Tracing the genetic signature of human brown adipocytes

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Abstract
Until recently, a functional brown adipose tissue was thought to be restricted to rodents and human infants. The last eight years have seen a re-emergence in the presence and activity of brown adipose tissue in adult humans. This study by Shinoda and co-workers[1] characterizes the cellular origins and molecular identity of adult human brown adipose tissue. Using RNA sequencing and genome wide expression analysis, the authors characterize that the clonally derived adipocytes isolated from brown fat of adult humans carry the genetic signature of recruitable thermogenic beige adipocytes. They further identify novel molecular markers that were highly enriched in the adult human brown adipocytes and rodent beige adipocytes. This study provides new approaches for BAT research and developing novel BAT targets.
Keywords: human brown adipocytes, obesity, beige adipocytes, BAT.

Introduction

The presence of active brown adipose tissue (BAT) depots in adult humans has sparked an interest in developing anti-obesity therapeutics targeting this tissue. Accidental discovery during ^{18}Fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) scanning for tumors revealed symmetrical uptake of glucose in non-tumor tissue depots[2], which were identified later on as brown adipose depots (Figure 1). Recent studies have shown that the adult brown adipose depots can be activated by cold exposure and β adrenergic-agonists[3-10]. This inducible nature of brown adipocytes has showed great promise in developing small molecule activators of towards anti-obesity therapeutics. Genetic analysis of human brown adipose revealed that it is more closely related to the rodent 'beige' adipocytes rather than the classical brown adipocytes[3-9]. Beige adipocytes are inducible subcutaneous white adipocytes that are derived from a smooth muscle lineage, very distinct from the skeletal muscle lineage of classical brown adipocytes, that can be induced to 'brown' in response to certain stimuli (such as cold or β adrenergic-agonists). However, some recent studies also report that cultured adipocytes derived from human brown adipose tissue express several classical mouse brown adipocyte selective markers[9,10]. This discrepancy could be due to a difference in the gene expression profile of this very heterogeneous tissue in adult humans; depending on which region the biopsies were from (i.e the neck region or the interior suprarenal region). Shinoda and coworkers in this follow-up study[9] carry out a global and unbiased genetic screen to identify the molecular markers that are unique to human brown adipocytes, which will help developing drug targets that will activate these human brown adipocytes in a highly selective way.

The Study

In this study, the authors established 65 clonally immortalized preadipocyte cell lines from BAT biopsies obtained from the supraclavicular region of two non-obese individuals. These lines were subjected to an in-vitro differentiation into brown adipocytes and from the 65 different cell lines, only 7 lines were capable of adipogenic differentiation. The authors reason that this is in accordance with previous reports, which suggest that only a small portion (6-10%) of cells retain their ability to differentiate in vitro. Further

screens were carried out using 3 of the 7 lines (Brown 1-3). The brown fat identity of these lines was confirmed by a gene expression analysis of the classical brown fat markers UCP1. These cell lines also respond to cAMP mediated induction of brown fat specific gene expression as well as show increased uncoupled mitochondrial respiration similar to the classical brown adipocytes. Next, to identify the gene signatures of these human brown adipocytes, these in-vitro differentiated cell lines were subjected to RNA-sequencing. The top 800 candidate genes were compared to mouse gene orthologs enriched in brown or beige adipocytes and human adipocytes derived from subcutaneous adipose tissue. Based on the hierarchical clustering, the authors conclude that the human brown adipocytes carry a molecular signature that closely resembles the mouse beige adipocytes, which is in accordance with previously published reports. Interestingly, the authors also report that the gene expression is similar between undifferentiated and differentiated human brown adipocytes, which suggests that the molecular signatures are determined at the precursor stage. The authors also identified two unique BAT marker genes, MTUS1 and KCNK3, in human clonally derived adipocytes that show a strong co-relation with the expression of mouse BAT genes. MTUS1 is a mitochondrial protein that has been shown to regulate cell proliferation and KCNK3 is a pH- dependent, voltage sensitive potassium channel. These genes are also inducible with cold exposure in supraclavicular BAT depots from subjects under prolonged cold exposure, suggesting sympathetic regulation of these genes. Interestingly, these genes are enriched only the supraclavicular and posterior mediastinum human BAT depots, but not in retroperitoneal BAT depots. In mice, both these genes were also induced with beta-adrenergic agonist treatment. siRNA knockdown of MTUS1 and KCNK3 in mouse beige adipocytes decreased the protein levels of UCP1 and expression of brown fat specific genes, which suggests a requirement of these genes in beige adipocyte differentiation and function.

