Could “Brown Adipose Tissue Failure” be a Cause of Metabolic Syndrome?

Authors:

Norihiko Kobayashi, Ph.D.
Masako Nakahara, Ph.D.
Masako Oka, Ph.D.
Kumiko Saeki M.D., Ph.D.

Department of Disease Control, Research Institute, National Center for Global Health and Medicine, Tokyo 162-8655, Japan

Correspondence to:
Kumiko Saeki M.D., Ph.D.,
Department of Disease Control, Research Institute, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku-ku, Tokyo 162-8655, Japan.
Telephone: 81-3-3202-7181;
Fax: 81-3-3207-1038;
E-mail: saeki@ri.ncgm.go.jp

Abstract

Human brown adipose tissue (BAT) is recognized as one of the most important target tissues in the drug discovery for the treatment of obesity-related metabolic disorders. It is suggested that the BAT improves glucose metabolism independently of its calorigenic capacity, probably *via* secreting factors. Although several molecules have been identified as BAT-derived glucose metabolism-improving hormones (i.e. BATkines), the crucial factor(s) remains undiscovered. The difficulty in discovering those crucial BATkines may be attributed to the fact that Rnase1 and a variety of chymotrypsin family peptidases are expressed at relatively high levels in murine BATs, which have been used as a material in BATkine hunting. In this review, we describe a new strategy for discovering novel BATkines by using brown adipocytes (BAs) derived from human pluripotent stem cells. We also discuss the possible mechanism of how human BAs are involved in the regulation of glucose metabolism.

Keywords: brown adipose tissue, human embryonic stem cells, human induced pluripotent stem cells, BATkine, glucose metabolism, metabolic syndrome.

1. Introduction/background

Brown adipose tissue (BAT) is a unique fat depot that shows high heat-producing capacity. Brown adipocytes (BAs) contain abundant cristae-rich mitochondria and multilocular lipid droplets. Under sympathetic nerve stimuli, BA produces heat by destructing the electron potential across the mitochondrial inner membrane generated by electron transport system (ETS). When uncoupling protein 1 (UCP1) is activated by lipolysis-derived free fatty acids under the adrenergic stimuli, the mitochondrial electron potential is destructed, thereby releasing electronic energy occurs as thermal energy. In small sized mammals with high body surface/weight ratios including mice, BAT-dependent thermogenesis is indispensable for maintaining body temperature. In addition to BATs, which are derived from myf5-positive myoblasts in dorsal regions of the dermomyotome [1], BAT-like thermogenic fat depots are detected in white adipose tissues (WATs), especially in inguinal regions in the close vicinity to the femoral sheath in cold acclimated mice. These fat depots are termed as Brite [2] or Beige [3]. Although BAT and Brite/Beige share a set of common gene expression profiles, each fat depot has a series of its selective gene markers [3-5]. While a single beige cell reportedly shows an equivalent calorigenic potential as single brown adipocyte [6, 7], it is suggested that the Brite depots of cold acclimated mice may not have a predominant influence on the capacity for nonshivering thermogenesis [6]. A consensus finding regarding the functional difference between BAT and Brite/Beige has not yet been obtained. Nevertheless, the unique structure of BATs, which are equipped with densely packed capillary networks similar to the case of endocrine tissues (Figure 1), imply that BATs may play unidentified roles as quasi-endocrine tissues.

The presence of BATs in adult humans was reported in 2009 [8-11]. After a dispute over whether humans had indeed BATs or they had nothing but Beige depots [3, 12], the existence of human BATs was re-confirmed in 2013 [13]. Thereafter, human BATs have been attracting increasing attention as an important target in the drug discovery for the treatment of metabolic disorders.

However, there are considerable amounts of hurdles in preparing high-quality research materials. In addition to the cost issue, there is a serious ethical concern in obtaining sufficient amounts of human BAT specimens via biopsy or surgery. Even in the case of murine BATs, an issue of "fragility" limits the progression of the researches. The fragileness of BAT may be attributed, at least in part, to its relatively high expressions of Rnase1 and chymotrypsin-related peptidases (Table 1). Thus, development of a novel tool for BA researches has long been awaited to proceed the studies of BATs.

We previously established a method for producing functional BAs from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) [14, 15]. Since glucose metabolism was significantly improved on the following day of transplantation [14], it seems that the glucose metabolism-improving capacity of BAs is independent of its thermogenesis potential. It has long been suggested that BAT secretes certain factor(s) to improve glucose metabolism. Although several candidates have been reported (reviewed by Wang et al. [16]), it seems that crucial BATkines involved in the improvement of glucose metabolism remain undiscovered. In this review, we would like to discuss a novel and effective strategy for discovering genuine BA-specific hormones.

2. The two waves of BAT regression in human life

2.1. BAT regression in neonatal period

There are two phases in the regression process of human BATs: physiological *versus* pathological. During the neonatal period, humans have a large BAT depot in the interscapular region that is equivalent to the murine interscapular BAT (iBAT), the major BAT depot in mice. In large-sized mammals including humans, however, iBAT disappears by the end of the neonatal period or at an early phase in the suckling stage. Since the regression of iBAT occurs in every individual without exception, it is considered as a physiological process. Although the mechanism and the reason for this regression remain elusive, a long-term existence of iBAT in large-sized mammals might exert adverse effects. For example, $ApoE^{-/-}$hypercholesterolemic mice reportedly undergo severer

atherosclerosis under cold acclimation due to accelerated UCP1-dependent lipolysis [17]. It is known that humans are more prone to undergo atherosclerosis than mice. Therefore, iBAT regression might rather be beneficial in the case of humans, reducing the risk of developing ischemic diseases. In any event, this early wave of BAT regression would not be a critical phenomenon in considering the etiology of metabolic disorders in adult life.

2.2. Age-related BAT regression in later life

Even after the disappearance of iBAT in the neonatal period, BATs remain to exist in cervical, supraclavical, axillary, paraaortic, perirenal and paravertebral regions. It is well known that adrenergic stimuli are required for the maintenance of histology and functions of BATs. Therefore, the distribution of BATs may reflect the local concentration of adrenalins, which are released from adrenergic nerve endings or uptaken from the circulating blood by adrenergic terminals [18]. Indeed, the regions where BATs are located receive intensive sympathetic innervation (Figure 2).

The distribution of active BATs can be visualized by a combination study of positron emission tomography (PET) using an ^{18}F-fluorodeoxyglucose probe and computed tomography (CT). A study on healthy volunteers preconditioned by cold stimuli (19˚C, 2 hour) has shown that active BAT decreases with age: more than 50 % of young individuals in their twenties showed active BATs while less than 30% and none of the individuals in their forties and sixties showed active BATs, respectively [19]. This study further showed that BAT-positive subjects lacked age-related body weight gains whereas BAT-negative subjects underwent middle-age spread [19]. Thus, it is strongly suggested that human BATs play indispensable roles in preventing age-related obesity. In other words, middle-age spread should not be a natural or physiological process, but rather, a pathological process that would lead to the development of metabolic disorders. Indeed, the incidence of metabolic syndrome by age shows the clearest correlation with the dynamics of BAT regression (Figure 3). Hence, the development of metabolic disorders could be explained, at least in part, the failure of

BAT functions. In the following section, we would like to discuss the possible mechanism for BAT-based metabolism regulation.

