論文の内容の要旨

論文題目　Roles of CDK5 regulatory subunit associated protein 1-like 1 in obesity and type 2 diabetes

（肥満・２型糖尿病における CDKAL1 の役割の検討）

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Background

Type 2 diabetes (T2D) and obesity are thought to develop by a combination of environmental and genetic factors. The prevalence of diabetes is 9% in adult population, and its prevalence continues to increase in many countries. A number of novel genetic susceptibility variants of T2D were identified by genome-wide association studies (GWAS), and Cdk5 regulatory associated protein 1-like 1 (*CDKAL1*) was one of such genes. Although Cdkal1 was recently shown to work as a $tRNA^{Lys}$ modifying enzyme and to be crucial in accurate translation and secretion of insulin from the pancreatic β cells, little is known about its role in adipocytes, which is also crucially relevant to the pathogenesis of T2D. Recently, common variant at *CDKAL1* was observed to be associated with body mass index (BMI) in East Asian populations. Meanwhile, a Meta-analysis of East Asians reported three novel loci in or near the genes associated with BMI, including *CDKAL1*. Unpublished data from our laboratory showed that, in 3T3-L1 adipocytes, Cdkal1 inhibits adipocyte differentiation and lipid accumulation by suppressing expression of PPARγ, possibly through activation of the WNT signal pathway.

Aim

To reveal the roles of Cdkal1 in adipose tissue *in vivo* using adipose-tissue-specific Cdkal1 transgenic (Tg) mice under the control of the aP2 promoter with FLAG-tag.

Methods

For generation of Cdkal1 transgenic mice, purified linear construct was microinjected into fertilized embryos via standard pronuclear injection techniques. For characterization of the Cdkal1 transgenic mice, we measured body weight, mass of adipose tissues as well as liver, analyzed the mice grossly and histologically, examined the gene expression, and conducted an oral glucose tolerance test (OGTT) and an insulin tolerance (ITT) test under both a normal chow and a high fat diet condition. The effect of Cdkal1 transgene on differentiation of primary cells from adipose tissue was examined. The effect of Cdkal1 overexpression via retroviral infection was also examined. To investigate whether modification of

$tRNA^{Lys}$ is involved in the action of Cdkal1 we observed in adipocytes, we performed a method to detect the modification of $tRNA^{Lys}$ using qPCR. To further investigate involvement of Cdkal1 in obesity and diabetes, we examined expression levels of Cdkal1 in db/db mice—the genetic mouse model for obesity and diabetes that lacks a functional leptin receptor. Finally, we investigated *CDKAL1* expression levels in subcutaneous adipose tissue of patients who underwent gastric surgery.

Results

At the mRNA levels, selective expression of the Cdkal1 transgene was confirmed in brown adipose tissue (BAT) as well as subcutaneous and epididymal white adipose tissues (scWAT and eWAT), but not in liver and spleen. Enhanced Cdkal1 bands were observed in scWAT. FLAG-tagged Cdkal1 bands were only observed in the transgenic mice but not in the wild type mice.

There was no apparent difference between Cdkal1 transgenic mice and the control mice in body weight during the observation period. Consistent with the growth curves, no apparent difference in the tissue weight of scWAT, eWAT and BAT of the two genotypes was observed. In histological analysis of H&E staining, we did not observe any significant difference between the genotypes. *Cdkal1* gene expression was increased in all adipose tissues but we did not observe significant changes in expression of other genes, including *Pparg*, *Fabp4*, and the WNT pathway target gene, *Wisp2*. The glucose tolerance test showed no statistically significant difference under this condition. There is a possibility that the effect of the transgene differs between adult and post-natal mice. Consequently, we looked at the tissues in the neonates. The results of tissue weight at Post-natal day 14 showed no significant difference detected between Cdkal1 transgenic neonates and the wild type neonates in body weight and mass of adipose or liver tissue.

