Original article

MOLECULAR METABOLISM

Genetic backgrounds determine brown remodeling of white fat in rodents

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ABSTRACT

Objective: Genetic background largely contributes to the complexity of metabolic responses and dysfunctions. Induction of brown adipose features in white fat, known as brown remodeling, has been appreciated as a promising strategy to offset the positive energy balance in obesity and further to improve metabolism. Here we address the effects of genetic background on this process.

Methods: We investigated browning remodeling in a depot-specific manner by comparing the response of C57BL/6J, 129/Sv and FVB/NJ mouse strains to cold.

Results: Surprisingly, 129/Sv and FVB/NJ mice showed distinct brown remodeling features despite their similar resistance to metabolic disorders in comparison to the obesity-prone C57BL/6J mice. FVB/NJ mice demonstrated a preference of brown remodeling in inguinal subcutaneous white adipose tissue (iWAT), whereas 129/Sv mice displayed robust brown remodeling in visceral epididymal fat (eWAT). We further compared gene expression in different depots by RNA-sequencing and identified Hoxc10 as a novel "brake" of brown remodeling in iWAT.

Conclusion: Rodent genetic background determines the brown remodeling of different white fat depots. This study provides new insights into the role of genetic variation in fat remodeling in susceptibility to metabolic diseases.

Keywords Genetic background; White adipose tissue; Brown remodeling; Hoxc10; Cold exposure; Browning brake

1. INTRODUCTION

Obesity is a worldwide public health problem [1—3] and a risk factor for many chronic diseases including type 2 diabetes, cardiovascular disorders, atherosclerosis, non-alcoholic fatty liver disease, and several forms of cancer [4,5]. Obesity arises as a consequence of excess fat storage and expansion of white adipose tissue (WAT) over energy expenditure. WAT functions primarily to store energy while brown adipose tissue (BAT) catabolizes free fatty acids and dissipates energy in the form of heat. Therefore, it is appealing to introduce BAT features into WAT, namely brown remodeling, to combat obesity and its co-morbidities.

With the discovery of brown remodeling, there has been growing interest in exploring this phenomenon to manage the consequences of obesity. Brown remodeling is characterized by the emergence of clusters of brown-like UCP1-positive multilocular cells (also referred to as beige or brite cells) interspersed within WAT. In rodents, brown remodeling can be induced by cold exposure or pharmacological treatments such as β-adrenergic receptor agonists and thiazolidinediones (PPARγ agonists) [6—10]. Recently activated human brown fat has been shown to have a similar expression profile to beige fat, and beige adipocytes have been identified in human WAT [11—13]. The origin of beige cells has been explained by either transdifferentiation, the direct conversion of white adipocytes into brown-like cells [7,14] or the differentiation of unique beige precursors [15,16]. Several studies in mouse models illustrate that brown remodeling contributes to an improved metabolic profile marked by protection against diet-induced obesity, improved glucose homeostasis and increased energy expenditure [6,17—21]. While brown remodeling of WAT offers a promising strategy to alleviate obesity, more knowledge is still needed to understand the genetic susceptibility of adipose plasticity to environmental cues.

The interaction of genetic and environmental factors plays a significant role in propagating obesity. It has been established that genetic background is a major contributing factor in the susceptibility to diet-induced obesity and type 2 diabetes. Among the commonly used inbred mouse strains, the C57BL/6J strain is more susceptible to obesity and type 2 diabetes while the 129/Sv strain is protected against these traits [22,23] as is the FVB/NJ strain, which is hyperactive and has higher body temperature [24—26].

Previous studies have integrated histological and quantitative trait loci analysis to identify potential browning genes and found that multiple loci on 8 different chromosomes function synergistically to increase *Ucp1* and *Pgc-1α* expression in retroperitoneal WAT in the A/J mice

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Received August 5, 2016 • Revision received August 16, 2016 • Accepted August 18, 2016 • Available online 26 August 2016

http://dx.doi.org/10.1016/j.molmet.2016.08.013

but not in the C57BL/6J strain [27–29]. However, there was no significant difference in *Ucp1* expression in BAT and epididymal WAT between the two strains [17]. These results highlight the contribution of genetic variation to strain-specific differences together with depot-dependent browning potential of WAT. Given the pressing need to identify genetic mediators of adipose tissue remodeling, in this study, we examined the browning response in the 3 commonly used mouse strains, C57BL/6J, 129/Sv and FVB/NJ, coupled with RNA sequencing to identify genes that account for the adipose plasticity upon chronic cold exposure.

2. METHODS

2.1. Animal studies

Five-week old male C57BL/6J (000664), 129/Sv (002448), FVB/NJ (001800) mice were purchased from Jackson Laboratory (Bar Harbor, ME) and exposed to 4 °C after two-week adaptation in the Columbia University animal barrier. Mice had ad libitum access to regular chow diet (PicoLab rodent diet 20 (LabDiet 5053)). Body temperature was measured by a subcutaneously implanted IPTT-300 transponder (DMDS). During chronic cold exposure, body temperature was measured in light phase at the same time on each day. Plasma glucose was measured with the One Touch Ultra II glucometer. The mice were gently handled by the same experienced animal technician to minimize stress-induced measuring variations. The Columbia University Animal Care and Utilization Committee approved all procedures.