3. Thermogenesis-independent functions of BATs

The biological significance of BATs was reported more than twenty years ago in BAT-depleted mice, which bore an *Ucp1* promoter-driven diphtheria toxin A (DTA)-expressing transgenes to induce prompt death in *Ucp1*-positive cells [20]. At 16 days of age, the BAT-depleted mice became obese with an equivalent severity to *ob/ob* mice even in the absence of hyperphagia. In the step of obesity progresses, they became hyperphagic and underwent severe glucose and lipid metabolic disorders [21] along with serious leptin resistance [22]. In the 21st century, however, gene-targeting approaches (i.e. knockout/knock-in mice) have become the standard for genetic engineering in animal experiments instead of transgenic approach because of the difficulty of maintaining the transgenes in a controlled state. Nowadays, the findings obtained from Ucp1-DTA-transgemic mice are scarcely referred. Nevertheless, a series of the works performed by using Ucp1-DTA-transgemic mice provided important findings in considering the biological significance of BATs. On the other hand, UCP1 KO mice, which were intolerant to acute cold stimuli, did not become obese when reared at room temperature even under high-fat diet [23] although they underwent obesity under thermoneutral (~30˚C) conditions without high-fat diet [24] and suffered from glucose metabolism disorder in later life [25]. Moreover, UCP1 KO mice showed sensitivity to single injection of leptin administration although they were more prone to undergo leptin resistance under repetitive leptin administrations than wild type mice [26]. Thus, there is a discrepancy between BAT-depleted mice and Ucp1 KO mice regarding obesity proneness and leptin resistance (Table 2).

There may be several interpretations for the discrepancy. Among those, the most likely explanation would be that BATs contribute to obesity prevention and glucose metabolism mainly in a thermogenesis-independent manner. In agreement with this, a clinical study showed that there was no correlation between core body temperature and obesity [27]. Moreover, transplantation of

hESC/hiPSC-derived BAs lowered fasting blood glucose levels and augmented glucose tolerance in oral glucose tolerance test (OGTT) as early as on the following day of transplantation (i.e. after 16 hours from transplantation) [14]. Since the BA-transplanted and control mice were similarly kept in starvation until the start of OGTT, the effect of food intake or body weight changes is not needed to be considered. The most appropriate explanation for the prompt effect of the transplantation would be that BAs secrete certain soluble factors (hereinafter collectively referred to as "BATkines") to improve glucose metabolism. In Figure 4, the hypothetic functions of BATkines are summarized. Up to now, a number of molecules including ANGPTL8, FGF21, IL6, VEGFA/B, BMPs, adiponectin, NGF and NRG4 have been reported as the candidate for BATkines (reviewed by Wang et al. [16]). However, it seems that the crucial glucose metabolism-improving factors have not been identified yet because the major producers of these molecules are other tissues than BAT. Even in the cases whose main producer is BAT (e.g. ANGPTL8, NRG4), their reported functions could not sufficiently explain the robust and prompt effects of transplanted BA on glucose metabolism improvement. The unsuccessful outcome in discovering genuine BATkines in the past indicates that the development of novel research tools is required for identifying crucial BATkines. In the following section, we would like to discuss the possibility of applying regenerative medicine technique as a new strategy for BATkine hunting.

4. Regenerative Medicine and Metabolic Disease Control

4.1. Fragility of murine BATs

In most of the previous studies, murine BAT samples were used for BATkine discovery. However, we need to take account of the considerable fragileness of BATs, which makes it difficult to obtain high-quality BA samples that correctly reproduce the functions and gene/protein expression profiles *in vivo*. The fragility of BATs may be attributed, at least in part, to their relatively high expressions of chymotrypsin family peptidases and Rnase1, which are primarily expressed in the pancreas (Table 1). Moreover, quality changes or degeneration generally occurs during the

process of cell preparation from living bodies (e.g. manipulation for tissue removal, enzymatic treatments for cell dissociation) as reported in hepatocytes and vascular endothelial cells [28-31]. In this regard, human pluripotent stem cells such as hESCs and hiPSCs have a great advantage that they can provide differentiated cells of interest without degeneration. In the following section, we would like to discuss the benefit of using hESC/hiPSC-derived BAs for BATkine hunting.

4.2. Exploration of BAT-derived hormones using hESCs

As already mentioned, we established a method for a directed differentiation of hESC/hiPSC into functional BA without exogenous gene transfer [14]. This method has an advantage over other methods that are based on exogenous gene transfer [32, 33] in that it is applicable to the analysis on gene expression dynamics during BA differentiation. Although it is known that BAs are derived from $En1^+$ cells in the somite [34] and $Myf5^+$ myoblasts in the dermomyotome [1], molecular events in earlier developmental process of BAs remain elusive. Since our method properly reproduces the *in vivo* events during BAT development ([14] and data not shown), gene/protein expression analyses using hESC-based differentiation system may provide useful information for early phases of BA differentiation. Currently, hiPSCs are the most commonly used human pluripotent stem cells. Nevertheless, we strongly recommend using hESCs instead because they are the natural counterpart of hiPSCs, and thus, provide information of the highest quality and reproducibility (data not shown).

The hESC-based differentiation system reproduces BA developmental process in fetal life. Whether or not there are alternative pathways to supply BAs in adult life remain controversial although the involvement of vascular components was suggested [35, 36]. It seems that age-related regression of BATs in humans is attributed to certain events during BA development in fetal life rather than insufficient BA supply from alternative pathways in adults. For example, the destruction of PRDM16 gene, which is involved in the commitment of myoblast to BAs [1], causes progressive degeneration of BAT in adult mice: Ucp1 mRNA levels were decreased by >90% at 6 months of age and severely whitened BATs were

detected at 11 months of age [37]. Since 11 months of age (ca. 25 years old in human age) corresponds to the time when human BAT detection rates begin to decrease, the failure in BAT maintenance may trigger the development of metabolic syndrome. Novel molecular targets in drug discovery for the treatment of metabolic syndrome may be obtained by elucidating the molecular mechanism for BAT maintenance in adult life. Our preliminary observations suggest that hESC-derived BAs secrete a variety of soluble factors, in which unidentified auto/paracrine factors and glucose metabolism-improving factors are included (Figure 5A). It is expected that the hESC-based BA differentiation system will provide a new strategy to elucidate the soluble factor-based regulatory network that is involved in BAT maintenance and glucose metabolism regulation (Figure 5B).

Collectively, the hESC-based BA differentiation system provides a useful research tool in 1) clarifying the molecular events in early-phase BA development, 2) discovering novel glucose metabolism-improving factors (e.g. insulin sensitizers, leptin sensitizers, insulin secretion stimulators), 3) identifying auto/paracrine factors involved in BAT maintenance in adult life. Elucidation of the mystery of BAT will make a large contribution to the therapeutic development of metabolic syndrome.