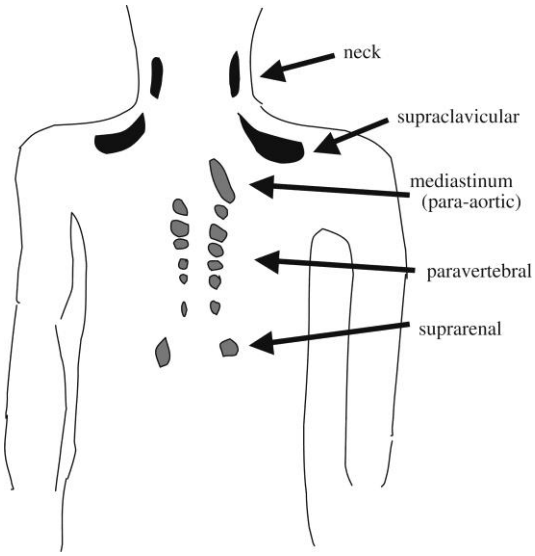

Figure 1: Distribution of brown fat depots in adult humans (adapted from [2])

Conclusion

In the recent quest for combating obesity, targeting the activation of brown fat to increase energy expenditure rather than targeting pharmacological control of appetite shows more promise and feasibility[5,6,11-14]. The recent discovery of presence of inducible brown adipose tissue in humans has seen a lot of discoveries in the last few years, right from characterizing the molecular markers to identifying the genetic lineage and identifying the developmental origins. Multiple groups have now confirmed that the adult human brown adipose tissue closely resembles the mouse inducible beige adipocytes that arise from a smooth muscle lineage unlike the classical brown adipose that arises from a Myf5+ skeletal muscle lineage[9,10,15,16]. The current study by Shinoda and co-workers reconfirm that human brown adipocytes closely resemble mouse beige adipocytes. One of the drawbacks of this study is the

limited sample population that was analyzed (n =2 individuals) and the regions of the biopsies. The study also further characterized biopsies from other BAT depots (i.e posterior mediastinum and retroperitoneal) and show that different depots carry a different gene expression profile. It would have been interesting to analyze brown adipocytes from obese individuals to identify genes that are differentially regulated between lean and the obese. Brown adipose tissue in humans is highly active in the newborns and its activity regresses as the individual grows. Further studies characterizing the gene signature of a younger population may help identify if the genetic signature changes with aging. This study identified some novel brown adipocyte markers that are highly enriched in human brown adipocytes and could be used as secondary markers to the classical ones.

Targeting BAT has been studied since the early 1930s where 2,4 Dinitrophenol (DNP), a mitochondrial uncoupler, which acts similar to UCP1, was widely used for weight loss[17]. Although it was effective in increasing the metabolic rate, it also resulted in deaths from hyperthermia (increased body temperature) and off-target effects, which led to its discontinuation for treatment. Pharmacological activation of brown adipose tissue still seems promising, but further studies are warranted in characterizing the nature of these adipocytes and long term effects of increasing energy expenditure to validate the feasibility of this approach.

