Background

Non-alcoholic fatty liver disease is the hepatic manifestation of the global epidemic of metabolic disease. It is tightly associated with obesity and type 2 diabetes, yet the precise mechanisms that drive its aetiology are not fully defined. Steroid hormones, including glucocorticoids and sex steroids, regulate metabolic phenotype; additionally, bile acids have recently been identified as potent metabolic regulators. AKR1D1 (5β-reductase), is predominantly expressed in the liver, and is a crucial regulator of steroid hormone clearance as well as bile acid synthesis. Its role in pathogenesis of metabolic disease has not been examined. We therefore developed systems to define the enzymology of human AKR1D1 in cell free assays and to determine the impact of manipulation of AKR1D1 expression and activity in human hepatocyte models.

Methods

B21 Rosetta bacteria cells were transformed with an AKR1D1 construct (pNIC-CTHF+AKR1D1) and recombinant protein extracted and purified. A high throughput assay was developed to determine AKR1D1 activity, substrate specificity and enzyme kinetics. 9 different substrates (7 steroids and 2 bile acid intermediates) were tested against the purified enzyme and activity was measured by NADPH reduction in a dose and time dependent manner. AKR1D1 mRNA expression was characterized in 4 different hepatoma cell lines (Hep3b, HepG2, C3A and Huh7.0) as well as primary cultures of human hepatocytes. In addition, over-expression was characterized in 4 different hepatoma cell lines (Hep3b, HepG2, C3A and Huh7.0) as well as primary cultures of human hepatocytes. Successful over-expression and knock down of AKR1D1 in HepG2 cells were also performed. AKR1D1 was highly expressed in HepG2 cells and expression decreased through differentiation, to levels that were similar to those seen in primary cultures of human hepatocytes. Successful over-expression and knock down of AKR1D1 were confirmed in HepG2 cells using real-time PCR. Importantly, changes in gene expression were paralleled by functional activity as measured by progesterone clearance.

Results

1. Substrate specificity of AKR1D1

2. Enzyme kinetics of AKR1D1

3. AKR1D1 inhibition by 5αR inhibitors

4. Genetic manipulation of AKR1D1

5. HepG2 differentiation by DMSO treatment

Conclusion

We have successfully manipulated AKR1D1 expression and activity that will serve as the platform for future studies to define its role in the regulation of metabolic phenotype within the liver.