5. Future prospects

Metabolic syndrome is a chronic disease. Therefore, long-term survival of transplanted hiPSC-derived BAs is required for the recovery from metabolic disorders. However, subcutaneously transplanted BAs could survive a relatively short duration of time (< 3-4 weeks) as reported in humans [14] and mice [38]. By contrast, intraperitoneally transplanted BATs could survive for sufficiently long time period (i.e. at least up to 16 weeks) to ameliorate metabolic disorders [38]. Thus, an intraperitoneal route should be taken in future clinical trials using hiPSCs-derived BAs for the treatment of metabolic syndrome. However, the intraperitoneal transplantation has a risk of peritonitis. In addition, the removal of transplanted grafts in case of tumor formation would be a highly complicated process because peritoneal adhesion inevitably occurs after transplantation. Therefore, the clinical study using hiPSC-derived BAs should be considered firstly in the case of subcutaneous transplantation.

Even the short-term survival of hiPSC-derived BAs may exert sufficient therapeutic effect, for example, via a transient amelioration of leptin resistance. The major cause of poor performance in dietotherapy is the presence of serious leptin resistance, due to which the subjects on diet suffer from unbearable sensation of hunger. At the very time period when subjects feel the most intolerable hunger, subcutaneously transplanted hiPSC-derived BAs may mitigate the sensation of hunger, assisting their willpower to continue dietotherapy. Even after the accomplishment of dietotherapy, additional subcutaneous transplantation of hiPSC-derived BAs may bring a desirable outcome, lowering the risk of rebound weight gain. Thus, even a limited application of hiPSC-derived BAs in the subcutaneous transplantation therapy may produce a positive result as an adjuvant or supportive therapy. Although there are several leptin resistance-ameliorating drugs, combination of the drug-based therapy and a regenerative medicine-based approach may further raise therapeutic performance. Since high concentrations of circulating leptin *per se* aggravates leptin resistance and the occurrence of leptin resistance precedes the development of metabolic disorders, searching for additional measures to ameliorate leptin resistance will provide improved results in the treatment of metabolic syndrome.

6. Conclusions

Increasing evidence suggests that BAT regression in adult life is a pathological process and is involved in the development of metabolic disorders. What proportion of patients with metabolic syndrome suffers from BAT failure is a matter to be addressed. For this aim, development of a feasible method for measuring active BATs amounts (e.g. serodiagnosis) is required. The hESC-derived BAs may provide an excellent tool for providing human BA-specific monoclonal antibodies that can be applied to serological diagnosis of BAT failure. New approaches by using regenerative medicine technique of may open the door for advanced therapeutic development of the treatment for metabolic syndrome.

References

1. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, Scimè A, Devarakonda S, Conroe HM, Erdjument-Bromage H, Tempst P, Rudnicki MA, Beier DR, Spiegelman BM. PRDM16 controls a brown fat/skeletal muscle switch. Nature 454: 961-967, 2008. DOI: 10.1038/nature07182.
2. Petrovic N, Walden TB, Shabalina IG, Timmons JA, Cannon B, Nedergaard J. Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. J Biol Chem 285: 7153-7164, 2010. DOI: 10.1074/jbc.M109.053942.
3. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerbäck S, Schrauwen P, Spiegelman BM. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150: 366-376, 2012. DOI: 10.1016/j.cell.2012.05.016
4. Waldén TB, Hansen IR, Timmons JA, Cannon B, Nedergaard J. Recruited vs. nonrecruited molecular signatures of brown, "brite," and white adipose tissues. Am J Physiol Endocrinol Metab 302: E19-E31, 2012. DOI: 10.1152/ajpendo.00249.2011
5. Jasper M. A. de Jong, Larsson O., Cannon B., Nedergaard J. A stringent validation of mouse adipose tissue identity markers. Am. J. Physiol. Endocrinol Metab. 308, E1085–E1105, 2015. DOI: 10.1152/ajpendo.00023.2015.
6. Shabalina IG, Petrovic N, de Jong JM, Kalinovich AV, Cannon B, Nedergaard J. UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. Cell Rep 5: 1196-1203, 2013. DOI: 10.1016/j.celrep.2013.10.044
7. Okamatsu-Ogura Y., Fukano K., Tsubota A., Uozumi A., Terao A., Kimura K., Saito M. Thermogenic ability of uncoupling protein 1 in beige adipocytes in mice. PLoS One. 8: e84229, 2013. DOI: 10.1371/journal.pone.0084229.
8. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo

FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR. Identification and importance of brown adipose tissue in adult humans. N. Engl. J. Med. 360, 1509–1517, 2009. DOI: 10.1056/NEJMoa0810780

9. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerba cḱ S, Nuutila P. Functional brown adipose tissue in healthy adults. N. Engl. J. Med. 360, 1518–1525, 2009. DOI: 10.1056/NEJMoa0808949

10. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, Schrauwen P, Teule GJ. Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med. 360, 1500–1508, 2009. DOI: 10.1056/NEJMoa0808718

11. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M. High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. Diabetes 58, 1526–1531, 2009. DOI: 10.2337/db09-0530.

12. Sharp LZ, Shinoda K, Ohno H, Scheel DW, Tomoda E, Ruiz L, Hu H, Wang L, Pavlova Z, Gilsanz V, Kajimura S. Human BAT possesses molecular signatures that resemble beige/brite cells. PLoS One 7: e49452, 2012. DOI: 10.1371/journal.pone.0049452

13. Cypess AM, White AP, Vernochet C, Schulz TJ, Xue R, Sass CA, Huang TL, Roberts-Toler C, Weiner LS, Sze C, Chacko AT, Deschamps LN, Herder LM, Truchan N, Glasgow AL, Holman AR, Gavrila A, Hasselgren PO, Mori MA, Molla M, Tseng YH. Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. Nat Med 19:635-629, 2013. DOI: 10.1038/nm.3112

14. Nishio M, Yoneshiro T, Nakahara M, Suzuki S, Saeki K, Hasegawa M, Kawai Y, Akutsu H, Umezawa A, Yasuda K, Tobe K, Yuo A, Kubota K, Saito M, Saeki K. Production of functional classical brown adipocytes from human pluripotent stem cells using specific hemopoietin cocktail without gene transfer. Cell Metab. 16: 394-406, 2012. DOI: 10.1016/B978-0-12-411619-1.00010-0.

15. Nishio M, Saeki K. Differentiation of human pluripotent stem cells into

highly functional classical brown adipocytes. Methods Enzymol 537: 177-197, 2014. DOI: 10.1016/B978-0-12-411619-1.00010-0

16. Wang GX, Zhao XY, Lin JD. The brown fat secretome: metabolic functions beyond thermogenesis. Trends Endocrinol Metab. 26: 231-237, 2015. DOI: 10.1016/j.tem.2015.03.002

17. Dong M, Yang X, Lim S, Cao Z, Honek J, Lu H, Zhang C, Seki T, Hosaka K, Wahlberg E, Yang J, Zhang L, Länne T, Sun B, Li X, Liu Y, Zhang Y, Cao Y. Cold exposure promotes atherosclerotic plaque growth and instability via UCP1-dependent lipolysis. Cell Metab. 18: 118-129, 2013. DOI: 10.1016/j.cmet.2013.06.003.

