Family with sequence similarity 13, member A modulates adipocyte insulin signaling and preserves systemic metabolic homeostasis

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学位論文の内容要旨

Family with sequence similarity 13, member A modulates adipocyte insulin signaling and preserves systemic metabolic homeostasis

Fam13a は脂肪細胞のインスリンシグナルを調節し、全身の代謝恒常性維持に寄与する

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Summary

The prevalence of obesity is increasing worldwide. Obesity is causally implicated in metabolic disease such as type 2 diabetes and is strongly associated with other pathological conditions including hypertension, hyperlipidemia, and atherosclerosis. Therefore, obesity is an established risk of cardiovascular disease, making it as a major global health issue. Adipose tissue expands under obese condition, and pathological adipocyte hypertrophy leads to increased production of fatty acids via lipolysis as well as inflammatory adipokines. Fatty acids activate inflammatory signaling in adipocytes and macrophages via Toll-like receptor 4, leading to chronic adipose tissue inflammation in obesity. In addition, excessive adipocyte expansion causes imbalanced vascularization and consequent hypoxia, which also triggers inflammation in adipose tissue. Chronic inflammation in adipose tissue is closely associated with obesity-related metabolic disorders at least in part through inducing adipocyte insulin resistance. Insulin is a major anabolic hormone in the body and tightly regulates energy metabolism through activation of skeletal muscle glucose uptake, inhibition of hepatic gluconeogenesis, and inhibition of adipocyte lipolysis. It has been shown that enhancing insulin signaling in adipocytes is sufficient to improve systemic metabolic homeostasis; therefore, adipocyte insulin resistance is a promising target in the treatment and prevention of obesity-related metabolic disorders. However, the detailed mechanisms underlying the obesity-mediated defect in insulin action in adipocytes remain unclear.

We have performed DNA microarray analysis using RNAs isolated from the WAT of lean and dietary-induced obese mice aiming at the identification of genes that could provide previously unknown mechanisms in adipocyte dysfunction associated with obesity. We searched for genes that are highly expressed in healthy adipocytes

and are down-regulated in obesity, and found Family with sequence similarity 13, member A (Fam13a), which has a highly conserved nucleotide sequence among various species but its biological function remains to be elucidated.

Fam13a was highly and preferentially expressed in adipose tissues, while its isoforms Fam13b and Fam13c were dominantly expressed in brain. Furthermore, Fam13a was predominantly expressed in the mature adipocytes of WAT as well as in differentiated 3T3-L1 adipocytes. Of note, Fam13a expression was substantially reduced in the WAT of obese mice fed a high-fat diet (HFD) for 14 weeks compared with that in lean mice. During obesity, adipose tissue is exposed to various stresses such as endoplasmic reticulum (ER) and oxidative stress. Treatment with thapsigargin or hydrogen peroxide significantly reduced Fam13a expression in 3T3-L1 adipocytes, suggesting that ER and/or oxidative stress might be causally involved in the reduction of Fam13a in WAT in obesity. We also found that Fam13a was highly expressed in human WAT.

We then investigated a role of Fam13a in adipocyte functions. Gene silencing of Fam13a significantly reduced insulin-mediated Akt activation in 3T3-L1 adipocytes without affecting their maturity, while overexpression of Fam13a accentuated it in HEK293 cells. These results strongly suggest that Fam13a directly modulates insulin signaling in a cell autonomous fashion. Since Fam13a contains two coiled-coil domains (CCDs) that is frequently involved in protein-protein interaction, we investigated a potential association of Fam13a with signaling molecules involved in the insulin signal cascade. Accordingly, we identified that Fam13a was associated with insulin receptor substrate 1 (IRS1) in 3T3-L1 adipocytes, while no significant association was detected for phosphatase and tensin homolog deleted on chromosome 10 or Akt. To investigate whether CCDs play a role in the association between

Fam13a and IRS1, we prepared expression constructs for CCD-deletion mutants of Fam13a. Deletion of CCD-2 or both CCD-1 and 2 abolished the binding of Fam13a to IRS1, indicating that CCD-2 is essential for the association of Fam13a with IRS1. In contrast to IRS1, Fam13a did not bind to IRS2. We further identified that treatment with NT157, an IRS inhibitor, abolished the enhanced insulin signaling induced by Fam13a, indicating that Fam13a modulates insulin signaling in an IRS-dependent manner.

Subsequently, we found that overexpression of Fam13a increased IRS1 expression in HEK293 cells, whereas gene silencing of Fam13a reduced it in 3T3-L1 adipocytes. CCDs-deletion abolished the Fam13a-mediated increase in IRS1 expression, suggesting that binding to IRS1 is essential for Fam13a to increase its expression. In consistent with no binding to IRS2, overexpression of Fam13a did not affect IRS2 expression in HEK293 cells. IRS1 is known to undergo proteasomal degradation, which is triggered by serine/threonine phosphorylation. Inhibition of proteasome by lactacystin increased IRS1 expression in MOCK-control cells, and abrogated the enhanced IRS1 expression induced by Fam13a. These results suggest that Fam13a enhances IRS1 expression by inhibiting its proteasomal degradation.

