論文の内容の要旨

論文題目　Co-Localization of NFIA and PPARγ Controls the Brown Fat Gene Program
(NFIA は褐色脂肪遺伝子エンハンサーにおいて選択的に PPARγ と共局在することで褐色脂肪の遺伝子プログラムを制御する)

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Obesity and its complications—such as diabetes, cardiovascular diseases and strokes— amount to a world-wide epidemic. While white adipose tissue (WAT) stores energy in the form of lipids and expands in obesity, brown adipose tissue (BAT) is specialized to dissipate energy by means of the uncoupling protein-1 (UCP1) on the mitochondrial inner membrane. In humans, brown fat activity is inversely correlated with body mass index, and several pilot studies have shown that therapeutic interventions such as chronic cold exposure and β3 agonist administration successfully recruit human brown fat and increase systemic energy expenditure. Thus, stimulating development and/or function of brown fat would be a novel strategy for the treatment of obesity and its complications.

Lineage tracing technology has demonstrated that brown fat and skeletal muscle share a common progenitor, but brown fat and white fat do not. Both brown fat and skeletal muscle derive from a Myf5-positive precursor, and a transcriptional cofactor PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16) works as a cell-fate switch for driving the brown fat gene program. However, our understanding of the global landscape of brown fat development is still in an early stage, and much remains elusive.

Here, we identified nuclear factor I-A (NFIA) as a novel transcriptional regulator of brown fat by a genome-wide open chromatin analysis of murine brown and white fat followed by motif analysis of brown-fat-specific open chromatin regions. Specifically, we performed formaldehyde-assisted isolation of regulatory elements coupled with high-throughput sequencing (FAIRE-seq) on murine brown and white fat. We found that the binding motif for the NFI transcription factor was most enriched within brown-fat-specific open chromatin regions. Of the four isoforms of the NFI family (NFIA, B, C and X), we found that NFIA was highly expressed in brown fat in compared to white fat or skeletal muscle, both *in vitro* and *in vivo*.

Introduction of NFIA into myoblasts results in brown adipocyte differentiation. Introduction of NFIA into white adipocytes also result in robust induction of brown-fat-specific genes, while the effect of NFIA on lipid accumulation and on gene expression of general adipocyte genes was modest. Conversely, knockdown of NFIA in brown adipocytes results in significantly decreased expression of *Ucp1* with relatively preserved adipocyte differentiation.

Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) revealed that NFIA binds to the enhancers of the master regulator *Pparg* and the brown-fat-specific genes such as *Ucp1*. Indeed, the NFIA binding signal was highly enriched near BAT-selective genes compared with that signal near WAT-selective genes. Further, NFIA and the adipogenic master regulator, PPARγ, co-localize at the brown-fat-specific enhancers. Moreover, the binding of NFIA precedes and facilitates the binding

of PPARγ, leading to increased chromatin accessibility and active transcription.

The brown fat of NFIA knockout mice displays significantly decreased expression of Ucp1 mRNA and UCP1 protein compared to wild type control. By ChIP-qPCR analysis of those tissues, we also observed that the binding of PPARγ to the Ucp1 enhancer was severely impaired. Transcriptome analysis using RNA-sequencing showed that impaired expression of the brown-fat-specific genes and reciprocal elevation of muscle genes on the genome-wide scale.

Finally, to explore the possible role of NFIA in human brown fat, we analyzed the perirenal brown fat of human patients with pheochromocytoma and with non-functional adrenal tumors. We observed that NFIA expression was higher in patients with pheochromocytoma compared with those who had non-functional adrenal tumors. Furthermore, expression levels of brown-fat-specific genes such as *UCP1* were positively and significantly correlated with *NFIA* expression

These results indicate that NFIA is a key transcriptional regulator of brown fat and exerts its effects by co-localizing with PPARγ at cell-type-specific enhancers.