18. Dib J, Lapsha VI, Gurin VN. Catecholamine concentration in adrenergic plexuses of the spleen and intestine during cold-induced and emotional stress. Neurophysiology 22: 261–266, 1990. PMID: 2398930

19. Yoneshiro T, Aita S, Matsushita M, Okamatsu-Ogura Y, Kameya T, Kawai Y, Miyagawa M, Tsujisaki M, Saito M. Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. Obesity 19: 1755-1760, 2011. PMID: 24897476

20. Lowell BB, S-Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 366: 740-742, 1993. DOI: 10.1038/366740a0

21. Hamann A, Flier JS, Lowell BB. Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes, and hyperlipidemia. Endocrinology 137:21-29, 1996. DOI: 10.1210/endo.137.1.8536614.

22. Mantzoros CS, Frederich RC, Qu D, Lowell BB, Maratos-Flier E, Flier JS. Severe leptin resistance in brown fat-deficient uncoupling protein

promoter- driven diphtheria toxin A mice despite suppression of hypothalamic neuropeptide Y and circulating corticosterone concentrations. Diabetes 47:230-238, 1998. PMID: 9519718

23. Enerbäck S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature. 387:90-94, 1997. DOI: 10.1038/387090a0

24. Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. Cell Metab 9:203-209, 2009. DOI: 10.1016/j.cmet.2008.12.014

25. Thoonen B, Hindle A, Miranda AB, Yao V, Tainsh R, Buys E, Bloch K, Scherrer-Crosbie M. Brown Adipocyte Dysfunction in UCP1 Knockout Mice leads to Age-Dependent Development of Type 2 Diabetes. FASEB J. vol. 29 no. 1 Supplement 995.13, 2015. http://www.fasebj.org/content/29/1_Supplement/995.13?related-urls=yes&legid=fasebj;29/1_Supplement/995.13

26. Okamatsu-Ogura Y, Nio-Kobayashi J, Iwanaga T, Terao A, Kimura K, Saito M. Possible involvement of uncoupling protein 1 in appetite control by leptin. Exp Biol Med (Maywood) 236:1274-1281, 2011. DOI: 10.1258/ebm.2011.011143.

27. Heikens MJ, Gorbach AM, Eden HS, Savastano DM, Chen KY, Skarulis MC, Yanovski JA. Core body temperature in obesity. Am J Clin Nutr 93:963-967, 2011. DOI: 10.3945/ajcn.110.006270.

28. Nakamura N, Saeki K, Mitsumoto M, Matsuyama S, Nishio M, Saeki K, Hasegawa M, Miyagawa Y, Ohkita H, Kiyokawa N, Toyoda M, Akutsu H, Umezawa A, Yuo A. Feeder-free and serum-free production of hepatocytes, cholangiocytes, and their proliferating progenitors from human pluripotent stem cells: application to liver-specific functional and cytotoxic assays. Cell Reprogram 14: 171-185, 2012. DOI: 10.1089/cell.2011.0064.

29. Nishio M, Nakahara M, Sato C, Saeki K, Akutsu H, Umezawa A, Tobe K, Yasuda K, Yuo A, Saeki K. New categorization of human vascular endothelial cells by pro- vs anti-proliferative phenotypes. World J Transl Med 4: 88-100, 2015a. http://www.wjgnet.com/esps/NewsArticleDetail.aspx?id=20824

30. Nishio M, Nakahara M, Saeki K, Fujiu K, Iwata H, Manabe I, Yuo A, Saeki K. Pro- vs anti-stenotic capacities of type-I versus type-II human iPS-derived endothelial cells. World J Transl Med 4:113-122, 2015b. http://www.wjgnet.com/esps/NewsArticleDetail.aspx?id=20826

31. Nishio M, Nakahara M, Yuo A, Saeki K. Human pluripotent stem cells: Towards therapeutic development for

the treatment of lifestyle diseases. World J Stem Cells 8: 56-61, 2016. DOI: 10.4252/wjsc.v8.i2.56.

32. Ahfeldt T, Schinzel RT, Lee YK, Hendrickson D, Kaplan A, Lum DH, Camahort R, Xia F, Shay J, Rhee EP, Clish CB, Deo RC, Shen T, Lau FH, Cowley A, Mowrer G, Al-Siddiqi H, Nahrendorf M, Musunuru K, Gerszten RE, Rinn JL, Cowan CA. Programming human pluripotent stem cells into white and brown adipocytes. Nat Cell Biol. 14:209-219, 2012. DOI: 10.1038/ncb2411

33. Kishida T, Ejima A, Yamamoto K, Tanaka S, Yamamoto T, Mazda O. Reprogrammed Functional Brown Adipocytes Ameliorate Insulin Resistance and Dyslipidemia in Diet-Induced Obesity and Type 2 Diabetes. Stem Cell Reports 5: 569-581, 2015. DOI: 10.1016/j.stemcr.2015.08.007.

34. Atit R, Sgaier SK, Mohamed OA, Taketo MM, Dufort D, Joyner AL, Niswander L, Conlon RA. Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. Dev Biol 296: 164-176, 2006. DOI: 10.1016/j.ydbio.2006.04.449

35. Tran KV, Gealekman O, Frontini A, Zingaretti MC, Morroni M, Giordano A, Smorlesi A, Perugini J, De Matteis R, Sbarbati A, Corvera S, Cinti S. The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. Cell Metab 15:222-229, 2012. DOI: 10.1016/j.cmet.2012.01.008

36. Gupta RK, Mepani RJ, Kleiner S, Lo JC, Khandekar MJ, Cohen P, Frontini A, Bhowmick DC, Ye L, Cinti S, Spiegelman BM. Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. Cell Metab 15:230-239, 2012. DOI: 10.1016/j.cmet.2012.01.010.

37. Harms MJ, Ishibashi J, Wang W, Lim HW, Goyama S, Sato T, Kurokawa M, Won KJ, Seale P. Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. Cell Metab 19: 593-604, 2014. DOI: 10.1016/j.cmet.2014.03.007.