It has been reported that protein phosphatase 2A (PP2A) protects IRS1 against excessive serine/threonine phosphorylation and consequent proteasomal degradation. On the other hand, Fam13a has been reported to associate with PP2A and modify Wnt/β-catenin signaling; therefore, we explored the Fam13a-association with PP2A, and found that Fam13a bound to PP2A independently of its CCDs. Pharmacological inhibition of PP2A by okadaic acid reduced IRS1 expression and notably, it abolished the Fam13a-mediated increase in IRS1 expression. These data collectively indicate that Fam13a recruits PP2A with IRS1, leading to the protection of IRS1 from

proteasomal degradation, which consequently accentuates insulin signal cascade in adipocytes.

To analyze the role of Fam13a in systemic metabolic homeostasis, we generated mice with a targeted deletion of Fam13a (Fam13a−/−). Fam13a−/− mice showed body weight and adiposity similar to those in wild-type (WT) mice under normal dietary conditions. Body fat distribution in normal chow (NC)-fed Fam13a−/− mice did not differ from that in WT mice both in male and female. Nevertheless, modest but significant impairments in insulin sensitivity and glucose tolerance were detected in Fam13a−/− mice. Expression of inflammatory cytokines in WAT was similar between WT and Fam13a−/−mice, suggesting that impaired insulin sensitivity in Fam13a−/− mice is not associated with WAT inflammation. Consistent with a role of Fam13a in IRS1 expression *in vitro*, loss of Fam13a caused reduction in IRS1 in WAT but not in skeletal muscle or liver in mice. These results suggest that Fam13a is crucially involved in the regulation of IRS1 expression in adipocytes, and therefore modifies systemic metabolic health even under normal dietary condition.

When fed a high-fat diet (HFD), body weight, adiposity, and fat distribution were similar between WT and Fam13a−/− mice both in male and female. Nevertheless, HFD-fed Fam13a−/− mice showed exacerbated insulin resistance and glucose intolerance compared with those in WT mice. Chronic inflammation in WAT of HFD-fed Fam13a−/− mice appeared to be deteriorated despite similar adiposity. Of note, insulin signaling in WAT was significantly impaired in association with reduced IRS1 expression in HFD-fed Fam13a−/− mice. IRS1 expression in skeletal muscle and liver in Fam13a−/− mice did not differ from that in WT mice fed a HFD. Insulin potently inhibits lipolysis and promotes fat storage in WAT at least in part by decreasing expression of adipose triglyceride lipase (ATGL), a rate-limiting lipolytic

enzyme that catalyzes the first step of fat breakdown. We found that adipocyte size was modestly reduced, while serum levels of free fatty acid (FFA) were higher in HFD-fed Fam13a−/− mice. Serum FFA levels primarily depend on the rate of lipolysis in WAT; therefore, increased serum FFA in conjunction with a reduction in adipocyte size suggests enhanced lipolysis in WAT of HFD-fed Fam13a−/− mice. Consistently, HFD-fed Fam13a−/− mice showed higher ATGL expression in WAT than in WT mice fed a HFD. These results collectively suggest that loss of Fam13a impairs insulin signaling in WAT in association with IRS1 reduction, resulting in enhanced lipolysis, which leads to exacerbated adipose tissue inflammation, higher circulating FFA, and subsequent deterioration of systemic metabolic disorders under high-fat dietary conditions.

We then generated mice with a targeted activation of Fam13a in adipocytes (aP2-Fam13a-Tg) to further analyze the Fam13a function in adipocytes. aP2-Fam13a-Tg mice showed higher insulin sensitivity and better glucose tolerance than in WT mice despite similar body weight and body fat distribution even while on a normal chow diet. Insulin signaling was accentuated in the WAT of aP2-Fam13a-Tg mice relative to WT mice fed NC. Expression of inflammatory cytokines, adiponectin, and leptin in WAT were not different between these mice. When fed a HFD, male aP2-Fam13a-Tg mice exhibited a slight increase in body weight and body fat ratio compared with WT mice, though it was not statistically significant. Body fat distribution was similar among HFD-fed WT and aP2-Fam13a-Tg mice both in male and female. Obesity-related insulin resistance and glucose intolerance were ameliorated in aP2-Fam13a-Tg mice fed a HFD in association with preserved insulin signaling in WAT. In contrast to Fam13a−/− mice, adipocyte size was modestly but significantly increased in conjunction with lower serum FFA levels in HFD-fed aP2-