References

1. Shinoda K, Luijten IH, Hasegawa Y, et al. Genetic and functional characterization of clonally derived adult human brown adipocytes. Nat Med. 2015;21(4):389-394. http://dx.doi.org/10.1038/nm.3819 PMid:25774848 PMCid:PMC4427356

2. Nedergaard J, Bengtsson T, Cannon B. Unexpected evidence for active brown adipose tissue in adult humans. Am J Physiol Endocrinol Metab. 2007;293(2):E444-52. http://dx.doi.org/10.1152/ajpendo.00691.2006 PMid:17473055

3. Cannon B, Nedergaard J. Yes, even human brown fat is on fire! J Clin Invest. 2012;122(2):486-489. http://dx.doi.org/10.1172/JCI60941 PMid:22269320 PMCid:PMC3266796

4. Cannon B, Nedergaard J. Brown adipose tissue: Function and physiological significance. Physiol Rev. 2004;84(1):277-359. http://dx.doi.org/10.1152/physrev.00015.2003 PMid:14715917

5. Cereijo R, Giralt M, Villarroya F. Thermogenic brown and beige/brite adipogenesis in humans. Ann Med. 2014:1-9.

6. Diaz MB, Herzig S, Vegiopoulos A. Thermogenic adipocytes: From cells to physiology and medicine. Metabolism. 2014;63(10):1238-1249. http://dx.doi.org/10.1016/j.metabol.2014.07.002 PMid:25107565

7. Kuhn E, Binart N, Lombes M. Brown, white, beige: The color of fat and new therapeutic perspectives for obesity. Ann Endocrinol (Paris). 2012;73 Suppl 1:S2-8. http://dx.doi.org/10.1016/S0003-4266(12)70009-4

8. Lidell ME, Betz MJ, Enerback S. Two types of brown adipose tissue in humans. Adipocyte. 2014;3(1):63-66. http://dx.doi.org/10.4161/adip.26896 PMid:24575372 PMCid:PMC3917936

9. Sharp LZ, Shinoda K, Ohno H, et al. Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS One. 2012;7(11):e49452. http://dx.doi.org/10.1371/journal.pone.0049452 PMid:23166672 PMCid:PMC3500293

10. Wu J, Bostrom P, Sparks LM, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell. 2012;150(2):366-376. http://dx.doi.org/10.1016/j.cell.2012.05.016 PMid:22796012 PMCid:PMC3402601

11. Lee P, Werner CD, Kebebew E, Celi FS. Functional thermogenic beige adipogenesis is inducible in human neck fat. Int J Obes (Lond). 2014;38(2):170-176.http://dx.doi.org/10.1038/ijo.2013.82 PMid:23736373

12. Rachid TL, Penna-de-Carvalho A, Bringhenti I, Aguila MB, Mandarim-de-Lacerda CA, Souza-Mello V. Fenofibrate (PPARalpha agonist) induces beige cell formation in subcutaneous white adipose tissue from diet-induced male obese mice. Mol Cell Endocrinol. 2015;402:86-94. http://dx.doi.org/10.1016/j.mce.2014.12.027 PMid:25576856

13. Okla M, Ha JH, Temel RE, Chung S. BMP7 drives human adipogenic stem cells into metabolically active beige adipocytes. Lipids. 2015;50(2):111-120. http://dx.doi.org/10.1007/s11745-014-3981-9 PMid:25534037

14. Wu J, Cohen P, Spiegelman BM. Adaptive thermogenesis in adipocytes: Is beige the new brown? Genes Dev. 2013;27(3):234-250. http://dx.doi.org/10.1101/gad.211649.112 PMid:23388824 PMCid:PMC3576510

15. Seale P, Bjork B, Yang W, et al. PRDM16 controls a brown fat/skeletal muscle switch. Nature. 2008;454(7207):961-967. http://dx.doi.org/10.1038/nature07182 PMid:18719582 PMCid:PMC2583329

16. Seale P, Kajimura S, Spiegelman BM. Transcriptional control of brown adipocyte development and physiological function--of mice and men. Genes Dev. 2009;23(7):788-797. http://dx.doi.org/10.1101/gad.1779209 PMid:19339685 PMCid:PMC2763499

17. Harper JA, Dickinson K, Brand MD. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. Obes Rev. 2001;2(4):255-265. http://dx.doi.org/10.1046/j.1467-789X.2001.00043.x PMid:12119996