38. Stanford KI, Middelbeek RJ, Townsend KL, An D, Nygaard EB, Hitchcox KM, Markan KR, Nakano K, Hirshman MF, Tseng YH, Goodyear LJ. Brown adipose tissue regulates glucose homeostasis and insulin sensitivity. J Clin Invest 123: 215-223, 2013. DOI: 10.1172/JCI62308

39. Dekaban AS. Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. Ann Neurol 4:345-356, 1978. DOI: 10.1002/ana.410040410

40. Sawabe M, Saito M, Naka M, Kasahara I, Saito Y, Arai T, Hamamatsu A, Shirasawa T. Standard organ weights among elderly Japanese who died in the hospital, including 50 centenarians. Pathology International 56: 315-323, 2006. DOI: 10.1111/j.1440-1827.2006.01966.x

41. Hildrum B, Mykletun A, Hole T, Midthjell K, Dahl AA. Age-specific prevalence of the metabolic syndrome defined by the International Diabetes Federation and the National Cholesterol Education Program: the Norwegian HUNT 2 study. BMC Public Health. 7: 220, 2007. DOI: 10.1186/1471-2458-7-220

42. Friend A, Craig L, Turner S. The prevalence of metabolic syndrome in children: a systematic review of the literature. Metab Syndr Relat Disord. 11:71-80, 2013. DOI: 10.1089/met.2012.0122

43. Wu C, Orozco C, Boyer J, Leglise M, Goodale J, Batalov S, Hodge CL, Haase J, Janes J, Huss JW 3rd, Su AI. BioGPS: an extensible and customizable portal for querying and organizing gene annotation resources. Genome Biol. 10: R130, 2009. DOI: 10.1186/gb-2009-10-11-r130

44. Lattin JE, Schroder K, Su AI, Walker JR, Zhang J, Wiltshire T, Saijo K, Glass CK, Hume DA, Kellie S, Sweet MJ. Expression analysis of G Protein-Coupled Receptors in mouse macrophages. Immunome Res 4: 5, 2008. DOI: 10.1186/1745-7580-4-5.

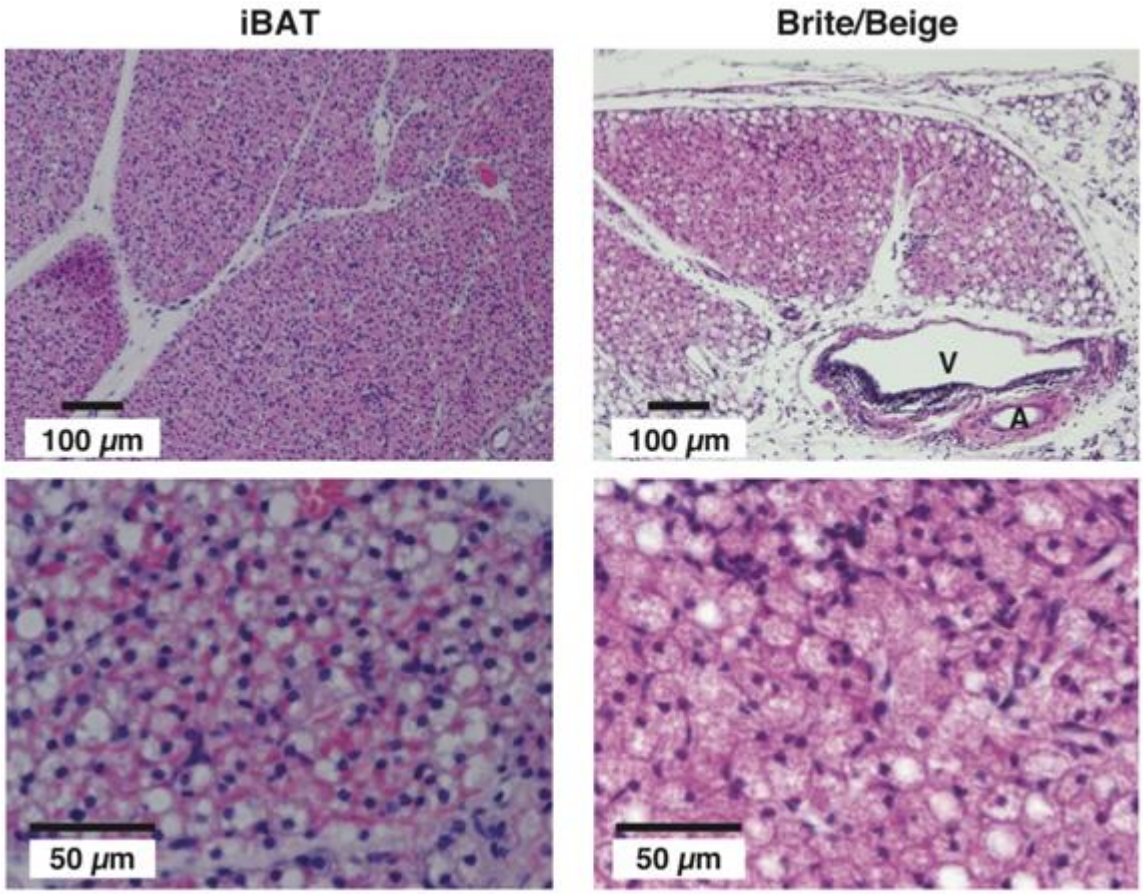

Figure 1. Histological findings of murine BAT and Brite/Beige depots

Hematoxylin and eosin (HE) staining of the slices of the inguinal Brite/Beige depots and those of interscapular BAT (iBAT) of ICR strain mice that were reared at room temperature. Note that iBAT has densely packed capillary networks as in the case of endocrine tissues. In the upper left panel, "A" and "V" indicate the femoral artery and femoral vein, respectively.

