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(Form 3)

Summary of Doctoral Thesis

Rashid, Misbah

Control of adipose tissue inflammation by hypothalamic SF1 neurons

Obesity is marked by dysfunctional adipose tissue that is accompanied by inflammation. Adipose tissue inflammation is characterized by infiltration of pro-inflammatory macrophages and secretion of inflammatory cytokines.

The hypothalamic ventromedial nucleus (VMH) plays a crucial role in the regulation of body weight and adiposity and is one of the important brain regions to regulate energy balance. Steroidogenic factor 1-expressing neurons (SF1 neurons) in the VMH play an essential role in controlling energy metabolisms of peripheral tissues, including brown adipose tissue (BAT) and white adipose tissue (WAT). Various previous studies revealed the roles of SF1 neurons in BAT thermogenesis, WAT browning, and resistance to diet-induced obesity. However, whether obesity-induced inflammatory responses in adipose tissues are regulated by SF1 neurons remains unclear.

In this study, I investigated the roles of SF1 neurons in the inflammatory responses in high-fat diet (HFD)-induced obese mice. To accomplish this, I modulated SF1 neurons in the VMH and investigated inflammatory responses in adipose tissues, as well as the changes in energy metabolism.

First, I ablated SF1 neurons in the VMH by injecting adeno-associated viruses (AAVs) with Cre recombinase-dependent diphtheria toxin-A (DTA) expression into SF1-Cre mice. In the absence of SF1 neurons, I found that body weight was increased without affecting food intake when mice were fed with HFD for 8 weeks. Both total and basal energy expenditure and locomotor activity during the dark phase were decreased in the mice without SF1 neurons. SF1 neuron-ablated mice showed glucose intolerance and lipid oxidation impairment. These results indicate that ablation of SF1 neurons

reduces energy expenditure and fat oxidation. Notably, ablation of SF1 neurons aggravates obesity-induced inflammatory responses in specific adipose tissues. I found that expression of macrophage markers and inflammatory cytokines was substantially increased in inguinal white adipose tissue (ingWAT) but not in not in epididymal WAT (epiWAT) in HFD-fed obese mice. Immunohistochemistry and transcriptome analysis of ingWAT confirmed these findings, revealing that SF1 neuron ablation enhanced the expression of many inflammation-related genes. Ablation of SF1 neurons significantly decreased the expressions of thermogenesis and mitochondria-related genes as well as increased the gene expressions of macrophage markers in BAT in these mice. These results suggest that SF1 neuron ablation in the VMH exacerbates HFD-induced inflammatory responses in ingWAT and thermogenic function in BAT but had little effect in epiWAT. Thus, the effects of SF1 ablation are dependent on the type of adipose tissues.

Next, I examined whether SF1 neurons played a role in ameliorating obesity-induced inflammatory responses upon activation. I activated SF1 neurons by a chemogenetic method. Chemogenetic receptor hM3Dq, when expressed in neurons, can activate neurons in the presence of a specific ligand, clozapine-N-oxide (CNO). I used AAV to selectively express hM3Dq in SF1 neurons in SF1-Cre mice and administered CNO through drinking water. These mice were fed with HFD for 4 weeks along with CNO to achieve simultaneous activation of SF1 neurons and obesity development. Mice with chronic activation of SF1 neurons showed a similar body weight increase and food intake when compared with control mice. However, the chronic activation of SF1 neurons substantially suppressed the gene expressions of inflammatory cytokines and macrophage markers in ingWAT but not in epiWAT in HFD-fed obese mice. Intriguingly, the expressions of thermogenesis and mitochondria-related genes in BAT were significantly increased by the chronic activation of SF1 neurons.

Finally, I examined whether chronic activation of SF1 neurons suppresses HFD-

induced inflammatory response in ingWAT in mice that already developed obesity before stimulation of the SF1 neurons. Mice were first fed with HFD for 8 weeks without stimulation of SF1 neurons and then SF1 neurons in the VMH were activated for 4 weeks by CNO administration with continued HFD feeding. Even under this condition, the chronic activation of SF1 neurons reduced the inflammatory gene expressions in ingWAT. These results suggest that chronic activation of SF1 neurons suppresses inflammatory responses in adipose tissue in mice that already developed obesity.

