（様式４）

学 位 論 文 の 内 容 の 要 旨

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（学位論文のタイトル）

Histone deacetylase 3 inhibitor alleviates cerebellar defects in perinatal hypothyroid mice by stimulating histone acetylation and transcription at thyroid hormone responsive gene loci

（ヒストン脱アセチル化酵素3阻害剤は甲状腺ホルモン応答遺伝子のヒストンアセチル化と転写を活性化して周産期甲状腺機能低下症による小脳障害を軽減する）

（学位論文の要旨）2,000字程度、Ａ４判

Introduction

Thyroid hormones play a pivotal role in cerebellar organogenesis in both mice and humans. Perinatal hypothyroidism results in multiple cerebellar defects. Thyroid hormone receptors (TR) suppresses transcription of the target gene in the absence of ligand and induced transcription when thyroid hormones are present. Histone acetylation is highly correlated with transcriptional regulation factor including TR. Deacetylation of histone tails by HDAC3 is responsible for transcriptional repression by unliganded TR. Therefore, it is expected that enzymatic activity of HDAC3 is responsible for cerebellar developmental defects in hypothyroidism. In this study, we attempted to rescue cerebellar defects in perinatal hypothyroid mice by abrogating the enzymatic activity of HDAC3 using a specific inhibitor RGFP966.

Materials and methods

Pregnant C57BL/6J mice and pups were treated with 250 ppm of PTU in drinking water from E14 to PND21. Male littermate pups were divided into three groups to minimize maternal or environmental effects and were given daily subcutaneous injection from PND3 to PND21. One group was given RGFP966, vehicle control group was injected with corresponding amount of DMSO. Last group was administered with T4 to rescue PTU-induced hypothyroidism. On PND7 and PND14, some mice were fixed by perfusion with 4% paraformaldehyde, and cerebella were subjected to cryosectioning. Sagittal sections were stained

with hematoxylin and eosin. From PND3 to PND12, surface righting reflex was tested, and negative geotaxis test was performed on PND7. Rotarod test was performed on PND21. Total RNA was extracted from mouse cerebella on PND7 and PND14. Quantitative PCR was performed using THUNDERBIRD SYBR qPCR Mix. On PND14, mice cerebella were snap-frozen in liquid nitrogen and crushed in frozen mortars on liquid nitrogen. The crushed samples were subjected to cross-linking in 1% paraformaldehyde and subsequent ChIP assay.

Results

We first confirmed that RGFP966 relieved transcriptional repression by unliganded TR in a luciferase-based transcriptional reporter assay. These results led us to study *in vivo* effects of this inhibitor on hypothyroid subjects. We induced hypothyroidism and consequent cerebellar defects in perinatal male mice by propylthiouracil treatment and tried to rescue the phenotypes by postnatal administration of RGFP966. Perinatal hypothyroidism resulted in reduced body weight, and RGFP966-treated hypothyroid mice gained more weight than vehicle-treated hypothyroid mice. The ratio of cerebellar weight to whole brain weight was significantly increased in the inhibitor group compared to vehicle group. Treatment with RGFP966 also improved both size and morphological appearances of cerebella in perinatal hypothyroid mice, indicating the mitigation of cerebellar defects. RGFP966 group mice also returned to proper position faster in surface righting test, turn to face up the slope faster on negative geotaxis test, and were able to stay longer on rotarod test. On PND7, changes in mRNA levels were not statistically significant for many of the genes tested. On PND14, mRNA levels of four genes known to be stimulated by thyroid hormone were significantly increased. Finally, RGFP966 treatment also significantly increased the levels of histone acetylation at the regions containing TREs in the promoter of *Pcp2* and *Hr* genes in hypothyroid mouse cerebellum.

Discussion

We found that multiple defects in perinatal hypothyroid mice were alleviated by the postnatal administration of RGFP966. These findings suggest that our procedure works properly as a rescue experiment for hypothyroid subjects. There are some limitations to this study. First, only one dosage of RGFP966 was administered. It was almost impossible to add more groups to this study because comparisons should have been made among male littermates, although studying the dose-dependent effect of RGFP966 would be important. It was very rare to get more than four males within one litter. Detailed studies of adverse effects

would be also important since euthyroid mice treated with the same dosage of RGFP966 showed slight but significant motor coordination disturbances. Next, TR is not the only transcription factor that mediates the repression by HDAC3. Therefore, some of the effects of RGFP966 would have been achieved through other transcription factors independently of TR. Finally, the degrees of behavioral abnormality, those of mRNA levels and those of histone acetylation levels, do not perfectly correlate with each other. Studies with multiple dosages of RGFP966 and other transcription factors might give some more findings as mentioned above. In addition, other types of histone modifications and DNA methylation might be involved as well.

Summary

Enzymatic activity pharmacological inhibition of HDAC3 by RGFP966 alleviated the cerebellar morphological defects in perinatal hypothyroid mice. Functionally, mice treated with RGFP966 exhibited better motor coordination than hypothyroid control mice. The inhibitor treatment increased mRNA levels of TR-target genes in hypothyroid cerebellums. In addition, the increases in mRNA levels were associated with facilitated histone acetylation status in the loci of TR-target genes. These results demonstrate important roles of HDAC3 in the cerebellar developmental defects induced by perinatal hypothyroidism. The results of the present study suggest that HDAC3 inhibitor might serve as a novel therapeutic strategy for cerebellar developmental defects.