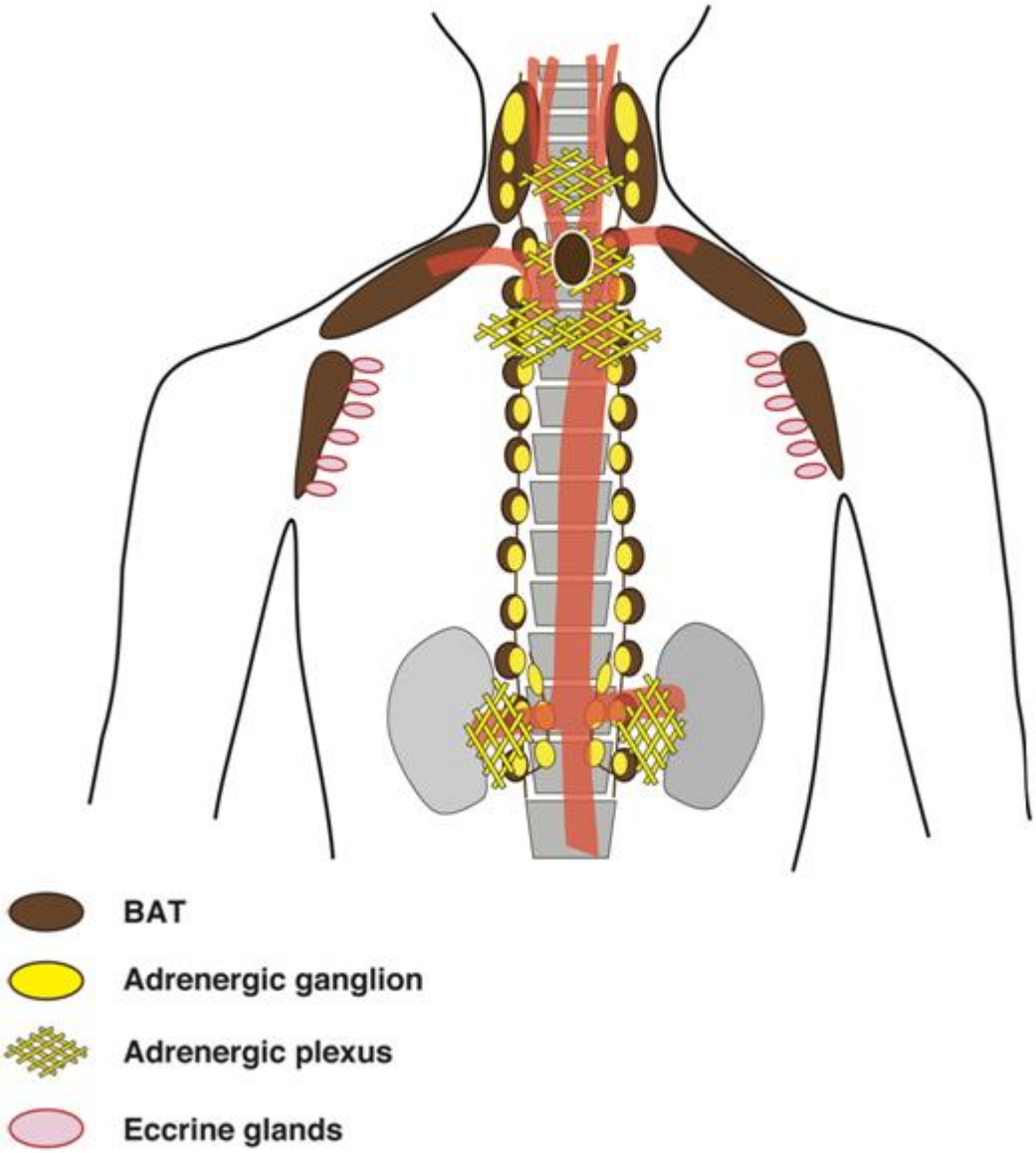

Figure 2. BATs distribute in the regions that receive intensive sympathetic innervation

The regions where BATs are distributed (brown ovals) and the areas which receive intensive sympathetic innervation *via* adrenergic ganglia (yellow ovals) or adrenergic plexuses (yellow meshes) are shown. The superior/meddle/inferior cervical ganglia and the thoracic ganglia of the sympathetic trunk have a close relationship with cervical/supraclavicular BATs and paravertebral BATs, respectively. On the other hand, the branchial/pharyngeal/cardiac plexuses and the renal plexus innervate the paraaortic BATs and perirenal BATs, respectively. Axillary regions are the areas that have the most abundant eccrine glands (pink ovals), which receive intensive sympathetic innervation.

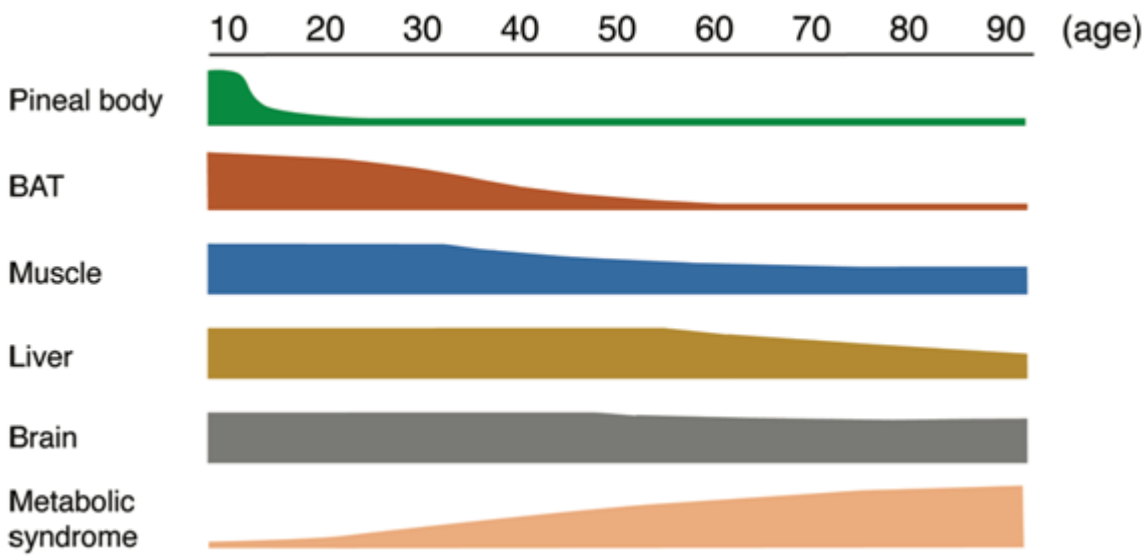

Figure 3. Age-related regression of the relative volume of the tissue

Dynamics of relative tissue weights (per body weight) [39, 40] and the incidence of metabolic syndrome [41, 42] during the span of human life are illustrated. Among the major insulin-targeted tissues including skeletal muscle, liver, brain, the incidence of metabolic syndrome shows the highest correlation with the dynamics of the regression of BATs.

