

Epigenetic Regulator Silencing by siRNA for the Treatment of Metabolic Diseases

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Background*: Non-alcoholic fatty liver disease (NAFLD) is a growing health problem worldwide, ranging from non-alcoholic fatty liver (NAFL) to the more severe metabolic non-alcoholic steatohepatitis (NASH). Although many studies have elucidated the pathogenesis of NAFLD, the epigenetic regulatory mechanism from NAFL to NASH remains incompletely understood.

Methods*: We employed a methionine- and choline-deficient (MCD) diet–induced NASH mouse model combined with genome-wide RNA sequencing (RNA-seq) to identify transcriptional alterations. Biochemical assays, histological evaluation, and cellular analyses were conducted to validate disease severity and molecular changes. In addition, siRNA-mediated knockdown experiments were performed in hepatocyte cultures to assess the functional role of candidate genes.

Results*: We show that mice lacking the one allele of the *Mll4* gene are resistant to hepatic steatosis, inflammation, and fibrosis in NASH conditions compared to wild-type controls. Transcriptome analysis of the livers of control and *Mll4*$^{+/-}$ mice identified pro-inflammatory genes regulated by the nuclear factor kappa B (NF-κB) signaling pathway as major target genes of MLL4. We show that MLL4 binds to p65 and that MLL4 is required for NF-κB transactivation. Myeloid-specific *Mll4* knockout mice showed an almost complete block of NASH, while hepatocyte-specific *Mll4* knockout mice showed mild inhibition of steatosis. Importantly, we show that histone H3-lysine 4 methylation mediated by the MLL4-complex plays a critical role in promoting the expression of *Ccl2* in hepatocytes and M1 marker genes in macrophages.

Conclusion*: Our results demonstrate that MLL4, through the NF-κB-MLL4 regulatory axis, exacerbates steatohepatitis in the context of an inflammatory response and represents a potential therapeutic target for NASH.