Cdkn2a deficiency promotes adipose tissue browning

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ABSTRACT

Objectives: Genome-wide association studies have reported that DNA polymorphisms at the CDKN2A locus modulate fasting glucose in human and contribute to type 2 diabetes (T2D) risk. Yet the causal relationship between this gene and defective energy homeostasis remains elusive. Here we sought to understand the contribution of Cdkn2a to metabolic homeostasis.

Methods: We first analyzed glucose and energy homeostasis from Cdkn2a-deficient mice subjected to normal or high fat diets. Subsequently Cdkn2a-deficient primary adipose cells and human-induced pluripotent stem differentiated into adipocytes were further characterized for their capacity to promote browning of adipose tissue. Finally CDKN2A levels were studied in adipocytes from lean and obese patients.

Results: We report that Cdkn2a deficiency protects mice against high fat diet-induced obesity, increases energy expenditure and modulates adaptive thermogenesis, in addition to improving insulin sensitivity. Disruption of Cdkn2a associates with increased expression of brown-like/beige fat markers in inguinal adipose tissue and enhances respiration in primary adipose cells. Kinase activity profiling and RNA-sequencing analysis of primary adipose cells further demonstrate that Cdkn2a modulates gene networks involved in energy production and lipid metabolism, through the activation of the Protein Kinase A (PKA), PKG, PPARGCA1 and PRDM16 signaling pathways, key regulators of adipocyte beiging. Importantly, CDKN2A expression is increased in adipocytes from obese compared to lean subjects. Moreover silencing CDKN2A expression during human-induced pluripotent stem cells adipogenic differentiation promoted UCP1 expression.

Conclusion: Our results offer novel insight into brown/beige adipocyte functions, which has recently emerged as an attractive therapeutic strategy for obesity and T2D. Modulating Cdkn2a-regulated signaling cascades may be of interest for the treatment of metabolic disorders.

Keywords Obesity; Type 2 diabetes; Insulin sensitivity; Energy expenditure; cdkn2a; Genome-wide association study; Adipose tissue browning

1. INTRODUCTION

Obesity is the main risk factor for type 2 diabetes (T2D) and is due to an imbalance between energy intake and energy expenditure. Current anti-obesity drugs affecting energy intake or intestinal lipid absorption cause important and troublesome side effects for the patient, which limits their use. On the other hand, bariatric surgery has proven efficient for obesity and for diabetes remission with a dramatic effect on insulin resistance, although long-term complications and obesity relapse may arise. Therefore, understanding the signaling pathways that control fat storage and energy expenditure may open alternative avenues against obesity and linked T2D.

In animal models, genes such as E2f1 and Sertad2 (TRIP-Br2) have been shown to prevent fat accumulation and protect against diet-induced obesity (DIO), but they also improve insulin action in metabolic organs including adipose tissue [1–3]. Two major types of

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While rodent and human studies have highlighted the potential role of Cdkn2a on insulin secretion [16,26], the contribution of Cdkn2a to the control of insulin sensitivity is still elusive.

To decipher the link between Cdkn2a, obesity and insulin resistance, we analyzed the effect of Cdkn2a deficiency in mice on metabolic homeostasis [27]. Here, we demonstrate that the genetic deletion of Cdkn2a promotes energy expenditure and improves insulin sensitivity during DIO. We found that the Cdkn2a locus modulates signaling pathways in inguinal WAT (ingWAT), where it controls beige adipocyte development. Importantly, we observed that Cdkn2a expression is increased in adipocytes from obese patients. Cdkn2a silencing during human-induced pluripotent stem cells adipogenic differentiation promoted UCP1 expression. These data suggest that Cdkn2a plays a key role in energy metabolism by regulating a white-to-brown fat transition.

2. MATERIAL AND METHODS

2.1. Materials and oligonucleotides

Chemicals, unless stated otherwise, were purchased from Sigma—Aldrich. Anti-UCP1 antibody was purchased from Abcam, and anti-PKA substrate antibody was from Cell Signaling Technology. The oligonucleotides sequences used for various experiments are listed in Table S5.

2.2. Animals experiments

Cdkn2a+/+ and Cdkn2a−/− mice were previously described [27] and were maintained on a mixed background according to European Union guidelines for use of laboratory animals. In vivo experiments were performed in compliance with the French ethical guidelines for studies on experimental animal (Animal house agreement no. B 59-35015, Authorization for Animal Experimentation no. 59-350294, project approval by our local ethical committee no. CEEA 482012). All experiments were performed with healthy male mice. Animals with detectable tumors were excluded from the study. Mice were housed under a 12 h light—dark cycle and given a regular chow (A04; Safe). For HFD studies, 8-week old mice were placed on D12492 diet (60% cal/fat; Research Diet Inc.) for 13 weeks. Metabolic data were collected between 5 and 8 weeks on a 12 h light—dark cycle. Mice were housed individually and maintained at 23 °C under a 12 h light/12 h dark cycle. Food and water were available ad libitum. Mice were sacrificed by cervical dislocation and tissues were harvested and snap-frozen for further processing.

2.3. RNA extraction, measurements, and profiling

Total RNA was extracted from rodent cells and tissues using trizol reagent (Life Technologies) as described previously [1,28]. mRNA expression was measured after reverse transcription by quantitative...
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