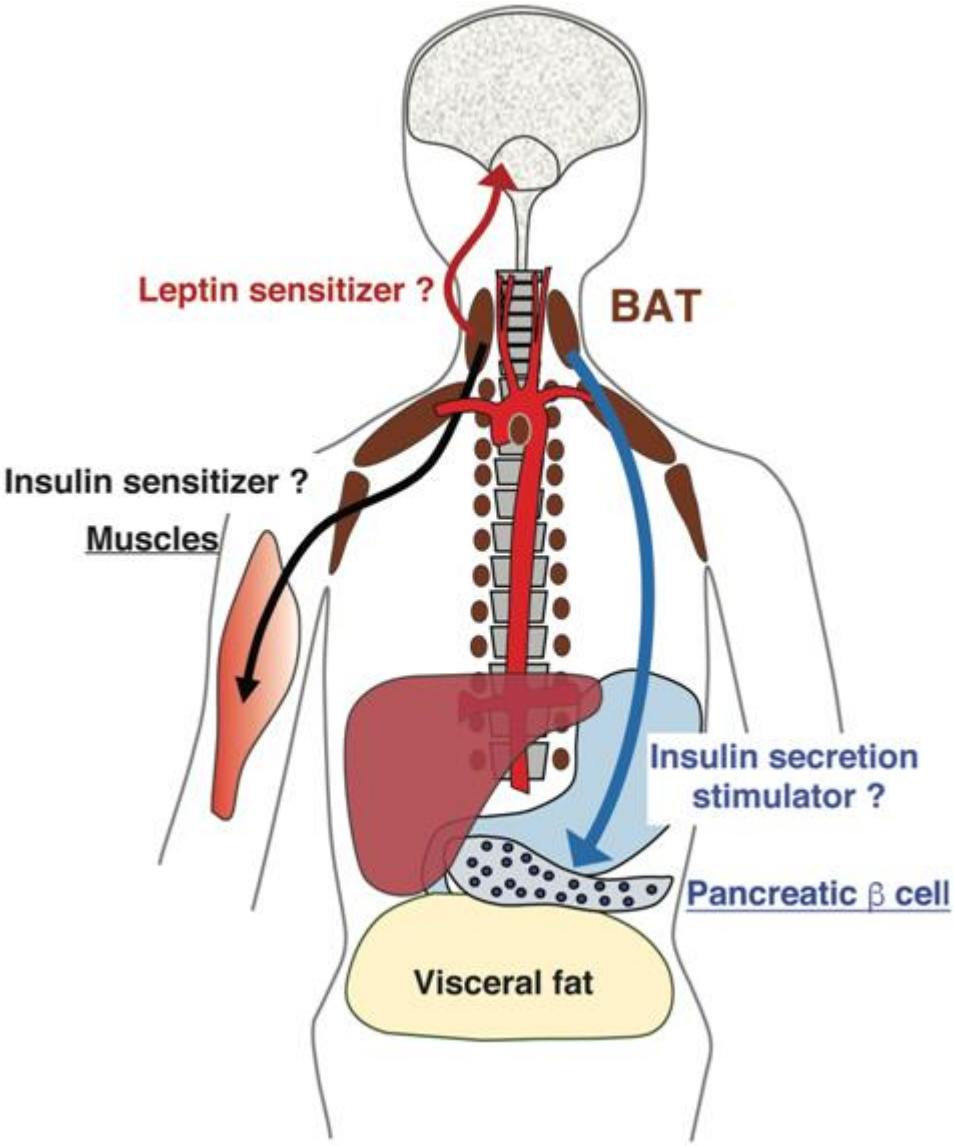

Figure 4. Hypothesized roles for BATkines in glucose metabolism regulation

Discrepancy in the severity of glucose metabolism disorders and leptin resistance between BAT-depleted mice and Ucp1 knockout mice (Table 1), as well as prompt glucose metabolism-improvement (~ 16 hrs) in hESC-derived BA-transplanted mice [14] suggests that the BAT regulates glucose metabolism independently of its thermogenic capacity but via secreting factors (i.e. BATkines). Although several molecules have been reported as the candidate of glucose metabolism-improving BATkines (reviewed by Wang et al. [16]), crucial factors that play the major roles in glucose metabolism improvement via leptin sensitization, insulin sensitization and insulin secretion remain undiscovered.

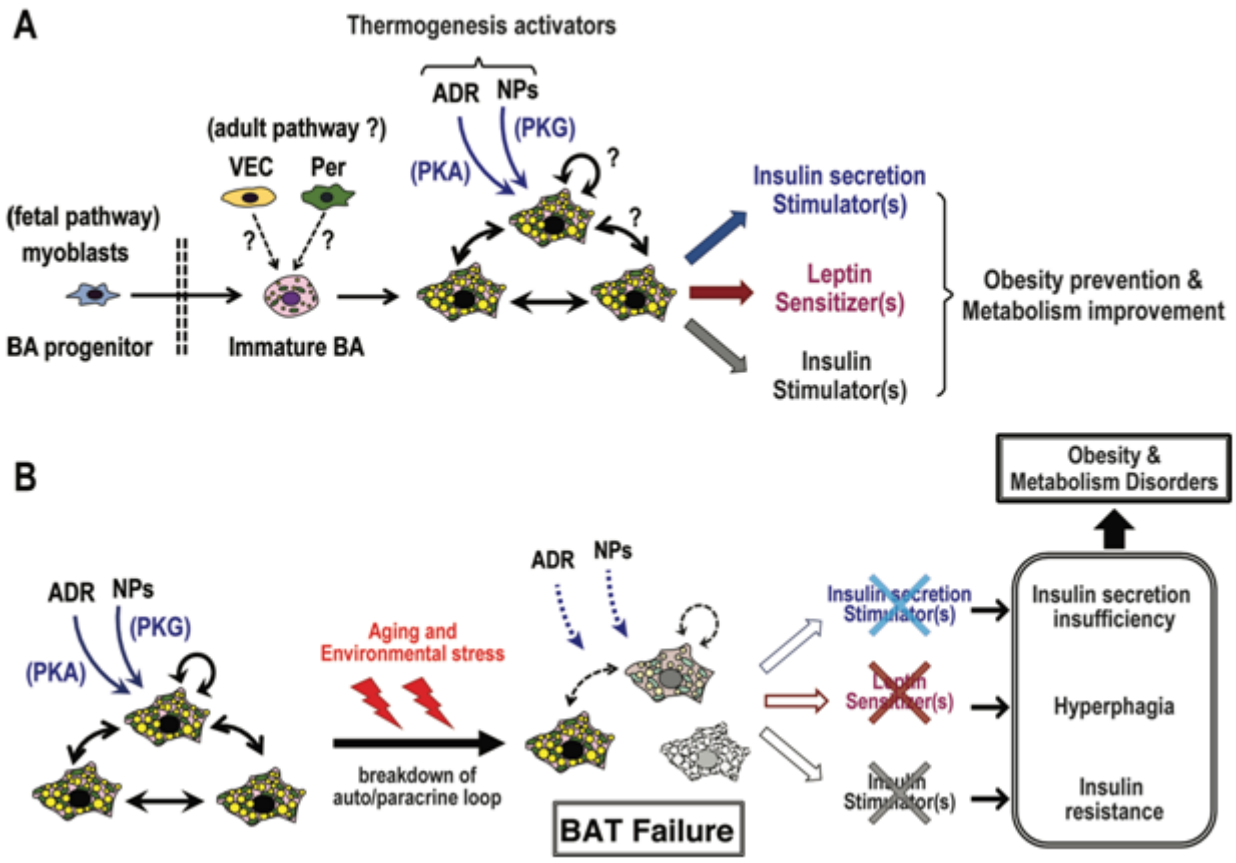

Figure 5. A hypothesis: BAT failure triggers the development of metabolic disorders

A conceived model for the maintenance of BAs in adult life (A) and a hypothesized mechanism for the development of obesity and metabolic disorders as a result of BAT failure (B). BAs are known to be derived from *myf5*$^{+}$ myoblasts by the action of PRDM16 during fetal life. Although the presence of alternative pathways to supply BA progenitors in adult life remains controversial, Immature BAs have relatively a large potential for self-renewal. It is also known that Prdm16 knockout mice undergo severe age-related BAT regression in adult life. Therefore, developing the method for maintaining self-renewing capacity of immature BAs that were produced from *myf5*$^{+}$ myoblasts may provide the most effectual strategy for the control of metabolic disorders. Currently, the mechanism of the age-related BAT regression, which is caused by age-dependent BA degenerations, is not understood. The hESC-based differentiation system, which is expected to provide an excellent tool for the analyses, will make a large contribution to therapeutic development for the treatment of metabolic syndrome.

Abbreviations: BA, brown adipocyte; VEC, vascular endothelial cells; Per, pericytes; ADR, adrenalin; NPs, cardiac natriuretic peptides; PKA, protein kinase A; PKG, protein kinase G.