Overall, my findings indicate that SF1 neurons play a critical role in controlling inflammatory responses in specific adipose tissues in HFD-fed obese mice, in addition to their roles in the regulation of the body weight, lipid, and glucose metabolism. These findings demonstrate that SF1 neurons in the VMH mitigate the obesity-induced inflammatory responses differently in various adipose tissues and provide the first evidence to reveal a connection between the VMH and inflammatory responses in the adipose tissues.

Form8・Separate Sheet
（様式８・別紙１）

Results of the doctoral thesis screening

博士論文審査結果

Name in Full
氏　　名　Rashid, Misbah

Title
論文題目　Control of adipose tissue inflammation by hypothalamic SF1 neurons

本論文は、中枢神経系が脂肪組織でのマクロファージの炎症反応にどのような調節作用を及ぼすかを個体レベルで初めて明らかにした研究である。インスリン抵抗性等の肥満症は、脂肪組織において中性脂肪の過剰蓄積に伴いマクロファージが蓄積し、炎症性サイトカインの分泌等を介して引き起こされる。視床下部腹内側核に存在するSF1(steroidogenic factor 1)発現ニューロンは、摂食、エネルギー消費、糖代謝を調節する。出願者は、SF1発現ニューロンにDTA（diphtheria toxin-A）を発現させ、SF1発現ニューロンを破壊したマウス、及び、逆にSF1発現ニューロンをDREADD(designer receptors exclusively activated by designer drugs)法によって慢性的に活性化したマウスを作出し、高脂肪食摂取によって引き起こされる脂肪組織の炎症反応に及ぼす効果を調べた。

最初に、SF1発現ニューロンを破壊したマウスに高脂肪食を摂取させた結果、対照マウスと比較して体重が有意に増加し、エネルギー消費、特に脂肪酸化能が低下した。摂食量に差はなかったが、グルコース負荷による耐糖能も悪化していた。また、SF1ニューロンを破壊して8週間後の鼠径部脂肪組織において、マクロファージマーカー（F4/80、CD11c、CD206）やサイトカインのmRNA発現量が増加していた。免疫組織学的解析の結果、マクロファージのマーカータンパク質発現量が鼠径部脂肪組織で増加しており、脂肪細胞周囲にマクロファージが集積することによる"crown-like structure"も増加していた。RNA-Seq解析から、炎症反応関連遺伝子のmRNA発現量が多数増加していることもわかった。熱産生を担う褐色脂肪組織では、マクロファージマーカーmRNA発現量が増加し、熱産生やミトコンドリア関連遺伝子の発現量が低下した。一方、副睾丸脂肪組織で変化はなかった。

次に、マウスに高脂肪食を摂取させながら、DREADD法により視床下部腹内側核SF1発現ニューロンを4週間活性化させ続けた。本法により、視床下部腹内側核SF1発現ニューロンにおいてcFos発現量が増加した。摂食量に変化はなかったが、体重は低下傾向であった。鼠径部脂肪組織においてマクロファージマーカー、炎症性サイトカインのmRNA発現量が低下し、褐色脂肪組織では熱産生、ミトコンドリア関連遺伝子のmRNA発現量が増加した。副睾丸脂肪組織に変化はなかった。さらに、既に肥満となったマウスにおいても、SF1発現ニューロンの効果が惹起されるか否かを調べるため、8週間高脂肪食をマウスに摂取させた後、4週間、SF1発現ニューロンを慢性的に活性化させた結果、体重、摂食量に変化がないにも関わらず、鼠径部脂肪組織においてマクロファージマーカー、炎症性サイトカインのmRNA発現が有意に低下した。

以上の結果は、視床下部腹内核SF1発現ニューロンが、高脂肪食誘導性肥満マウスの鼠径部脂肪組織において、マクロファージによる炎症反応に調節作用を及ぼすことを明らかにした画期的な知見である。また、脂肪組織によりその効果が異なることも明らかにした。以上の研究結果から、全員一致で本論文が博士学位論文に相応しいと結論した。