2.2. RNA analysis

One entire side of each respective fat depot was used to isolate total RNA by using RNeasy Lipid Tissue kit (QIAGEN) following the manufacturer's instructions. 1 μg RNA was subjected to cDNA synthesis by using High-capacity cDNA Reverse Transcription kit (Applied Biosystems). qPCR was performed on the CFX96 Real-Time PCR system (Bio-Rad) by using GoTaq qPCR Master Mix (Promega). The relative gene expression levels were calculated by ddCt method with TBP as the reference gene. qPCR primer sequences are available upon request.

2.3. RNA-seq analysis

mRNA was enriched by poly-A pull-down from 1 μg total RNA and used to generate proceed library with using Illumina TruSeq RNA prep kit. Libraries were then sequenced using Illumina HiSeq2000 at Columbia Genome Center. Sequencing reads were aligned to the mouse genome (UCSC/mm9) using Tophat [30] with 4 mismatches and 10 maximum multiple hits. Differential gene expression across strains and tissues was found using cuffdiff [31] with cutoff FPKM > 5, FDR < 0.05 and fold-change > 2. K-means clustering, heatmaps, and scatter plots were generated in R [32]. Genes coding for transcription factors were identified among other regulated genes using "Animal TFDB" database [33].

2.4. *In vitro* cell culture analysis

The C3H/10T1/2 cell line was purchased from ATCC and cultured and differentiated as described [34]. Myc-tagged Human *HOXC10* cDNA was cloned into pTRIPZ inducible expression vector (Thermo Open Biosystems) [35] and used to generate stable cell line into C3H/10T1/2 cell under selection by 1 μg/mL puromycin. On Day 7 of differentiation, HOXC10 overexpression was induced by adding 0.1 μg/mL doxycycline. Cells were treated with isoproterenol (10 μM) and CL-316,243 (1 μg/mL) for 4 h before harvesting for RNA analysis. HOXC10 expression was validated by probing with anti-Hoxc10 antibody HoxC10 (L-17) (Santa Cruz).

2.5. Morphological analysis

Fat tissues were fixed in 10% formalin, embedded in paraffin, and stained with Hematoxylin and Eosin (H&E). Lipid content in differentiated C3H/10T1/2 adipocytes was assessed by Oil Red O staining.

2.6. Statistical analysis

All values were presented as means ± standard error of means (SEM). Unpaired 2-tailed Student's *t* tests were used to evaluate statistical significance and $p < 0.05$ was declared as a statistically significant change.

3. RESULTS

3.1. Genetic background-dependent metabolic responses to cold challenge

To test whether genetic background impacts thermogenic response as well as physiological adaptation to cold, we compared the cold response among 129/Sv, C57BL/6J and FVB/NJ strains. During an 8-day cold challenge at 4 °C, both C57BL/6J and 129/Sv mice lost body weight (5.5% and 9.3%, respectively) while, surprisingly, FVB/NJ mice were able to maintain their body weight (Figure 1A–B). We also monitored their cold responses by measuring body temperature. 129/Sv and FVB/NJ mice were better to maintain their body temperature than C57BL/6J mice during both acute (Figure 1C) and prolonged (Figure 1D) cold challenge, indicating higher thermogenesis in these two strains. During cold challenge, body weight change is determined by the compromise between thermogenesis-induced fat consumption and food intake. The food intake of FVB/NJ mice was significantly higher than that of C57BL/6J and 129/Sv mice (Figure 1E), in support of their maintenance of body mass and preservation of body temperature. 129/Sv mice had less food intake compared to C57BL/6J mice, but also experienced elevated thermogenesis, accounting for their greater body weight loss during cold challenge. Lastly, we measured the blood glucose levels at the end of the cold challenge. The 129/Sv strain had much lower glucose levels than the other stains (Figure 1F). Taken together, these data indicate that genetic backgrounds determine physiological responses to cold exposure, consistent with previous work [17,27,36]

3.2. Distinct brown remodeling in subcutaneous WAT

Prolonged cold exposure is the classic method of inducing brown remodeling of subcutaneous WAT. Given their distinct cold responses, we hypothesized that the subcutaneous WAT from the three strains would have different extents of brown remodeling. Indeed, histological examination of their inguinal fat depots (iWAT) showed a strikingly higher prevalence of multilocular adipocytes, that is an increased browning morphology, in FVB/NJ mice than in C57BL/6J mice (Figure 2A). To our surprise, we observed fewer multilocular adipocytes in iWAT of 129/Sv mice than in C57BL/6J mice (Figure 2A) regardless of their superior defense of temperature loss in the cold (Figure 1D).

Consistent with the morphological analysis, the expression of representative brown genes and brown gene regulators was significantly higher in FVB/NJ mice as compared to C57BL/6J. Indeed, there was a significantly increased expression of brown markers (e.g., *Ucp1, Elovl3, Dio2, Cidea*), mitochondrial respiratory chain enzymes (e.g., *Cox7a1, Cox8b*), and genes involved in fatty acid oxidation pathway (e.g., *Cpt1b*) as well as regulatory genes involved

