学位論文内容の要約

Summary of the dissertation

Study on the role of gut microbiota and intestinal environment in adipose tissue development in preweaning mice
(乳子期マウスの脂肪組織発達における腸内細菌および腸内環境の役割に関する研究)

【Preface】

The rising prevalence of obesity and related metabolic disorders, such as diabetes, hyperlipidemia, and cardiovascular diseases, has emerged as a significant global issue that requires urgent attention. In response to the growing demand for safer and more effective treatments, a novel therapeutic approach that augments energy expenditure through thermogenic adipocytes has gained attention as a promising strategy for obesity treatment.

Adipose tissue in mammals is mainly classified into two types: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT stores excess energy as lipids, and its enlargement caused by obesity induces systemic insulin resistance, leading to related metabolic diseases. In contrast, BAT activation increases whole-body energy expenditure dependently on uncoupling protein 1 (UCP1) activity and prevents obesity. The activation of UCP1 and its gene expression in BAT are induced by sympathetic stimulation, such as cold exposure. When cold exposure is prolonged, UCP1 expression is induced in specific WAT depots (WAT browning), and adipocytes expressing UCP1 are referred to as beige adipocytes.

BAT mass declines with aging in humans; thus, ways to not only activate thermogenic adipocytes but also increase their number are required. Beige adipocytes have thermogenic functions comparable to those of brown adipocytes and regulate whole-body metabolism by secreting cytokines, metabolites and exosomes. Furthermore, human BAT is primarily composed of beige adipocytes rather than brown adipocytes. These observations highlight the importance of identifying ways to promote beige adipocyte induction. Interestingly, beige adipocytes are also transiently induced in WAT during the early postnatal period in mice, yet the underlying mechanisms remain largely unknown. Therefore, this study aimed to elucidate the mechanisms underlying beige adipocyte induction during the postnatal period in mice, which could provide novel insights into increasing thermogenic adipocytes to combat obesity.

【Chapter I】

The association between beige adipocyte induction and gut microbiota formation in preweaning mice was investigated. To examine the postnatal development of WAT and gut microbiota, tissues were collected every 5 days from postnatal day 7 to day 32. UCP1-expressing beige adipocytes were induced in WAT, reaching a peak at postnatal day 17, but subsequently disappeared after weaning. The composition of gut microbiota differed among postnatal days, and the abundance of Porphyromonadaceae and Ruminococcaceae was positively and negatively correlated with the gene expression of the beige marker, respectively. To confirm the role of gut microbiota in beige adipocyte induction, pups' gut microbiota was depleted by maternal antibiotic (Abx) treatment. The level of *Ucp1* mRNA and the protein level of UCP1 significantly decreased in the Abx group. In addition, histological analysis revealed fewer multilocular adipocytes in the Abx group, indicating the suppression of beige adipocyte induction. Furthermore, alteration of gut microbiota by maternal high-fat diet (HFD) feeding inhibited beige adipocyte induction. This inhibition also occurred after the transplantation of microbiota from pups of the maternal HFD group. In summary, depletion and alteration of gut microbiota suppressed postnatal WAT browning, highlighting the critical role of the establishment of gut microbiota during the postnatal period. These findings may provide a comprehensive understanding of the association between gut microbiota and adipose tissue development during this stage.

【Chapter II】

The mechanism by which gut microbiota influences the induction of beige adipocytes was investigated, focusing on gut microbiota-derived signaling molecules, including bile acids (BAs) and short-chain fatty acids (SCFAs).

BAs are synthesized from cholesterol to form primary BAs, which are then deconjugated by intestinal microbiota to form secondary BAs. BA profiles in the serum of the pups were examined using LC/MS. Maternal Abx and HFD altered BA profiles and reduced secondary BAs in pups, suggesting that postnatally formed microbiota induces beige adipocytes through secondary BAs. To confirm the role of bile acids, Takeda G protein-coupled receptor (TGR5), a BA receptor, knockout (KO) mice were generated using the improved genome-editing via oviductal nucleic acids delivery (i-GONAD) method. However, TGR5-KO pups did not significantly suppress beige adipocyte induction, indicating a minor role for BA-TGR5 signaling in postnatal WAT browning.

To further investigate the factors affecting postnatal WAT browning, the critical period of gut microbiota formation for beige adipocyte induction was determined. Antibiotics were

administered to the dams during the first 10 days or last 10 days of lactation. Consistent with the timing when the expression of beige markers peaked, maternal Abx treatment during late lactation significantly inhibited beige adipocyte induction in the pups. This result suggests that gut microbiota established during late lactation is crucial for WAT browning.

To gain further insights into the relationship between WAT browning and gut microbiota, RNA-seq analysis of the ileum and colon of the pups was performed. Genes involved in fatty acid metabolism, steroid biosynthesis, and cholesterol metabolism were significantly upregulated, whereas genes related to inflammation, such as response to type 1 interferon, defense response, and antiviral innate immune response, were downregulated by maternal antibiotics. Given that lipid metabolism in the intestine is regulated by farnesoid X receptor (FXR) signaling, the involvement of FXR signaling in beige adipocyte induction was assessed by administering FXR antagonist to the pups during the last 5 days of lactation. However, pharmacological inhibition of FXR did not affect beige adipocyte induction.

Furthermore, maternal Abx during late lactation reduced the cecal SCFA levels in the pups. Given that SCFAs have been demonstrated to play a pivotal role in modulating energy metabolism, microbiota-derived SCFAs may contribute to postnatal WAT browning. Unexpectedly, knockout of the SCFA receptors G protein-coupled receptor 43 (GPR43) and GPR41 did not influence WAT browning, implying that SCFA-GPR43/41 signaling is not critical to this mechanism.

【Conclusion】

This study demonstrated that gut microbiota development is essential for postnatal WAT browning in mice. In addition, to elucidate the mechanism by which gut microbiota influences the induction of beige adipocytes, the role of gut microbiota-derived signaling molecules was examined. By generating receptor-deficient mice using the i-GONAD method, it was clarified that the signaling pathways mediated by TGR5, GPR43, and GPR41 are minimally involved in this mechanism. Considering that postnatal WAT browning occurs independently of sympathetic nerve activity, these findings may contribute to the identification of a new sympathetic stimulation-independent pathway and underscore the necessity for further research to identify additional regulators that may potentially target beige adipocyte induction in obesity treatment.