ABSTRACT

The peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors involved in energy homeostasis. Their natural ligands are fatty acids and there are three different PPAR isoforms; PPARA, PPARG and PPARD. They are encoded by separate genes and have distinct functions, due to different tissue expression and affinity for ligands. PPARA controls genes involved in fatty acid oxidation, PPARG regulates genes important for fatty acid storage, and PPARD controls genes implicated in lipid oxidation and lipoprotein metabolism. Primates and humans treated with a PPARD agonist (GW501516) resulted in improved insulin sensitivity, increased HDL and decreased LDL cholesterol levels, making it a putative drug candidate for treatment of metabolic disease. PPARD has recently been assigned a beneficial role in macrophages, by inducing a switch from proinflammatory (M1) to antiinflammatory (M2) macrophages.

To characterize additional target genes of PPARD involved in the lipoprotein metabolism, the effect of PPARD activation on the apolipoprotein A-II (apoA-II) gene was investigated in human hepatoma cells. ApoA-II is one of the major proteins in the HDL particles. Treatment with GW501516 increased apoA-II promoter activity and mRNA levels in hepatoma cell lines. A site located at -737/-717 in the promoter was identified as the functional PPAR response element (PPRE). These results suggest that increased expression of the apoA-II gene is one of the reasons for the beneficial effects on lipoprotein metabolism after treatment with the PPARD agonist.

To investigate whether PPARs could regulate the alanine aminotransferase (ALT) genes, the effect of PPARA, G and D agonist treatment was studied. ALT activity in plasma is used as a marker for hepatotoxicity in humans. During a clinical trial with the PPARA ligand, AZD4619, the plasma ALT activity increased in some patients and *in vitro* studies showed that ALT1 protein and mRNA expression was induced by treatment with PPARA agonists in primary hepatocytes. Similarly, transient transfection of a promoter construct of ALT1 in HuH-7 cells showed increased activity mediated via a PPRE located at -574 after treatment with PPAR agonists. This study shows that the ALT1 gene is regulated by PPARs and that PPAR drugs might contribute to increased ALT activity in serum.

To explore regulation of the PPARD gene by posttranscriptional events, 5'- and 3'-RACE were performed on cDNA obtained from placenta, adipose tissue and pancreas. Both 5'- and 3'-alternative splicing of PPARD was identified. Coupled transcription/translation showed that the length and number of upstream AUGs in the 5'-UTR had a major impact on translational efficiency. Further, the promoter located upstream of exon one was verified as the major promoter, using reporter gene assays. A 3'-splice variant encoding a truncated PPARD protein, PPARD2, was shown to be a negative regulator of the full length receptor, PPARD1, in transient transfection assays.

To identify whether PPARD is regulated by microRNA (miRNA), the 3'-UTR was analysed *in silico*. Two putative miRNA target sites were identified in the PPARD 3'-UTR; miR-9 and miR-29. The miR-9 was verified as a functional miRNA targeting PPARD. However, PPARD mRNA levels remained unaffected by miR-9 expression, indicating that only the translation of PPARD was inhibited. Since both miR-9 and PPARD have been shown to play important roles in the inflammatory response of monocytes, the regulation of PPAR expression by miR-9 was investigated in these cells. A suppressive role of miR-9 on PPARD expression was identified in monocytes after LPS treatment but not in M1 or M2 macrophages, suggesting that the regulatory role of miR-9 on PPARD is exerted in monocytes, before differentiating into macrophages.

In summary, this thesis describes additional functions and ways of regulation of the ubiquitously expressed transcription factor PPARD with a major role in both health and disease.

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