Fam13a-Tg mice relative to WT mice, suggesting reduced lipolysis in the WAT of aP2-Fam13a-Tg mice. Consistently, expression of ATGL was significantly reduced in the WAT of HFD-fed aP2-Fam13a-Tg mice. Additionally, expression of inflammatory genes was modestly reduced in the WAT of HFD-fed aP2-Fam13a-Tg mice compared with WT mice fed a HFD. These data demonstrate that preserved expression of Fam13a in adipocytes sufficiently ameliorates adipose tissue insulin resistance and systemic metabolic disorders associated with obesity, and further suggest Fam13a as an attractive pharmacotherapeutic target in the treatment and/or prevention of metabolic disease associated with obesity.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨

受付番号	甲 第2768号	氏名	DONYTRA ARBY WARDHANA
論文題目 Title of Dissertation	Fam13aは脂肪細胞のインスリンシグナルを調節し、全身の代謝恒常性維持に寄与する Family with sequence similarity 13, member A modulates adipocyte insulin signaling and preserves systemic metabolic homeostasis		
審査委員 Examiner	主査 Chief Examiner 小川 渉 副査 Vice-examiner 古屋敷智之 副査 Vice-examiner 鈴木 亮		

（要旨は１，０００字～２，０００字程度）

In this study, the candidate has investigated the molecular mechanism of insulin resistance. The candidate searched for genes that are highly expressed in healthy adipocytes and are down-regulated in obesity, and found Family with sequence similarity 13, member A (Fam13a).Fam13a was highly expressed in adipose tissues. Fam13a expression was substantially reduced in the WAT of obese mice fed a high-fat diet (HFD). During obesity, adipose tissue is exposed to various stresses such as endoplasmic reticulum (ER) and oxidative stress. Treatment with thapsigargin or hydrogen peroxide significantly reduced Fam13a expression in 3T3-L1 adipocytes, suggesting that ER and/or oxidative stress might be causally involved in the reduction of Fam13a in WAT in obesity.

The candidate has shown that knockdown of Fam13a reduced insulin-mediated Akt activation in 3T3-L1 adipocytes, while overexpression of Fam13a accentuated insulin signaling in HEK293 cells. The candidate also has shown that Fam13a bind to IRS1, a key element of insulin signaling pathway, through its CCD-2 domain.. He has found that binding to IRS1 is essential for Fam13a to increase its expression. IRS1 is known to undergo proteasomal degradation, which is triggered by serine/threonine phosphorylation. Inhibition of proteasome by lactacystin increased IRS1 expression, and abrogated the enhanced IRS1 expression induced by Fam13a. These results suggest that Fam13a enhances IRS1 expression by inhibiting its proteasomal degradation. The candidate has shown that Fam13a recruits PP2A with IRS1, leading to the protection of IRS1 from proteasomal degradation, which consequently accentuates insulin signal cascade in adipocytes.

To analyze the role of Fam13a in systemic metabolic homeostasis, he generated mice with a targeted deletion of Fam13a (Fam13a−/−). Fam13a−/− mice showed body weight and adiposity similar to those in wild-type (WT) mice under normal dietary conditions. Body fat distribution in normal chow (NC)-fed Fam13a−/− mice did not differ from that in WT mice both in male and female. Nevertheless, modest but significant impairments in insulin sensitivity and glucose tolerance were detected in Fam13a−/− mice. Expression of inflammatory cytokines in WAT was similar between WT and Fam13a−/−mice, suggesting that impaired insulin sensitivity in Fam13a−/− mice is not associated with WAT inflammation. Consistent with a role of Fam13a in IRS1 expression *in vitro*, loss of Fam13a caused reduction in IRS1 in WAT but not in skeletal muscle or liver in mice. These results suggest that Fam13a is crucially involved in the regulation of IRS1 expression in adipocytes, and therefore modifies systemic metabolic health even under normal dietary condition. When fed a high-fat diet (HFD), body weight, adiposity, and fat distribution were similar between WT and Fam13a−/− mice. Nevertheless, HFD-fed Fam13a−/− mice showed exacerbated insulin resistance and glucose intolerance compared with those in WT mice. Chronic inflammation in WAT of HFD-fed Fam13a−/− mice appeared to be deteriorated despite similar adiposity. Of note, insulin signaling in WAT was significantly impaired in association with reduced IRS1 expression in HFD-fed Fam13a−/− mice. IRS1 expression in skeletal muscle and liver in Fam13a−/− mice did not differ from that in WT mice fed a HFD. Insulin potently inhibits lipolysis and promotes fat storage in WAT at least in part by decreasing expression of adipose triglyceride lipase (ATGL). He found that adipocyte size was modestly reduced, while serum levels of free fatty acid (FFA) were higher in HFD-fed Fam13a−/− mice. Serum FFA levels primarily depend on the rate of lipolysis in WAT; therefore, increased serum FFA in conjunction with a reduction in adipocyte size suggests enhanced lipolysis in WAT of HFD-fed Fam13a−/− mice.

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The candidate, having completed studies on the function of Fam 13a, with a specialty in its role of systemic insulin sensitivity, and having advanced the field of knowledge in the area of the pathogenesis of insulin resistance , is hereby recognized as having qualified for the degree of Ph.D.(Medicine)