Original Article

MOLECULAR METABOLISM

The long noncoding RNA Blnc1 orchestrates homeostatic adipose tissue remodeling to preserve metabolic health

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ABSTRACT

Objective: Long noncoding RNAs (lncRNAs) are emerging as powerful regulators of adipocyte differentiation and gene expression. However, their significance in adipose tissue metabolism and physiology has not been demonstrated *in vivo*. We previously identified Blnc1 as a conserved lncRNA regulator of brown and beige adipocyte differentiation. In this study, we investigated the physiological role of Blnc1 in thermogenesis, adipose remodeling and systemic metabolism.

Methods: We generated fat-specific Blnc1 transgenic and conditional knockout mouse strains and investigated how adipocyte Blnc1 levels are causally linked to key aspects of metabolic health following diet-induced obesity. We performed studies using cultured adipocytes to establish cell-autonomous role of Blnc1 in regulating adipocyte gene programs.

Results: Blnc1 is highly induced in both brown and white fats from obese mice. Fat-specific inactivation of Blnc1 impairs cold-induced thermogenesis and browning and exacerbates obesity-associated brown fat whitening, adipose tissue inflammation and fibrosis, leading to more severe insulin resistance and hepatic steatosis. On the contrary, transgenic expression of Blnc1 in adipose tissue elicits the opposite and beneficial metabolic effects, supporting a critical role of Blnc1 in driving adipose adaptation and homeostatic remodeling during obesity. Mechanistically, Blnc1 cell-autonomously attenuates proinflammatory cytokine signaling and promotes fuel storage in adipocytes through its protein partner Zbtb7b.

Conclusions: This study illustrates a surprisingly pleiotropic and dominant role of lncRNA in driving adaptive adipose tissue remodeling and preserving metabolic health.

Keywords lncRNA; Adipose tissue remodeling; Brown fat; White fat; Whitening; Inflammation; Obesity

1. INTRODUCTION

Adipose tissue is a remarkably versatile organ central to metabolic homeostasis. White adipose tissue (WAT) stores lipids, secretes endocrine factors, and integrates immune and metabolic signals [1–6], whereas brown adipose tissue (BAT) oxidizes fuels to generate heat and is emerging as a source of important endocrine factors [7–11]. WAT exhibits enormous plasticity in its storage capacity. In response to chronic overnutrition, WAT undergoes dramatic expansion through adipocyte hypertrophy and recruitment of newly generated adipocytes [3,12–14]. However, this healthy fat expansion often transitions into a pathological state of adipose tissue remodeling that is associated with activation of a proinflammatory immunological milieu in rodent and human obesity. Persistent adipocyte stress and injury activate wound repair response in adipose tissue, leading to fibrosis in WAT. The mechanisms underlying the adaptive healthy fat expansion and its switch to adipose tissue dysfunction in obesity remain elusive.

Brown fat contains high mitochondrial content and generates heat through uncoupling protein 1 (UCP1)-dependent and independent mechanisms [15]. Brown fat thermogenesis is critical for defense against cold temperature and obesity in rodents. Genetic and pharmacological activation of brown fat thermogenesis increased energy expenditure, reduced adiposity, and improved glucose and lipid parameters [16–18]. Recent studies demonstrate that brown fat undergoes pronounced whitening in obesity, in which brown adipocytes acquire molecular and morphological features of white adipocytes [19–21]. This aberrant remodeling of brown fat may contribute to impaired thermogenesis and metabolic dysfunction. Beyond thermogenesis, brown fat influences metabolic physiology through its secretion of endocrine factors such as Neuregulin 4 (Nrg4), which attenuates hepatic lipogenesis and liver injury [22–24]. Several transcriptional regulators have been implicated in the control of brown and beige fat development and maintenance, including PR Domain Containing 16 (PRDM16) [8,25,26], Early B-cell Factor 2 (EBF2) [27],

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Interferon Regulatory Factor-4 [28], Zinc-Finger Protein 516 [29], PPARγ coactivator-1α (PGC-1α), Euchromatic Histone-lysine N-methyltransferase 1 (EHMT1) [26,30] and zinc finger and BTB domain containing 7b (Zbtb7b) [31].

Recent studies have implicated long noncoding RNAs (lncRNAs) as a new class of non-protein regulators of adipocyte biology [32]. A notable feature of lncRNA expression is that many exhibit restricted tissue distribution and are highly regulated by developmental and hormonal signals [33–36]. Global transcriptomic analyses have led to the identification of highly inducible lncRNAs during brown and white adipocyte differentiation [37–39]. Among these, brown fat lncRNA 1 (Blnc1) was identified as a conserved BAT-enriched lncRNA that promotes differentiation of cultured brown and beige adipocytes. Blnc1 forms ribonucleoprotein transcriptional complexes with the transcription factors EBF2 and Zbtb7b and heterogeneous nuclear ribonucleoprotein U (hnRNPU) to stimulate thermogenic gene expression [31,39,40]. Despite this, the role of lncRNAs in adipose tissue remodeling and metabolic physiology has not been fully established.

Here we show that fat-specific inactivation of Blnc1 accelerates brown fat whitening and impairs homeostatic fat expansion during high-fat diet (HFD) feeding, leading to adipose tissue inflammation, insulin resistance and hepatic steatosis. Adipocyte-specific transgenic expression of Blnc1 elicited the opposite and beneficial metabolic effects. Mechanistically, Blnc1 cell-autonomously attenuates proinflammatory cytokine signaling in adipocytes through its protein partner Zbtb7b. This study illustrates a surprisingly powerful role of lncRNA in orchestrating adipocyte adaptation to obesity and maintaining systemic metabolic health.

