論文の内容の要旨

The Role of Methylation of Lysine 4 and Lysine 27 of Histone H3 in the Promoter Region of the Peroxisome Proliferator-Activated Receptor γ Gene in Adipocyte Differentiation

脂肪細胞分化における PPARγプロモーター領域のヒストン H3 リジン 4 とリジン 27 のメチル化の役割

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Background: Heritable change in the chromatin structure such as modifications of histone and DNA without nucleotide alteration — the epigenome — has been recognized to be crucial for differentiation and cell-type specific gene expression. Recent studies for histone modification on a genome-wide scale revealed the unexpected finding that the promoters of many developmental regulators in embryonic stem (ES) cells are characterized by co-existence of active histone H3 lysine 4 trimethylation (H3K4me3) and repressive histone H3 lysine 27 trimethylation

(H3K27me3), which is referred to as “bivalent modifications”. It is proposed that such bivalent modification is one of the key mechanisms by which stem cells maintain their stemness. It silences genes involved in differentiation in stem cells while keeping them poised for subsequent activation during differentiation. The bivalent domains of developmental genes are then “resolved” into either active or inactive histone modification during differentiation of stem cells.

PPARγ is a nuclear receptor which is the master regulator of adipogenesis and a molecular target for thiazolidinediones class of small molecules, which are widely prescribed to treat insulin resistance in obese patients. Numbers of factors are reportedly involved in adipogenesis such as PPARγ, C/EBPα, C/EBPβ, C/EBPδ, KLFs, EBFs, GATA factors, Wnt pathways, forming the transcription cascade. It is not, however, fully understood whether these factors explain the molecular mechanisms of adipocyte differentiation. For example, the mechanism of cell-type-specific activation of PPARγ and C/EBPα is still unclear. So we hypothesized that epigenetic mechanisms may be involved in the regulation of PPARγ expression and adipocyte differentiation.

In this study, we investigated histone modification pattern of H3K4 and H3K27 at the PPARγ promoters by using ChIP-qPCR in various cells with different potential to express PPARγ, such as embryonic stem (ES) cells, murine embryonic fibroblasts

(MEFs), 3T3-F442A, 3T3-L1, C3H10T1/2 and NIH-3T3 cells at their basal state and during differentiation. Furthermore, we performed retroviral overexpression and siRNA-mediated knockdown of histone-modifying enzymes to investigate functional relevance of change in H3K27 methylation in adipocyte differentiation.

Results: Bivalent modification (H3K4me3 (+), H3K27me3 (+)) was observed on the PPARγ1 promoter in ES cells and MEFs. Repressive H3K27me3 was lost and the bivalent domain was resolved to active histone modification (H3K4me3(+), H3K27me3(-)) during adipocyte differentiation of MEFs. This change was consistent with induction of PPARγ expression during adipogenesis. Active histone modification (H3K4me3 (+), H3K27me3 (-)) was observed at the PPARγ1 promoter already at the basal state in adipogenic 3T3-L1 cells and C3H10T1/2 cells. On the other hand, NIH-3T3 cells, which have very low potential to express PPARγ, have no either (H3K4me3(-), H3K27me3(-)). Interestingly, the loss of H3K27me3 modification on the PPARγ promoter during MEFs adipogenesis was independent of the DMI treatment — a combination cocktail of IBMX, dexamethasone and insulin — used to initiate adipogenesis in vitro. These data suggest that the resolution of the bivalent domain at the PPARγ1 promoter is more upstream event than the initiation of differentiation by the DMI treatment and may poise the cells for subsequent differentiation.

Recently, Utx and Jmjd3 were identified as demethylases of H3K27me3. Knockdown of Utx and Jmjd3 by siRNA in MEFs resulted in sustained maintenance of H3K27me3 on the PPARγ1 promoter during adipogenesis and significantly suppressed induction of PPARγ and triglyceride accumulation. The inhibition of adipogenesis by Utx/Jmjd3 knockdown was rescued by overexpression of PPARγ1, suggesting that suppression of PPARγ1 was, at least in part, responsible for suppression of adipogenesis by Utx/Jmjd3 knockdown. These data suggest that demethylation of H3K27 is an important process for expression of PPARγ and subsequent adipocyte differentiation of MEFs.

On the other hand, overexpression of Bmi1 — a core component in the Polycomb complexes — in 3T3-L1 cells caused forced acquisition of H3K27me3 and restored the bivalent domain at the PPARγ1 promoter, which resulted in suppression of PPARγ expression and adipocytes differentiation. We conducted deletion studies of Bmi1 and found that the Ring Finger domain of Bmi1 was necessary for the suppressive effect of Bmi1 on PPARγ and adipogenesis.

Conclusion: Our data indicate that epigenetic regulation of H3K27 methylation at the PPARγ1 promoter is an important process for adipocyte differentiation.