Plasticity of histone modifications around Cidea and Cidec genes with secondary bile in the amelioration of developmentally-programmed hepatic steatosis

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論文題目

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(Cidea, Cidec 遺伝子周辺におけるヒストン修飾の可塑性：発達期にプログラムされた肝脂肪変性が二次胆汁酸により改善する機序における関与)

論文の内容の要旨

[Introduction]

Increasing evidence supports the relationship between nutritional imbalances in the early developmental period and a predisposition for non-alcoholic fatty liver disease (NAFLD) in later life. We recently reported that undernourishment in utero primes hepatic steatosis under obesogenic diet and that treatment with tauroursodeoxycholic acid (TUDCA), a secondary bile acid, extremely improved the fat deposit. The cellular and molecular mechanisms behind are still illusive. Hence we aimed to study the epigenetic regulation in pathophysiology underlying developmental programming as well as marked recovery by TUDCA treatment.

[Materials and Methods]

Pregnant C57Bl/6NCr mice were purchased and were randomly divided into an ad libitum or normal nourished group (group NN dams; n=20) and a 40% caloric restriction or undernourished group (group UN dams; n=20). We used male offspring only and adjusted the number to 8 pups per litter. Pups were then fed the regular chow diet for 1 week (up to 9 weeks) followed by a high-fat diet (HFD) containing 60% lipids up to 22 weeks in order to mimic an obesogenic diet. Different experimental procedures were performed on 3 cohorts as follows: cohort 1 (NN and UN at 9 weeks; before HFD), cohort 2 (NN and UN at 17 weeks; after HFD), and cohort 3 (NN-Veh, NN-TU, UN-Veh, UN-TU at 22 weeks; HFD with or without the TUDCA treatment). In cohort 3, TUDCA was orally administered by gastric lavage at 0.5 g/kg body weight per day while pups were on HFD. For genetic expression analysis we performed Microarray using Affymetrix. As for epigenetic analysis, DNA methylation was assessed by methyl binding domain (MBD) whole genome sequencing and histone modification was carried out by Chromatin immunoprecipitation (ChIP) assay. All animal procedures were approved by the Institutional Animal Research Committee of Hamamatsu University School of Medicine (H20-014) and the standards of humane animal care by the criteria outlined in the “Guide for the Care and Use of Laboratory Animals”.

[Results]

Microarray gene expression analysis showed wide range of genetic changes from which

we have selected 9 genes of interest (GOI) by assessing the result in longitudinal comparison (before and after HFD) and cross-sectional comparison (with or without TUDCA treatment) after HFD. Thereby, in this study we focused on two genes, Cell Death-Inducing DNA Fragmentation Factor-Like Effectors A (Cidea) and C (Cidec). Gene enrichment analysis using DAVID Bioinformatics Resources 6.8 showed that Cidea and Cidec are most involved in function of lipid droplet and lipid particle pathway among 9 GOI. Indeed, they are enhancers of lipid droplet (LD) sizes in hepatocytes and showed the greatest up-regulation in expression by UN among 9 GOI that were completely recovered by TUDCA, concomitant with parallel changes in LD sizes. Then, we investigated significant differentially methylated sites on the full-length genes of Cidea and Cidec, using overlapping peaks by DNA MBD sequencing by next-generation sequencer. Neither maternal caloric restriction (UN) nor the TUDCA treatment had any effect on DNA methylation around entire 24kb Cidea genes and entire 11kb Cidec genes. We further investigated histone modifications around Cidea and Cidec genes by ChIP assay, concerning mono- and di- methylation of H3K9, H3K27, and H3K36, di-methylation of H3K4, tri-methylation of H3K9, H3K27 and H4K20, and acetylation of H3K9 and H4. TUDCA remodeled developmentally-induced histone modifications (di-methylation of H3K4, H3K27, or H3K36) around the Cidea and Cidec genes in UN pups only. Changes of these histone modifications may contribute to the markedly down-regulated expression of Cidea and Cidec genes in UN pups, which was observed in alleviation of hepatic fat deposition even under HFD. Intriguing results were observed when we performed ChIP assay in cohort 1 and 2, before and after HFD. H3K27 di-methylation was unaltered in Cidea although suppressed in Cidec in cohort-1 before HFD and it remained persistently suppressed through cohort 2 and 3 after HFD.

[Discussion]

UN in utero programmed an enhanced-expression of Cidea and Cidec, accumulating intrahepatic LDs and leading to deterioration of hepatic steatosis. Herein, plasticity of histone modification was observed underlying not only in pathophysiology, but also in the treatment with endogenous bile acid like TUDCA. Our results suggest that these chromatin programming might be occurring as early as in maternal in utero environment and triggered by HFD in adulthood. Moreover, these modifications can be restored by endogenous component even at established stage of hepatic steatosis.

[Conclusion]

In the present experimental model, we demonstrated the plasticity in the developmentally-programmed histone modifications around the specific genes of Cidea and Cidec, in the process of the amelioration of hepatic steatosis by TUDCA treatment.

The future prospect of this study shed lights on the chromatin plasticity as promising target of precision medicine for developmentally- programmed hepatic steatosis.