2. METHODS

2.1. Animal studies

All animal studies were performed according to procedures approved by the University Committee on Use and Care of Animals at the University of Michigan. Mice were maintained in 12/12 h light/dark cycles and fed regular rodent chow or high-fat diet (D12492, Research Diets). Wild type C57BL/6J mice (JAX stock #000664) and floxed STOP-Cas9 knockin mice (JAX stock #024857) was purchased from the Jackson Laboratory.

For the generation of Blnc-single-guide RNA transgenic (sgRNA-TG) mice, we designed two sgRNAs flanking the Blnc1 gene, which contains a single exon using a CRISPR design web tool (http://crispr.mit.edu/) [43]. Each sgRNA targeting Blnc1 was cloned at downstream of U6 promoter and the tandem U6-sgRNA cassette with two sgRNAs was pronuclear microinjected into the fertilized egg. Then, we crossed floxed STOP-Cas9 knockin mice with Adiponectin-CRE transgenic mice and Blnc1-sgRNA TG mice to generate floxed STOP-Cas9, Adiponectin-CRE and Blnc1 sgRNA triple TG mice, which contain the Blnc1 deletion allele in adipose tissue. For Blnc1 adipose specific transgenic mice, full-length Blnc1 sequence was cloned downstream of a murine aP2 promoter [44]. Transgenic mice were generated by pronuclear microinjection. Seven independent founders were identified and crossed with C57BL/6 mice to generate stable transgenic lines.

For cold acclimation, mice were maintained in a temperature-controlled chamber. The temperature decreased 4 °C every day until reaching 10 °C for five more days before tissue harvest. For histology, tissues were dissected and fixed in 10% formalin overnight at 4 °C and subjected to paraffin embedding and H&E staining. Sirius Red staining was processed as previously described [45]. Hydroxyproline level in the eWAT was measured using a Hydroxyproline Colorimetric Assay Kit (BioVision).

2.2. Cell culture

C3H10T1/2 cells expressing vector/Blnc1/Zbtb7b or scramble/Blnc1 shRNA were maintained in DMEM supplemented with 10% fetal bovine serum (FBS). Confluent preadipocytes were subjected for differentiation by adding an induction medium containing 0.5 mM IBMX, 125 μM indomethacin, 1 μM dexamethasone, 20 nM insulin and 1 nM T3. Cells were switched to differentiation medium (DMEM, 10% FBS, 20 nM insulin and 1 nM T3) after two days. TNFα treatment was performed in the fully differentiated adipocytes. Protein level of IL-6 and CCL5 in the conditional medium was measured using ELISA kits (R&D system).

2.3. Metabolic analyses

For Glucose Tolerant Test (GTT), mice were fasted overnight (16 h) and injected intraperitoneally (IP) with a glucose solution at a dose of 1.0 g/kg body weight. For Insulin Tolerant Test (ITT), mice were pre-fasted for 4 h and IP injected with insulin at a dose of 1 U/kg body weight. Blood glucose concentrations were measured before and 20, 45, 90 and 120 min after glucose or insulin injection. Liver triglyceride was extracted and measured as previously described [46]. Plasma insulin was measured using an ELISA kit (CrystalChem).

2.4. Microarray analysis

Total RNA was isolated from white adipose tissues of WT and Blnc1 Tg mice after HFD feeding. Gene expression profiling was performed using Mouse Gene ST 2.1 plates. We used a cutoff of normalized array values (log2-transformed values > 6.0) to enrich for transcripts present in white fat. We performed student's t-test to identify transcripts exhibiting significant differences of over 1.6-fold for Tg groups compared to WT. Gene Ontology analysis was performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, available at http://david.abcc.ncifcrf.gov).

2.5. Adipose tissue explant culture

Epididymal WAT (eWAT) from WT and Blnc1 Tg mice was dissected and transferred to a culture dish with 10 ml DMEM. Fat pad was cut into approximately 4 mm pieces and washed sequentially with 10 × volume PBS and DMEM. Equal number of fat pieces were transferred into six-well plates with serum-free M199 media (1 nM insulin, 1 nM dexamethasone), and cultured for 24 h before TNFα treatment. Following treatments, fat tissues were collected and processed gene expression and immunoblotting analyses.

2.6. Gene expression analyses

Total RNA from differentiated adipocytes was extracted using TRIzol method following manufacturer instructions. Total tissue RNA was isolated using PureLink RNA isolation kit (ThermoFisher). For RT-qPCR, 2 μg of RNA was reverse-transcribed using MMLV (Invitrogen) followed by qPCR using SYBR Green (Life Technologies). Relative mRNA expression was normalized to the expression of ribosomal protein 36B4.

2.7. Immunoblotting analysis

Total cell lysates were prepared in a lysis buffer containing 50 mM Tris–HCl (pH = 7.8), 137 mM NaCl, 10 mM NaF, 1 mM EDTA, 1% Triton X-100, 10% glycerol, and the protease inhibitor cocktail (Roche) after three freeze/thaw cycles. Tissue lysates were prepared by homogenizing in a buffer containing 50 mM Tris (pH = 7.6), 130 mM NaCl, 5 mM NaF, 25 mM β-glycerophosphoate, 1 mM sodium orthovanadate, 10% glycerol, 1% Triton X-100, 1 mM DTT, 1 mM PMSF and the protease inhibitor cocktail. The antibodies used are: anti-UCP1 (UCP11-A) from Alpha Diagnostic; anti-Tubulin (T6199,