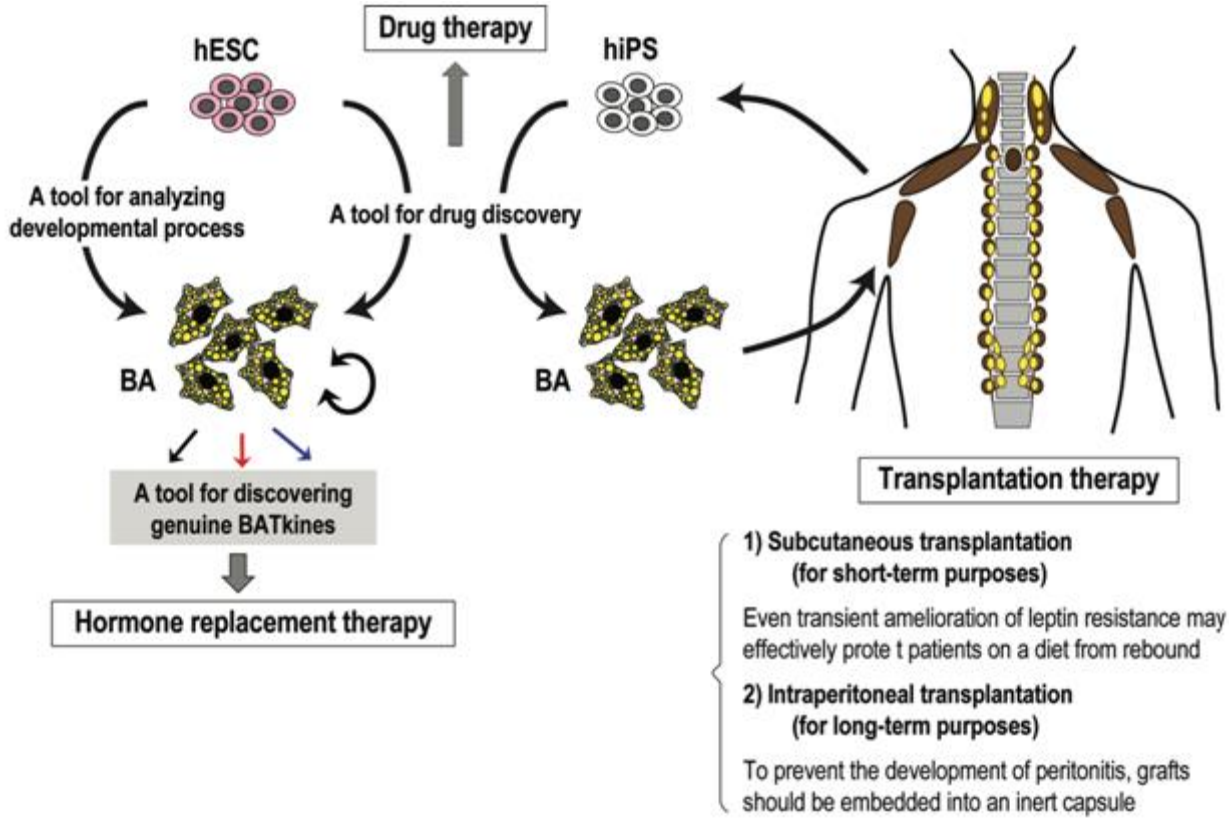

Figure 6. Future prospects in the hESC/hiPSC-derived BA-based therapeutic development

The method for a highly directed differentiation of hESCs into functional BA [14] provide an excellent tool for analyzing early-phase developmental process of BA, which remains elusive but is expected to identify new molecular targets for the drug discovery for metabolic syndrome. hESC-derived BAs also provide a feasible tool for BATkine hunting, which will contribute to the establishment of replacement therapies for BAT failure patients. The hiPSC-derived BAs will be applied to transplantation therapy. Although intraperitoneally transplantation, which has a risk of peritonitis, is required for long-term purposes, even a short-tern survival of the hiPSC-derived BA (3-4 weeks) can exert beneficial effects by ameliorating the unbearable sensation of hunger during the course of dietotherapy. The hESC/hiPSC-based differentiation system is also of use as a tool for drug discovery via screening small molecules that enhance BA differentiation, activate self-renewing of immature BAs or augment the production and secretion of glucose metabolism-improving BATkines.

Abbreviations: hESC, human embryonic stem cells; hiPSC, human induced pluripotent stem cells.

	Obesity	Glucose metabolism Disorder	Leptin resistance
BAT-depleted mice	very severe (early onset)	very severe	very severe
UCP1 knockout mice	mild (under thermoneutrality)	mild (under thermoneutrality)	sensitive ~ mild resistance

Table 1. Phenotype differences between BAT-depleted and UCP1 knockout mice

Phenotype differences between BAT-depleted mice [20] and UCP1 knockout (KO) mice [23] were summarized. BAT-depleted mice became obese as early as at postnatal day 16 and underwent severe obesity equivalent to *ob/ob* mice [20]. These mice suffered from severe glucose metabolism disorder [21] and serious leptin resistance [22]. On the other hand, UCP1 KO mice become obese [24] and undergo glucose metabolic discorders in later life [25] when they are reared at thermoneutral temperature (~30˚C). In addition, UCP1 KO mice are basically sensitive to leptin administration although they are more prone to undergo leptin resistance under repetitive leptin administrations than wild type littermates [26].

Gene symbol	Gene name	The place of BAT in descending order	Signal Values		The top three tissues
			BAT	Median	
Ctrl	chymotrypsin-like	2nd	6171.1	7.9	Pancreas > BAT > Spleen > ...
Cela1	chymotrypsin-like elastase family, member 1	2nd	10068.2	34.3	Pancreas > BAT > Spleen > ...
Cela2a	chymotrypsin-like elastase family, member 2A	2nd	11986.5	5.3	Pancreas > BAT > Spleen > ...
Cela3a	chymotrypsin-like elastase family, member 3A	2nd	30332.5	4.7	Pancreas > BAT > Intestine small > ...
Cela3b	chymotrypsin-like elastase family, member 3B	2nd	1601.1	4.9	Pancreas > BAT > Spleen > ...
Cpa1	carboxypeptidase A1, pancreatic	2nd	4960.4	4.6	Pancreas > BAT > Spleen > ...
Cpa2	carboxypeptidase A2, pancreatic	2nd	689.3	4.6	Pancreas > BAT > Spleen > ...
Cpb1	carboxypeptidase B1, tissue	2nd	8988.7	4.6	Pancreas > BAT > Spleen > ...
Rnase1	ribonuclease, Rnase A faminly, 1 (pancreatic)	3rd	2947.8	4.5	Pancreas > Prostate > BAT > ...

Table 2. Murine BAT expresses various pancreatic peptidases and a ribonuclease at relatively high levels

The information regarding gene expressions in the BAT in mice was retrieved by searching BioGPS database [43] using a dataset of GeneAtlas MOE430, gcrma [44].