Under a high fat diet, although the difference in body weight did not reach statistical significance, the body weight of Cdkal1 transgenic mice showed a moderate tendency to be lower compared to the wild type mice. We did not observe any difference in food intake during the observation period. Among the tissues we tested, the weight of scWAT of Cdkal1 transgenic mice was significantly lower than the wild type mice, and the BAT of transgenic mice had a moderate tendency to be smaller.
Histological analyses of H&E staining revealed that BAT and scWAT of Cdkal1 transgenic mice had less lipid droplet accumulation compared to wild type mice in both Line 52 and Line 66. Consistent with this, triglyceride content in BAT was reduced in Cdkal1 Tg mice. In gene expression studies, we observed increased levels of *Cdkal1*in BAT, scWAT and eWAT. We observed a small trend that *Fabp4* to be lower in scWAT, Type II iodothyronine deiodinase (*Dio2*) to be higher in BAT in Cdkal1 transgenic mice, but the differences of those genes did not reach statistical significance. Expression of other genes such as genes involved in lipid metabolism, inflammation and adipokines were also examined but there was no significant change as far as we tested. In metabolic studies, the glucose levels of Cdkal1 transgenic

animals were significantly lower than the wild type animals during the OGTT. Area under the curve was also significantly lower in Cdkal1 transgenic mice. There was a trend that Cdkal1 transgenic mice had lower insulin levels and the insulin resistance index, suggesting that Cdkal1 transgenic mice were more insulin sensitive. Consistent with the OGTT results, ITT experiments showed lower glucose levels in Cdkal1 transgenic mice. There were no obvious changes in fasting triglyceride levels or cholesterol levels.

In Cdkal1 transgene in *ex vivo* experiments, we observed overexpression of the *Cdkal1* transgene after differentiation (day 6), while the extent of the transgene overexpression is quite modest before differentiation (day 0). In this condition, we did not observe significant difference in expression levels of *Pparg*, *Fabp4*, and *Wisp2* and other genes such as fatty acid synthase (*Fasn*) on day 6.There was also no difference in lipid accumulation, judged by Oil red O staining, between cells isolated from Cdkal1 transgenic mice and the wild type mice. By contrast, if I overexpress Cdkal1 in primary adipocytes of wild-type mice by using retrovirus, we observed significantly reduced expression of *Pparg* and *Fabp4*, and elevated expression of *Wisp2* at day 6 and reduced lipid accumulation. These results were consistent with the effects of Cdkal1 overexpression in 3T3-L1 adipocyte cell line and the difference was speculated be due to the difference in the timing and/or the magnitude of the induction of Cdkal1 expression in the two methods.

In modification of $tRNA^{Lys}$ experiment, we observed 80% reduction of $tRNA^{Lys}$ modification index in Cdkal1-knockdown HEK 293T cells compared to the control, which was consistent with the published result. There was no change in the index in Cdkal1-overexpressing HEK293T cells. Similarly, neither Cdkal1-overexpressing 3T3-L1 cells nor adipose tissues from Cdkal1 transgenic mice showed significant difference in the modification index. These data suggest that the effects of Cdkal1 in adipocytes differentiation may be independent of its action on the modification of $tRNA^{Lys}$.

Db/db mice had a much heavier body weight and a higher glucose level compared to control mice at a gene expression level, inflammatory cytokine genes such as *Tnfa* and *Ccl2* were increased and expression of the adiponectin gene (*Adipoq*) and *Ucp1* were decreased. Remarkably, the Cdkal1 mRNA expression levels were significantly lower in BAT *db/db* mice compared to the wild type mice. Consistent with the mRNA levels, the western blot data showed that Cdkal1 protein levels were also significantly lower in BAT and scWAT of *db/db* mice compared to the control mice.

Finally, *CDKAL1* exhibited a significant inverse correlation with body mass index (BMI) and waist circumference in human adipose tissue.

Conclusion

We demonstrated that overexpression of Cdkal1 in adipose tissue have an impact on the mass and lipid accumulation of adipose tissues and systemic insulin sensitivity, and that the expression levels of Cdkal1 in adipose tissue is associated with adiposity in mice and human subjects. Cdkal1 in adipocytes may play a role in the development of diabetes and obesity.