (RAR Related Orphan Receptor) family.

These sterols are thought to be RORC agonists, regulating the expression of genes involved in lipid and glucose metabolism, as well as in the circadian clock genes. RORs are part of stabilizing loop of circadian clock and we hypothesize that the activation of RORC with non-polar sterols fine-tunes the expression of clock genes.

To investigate the downstream biological roles of the different cholesterol intermediates, we used the CRISPR-Cas9 on human HepG2 cells. We produced cell lines, each with a knockout (KO) of a different gene from the late part of cholesterol synthesis (CYP51, DHCR24, SC5DL and HSD17B7). Each cell line accumulates the upstream sterols and lacks intermediates downstream of the deleted enzyme. Transcriptome changes in KO cell lines were evaluated using microarrays and Nanopore sequencing and differentially expressed genes and pathways assessed using KEGG and TF enrichment analysis. Analysis revealed 102 differentially expressed genes altered in all KO cells, mostly associated with impaired cholesterol synthesis and related metabolic pathways. RORC target genes were altered to varying degrees, with the highest number of upregulation in SC5D (23) and HSD17B7 (62) KO cells. This suggests RORC activation through accumulated zymostenol and zymosterol. Each KO cell line differentially expressed a number of genes, indicating the specific signalling functions of the accumulated sterols which are still under investigation. The role of individual sterols from cholesterol synthesis in RORC-mediated fine tuning of the clock genes, the circadian phase and the period, is in progress.