Sci. Bull. (2015) 60(11):1033–1035
DOI 10.1007/s11434-015-0797-9

www.scibull.com
www.springer.com/scp

Letter

Life & Medical Sciences

Zic1 negatively regulates brown adipogenesis in $C_3H_{10}T_{1/2}$ cells

Hanlin Zhang · Yuanyuan Huang · Hyuek Jong Lee · Wanzhu Jin

Received: 4 February 2015 / Accepted: 10 April 2015 / Published online: 16 May 2015

Abstract Zinc finger in the cerebellum 1 (*Zic1*) is known to regulate neurogenesis and myogenesis in the developmental stage and widely used as one of the brown adipocyte-specific markers. In this study, we examined the effect of *Zic1* on brown adipogenesis. Overexpression of *Zic1* attenuated the lipid accumulation and the expressions of *PPARγ2* and *C/EBPα* in $C_3H_{10}T_{1/2}$ mesenchymal stem cells. The mRNA levels of BAT-specific thermogenic genes (*PRDM16*, *PGC-1α* and *UCP1*) and fatty acid oxidation regulatory genes (*PPARα*, *CPT1α*, *CPT1β* and *COX7α1*) were suppressed in *Zic1*-overexpressed cells. Moreover, overexpression of *Zic1* reduced the mitochondrial oxidative phosphorylation (OXPHOS) regulatory proteins including ATP5α, UQCRC2, SDHB and NDUFB5. These results indicate a potential role of *Zic1* in the regulation of brown adipogenesis via inhibiting adipogenesis, fatty acid oxidation and mitochondrial OXPHOS.

Keywords Zic1 · Brown adipogenesis · Fatty acid oxidation · OXPHOS

Electronic supplementary material The online version of this article (doi:10.1007/s11434-015-0797-9) contains supplementary material, which is available to authorized users.

H. Zhang
College of life Science, Anhui University, Hefei 230601, China

H. Zhang · Y. Huang · H. J. Lee (✉) · W. Jin
Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China
e-mail: leehj@ioz.ac.cn

Human and small mammals have mainly two different types of fat tissue, white adipose tissue (WAT) and brown adipose tissue (BAT) [1]. BAT arises from progenitor cell that shares common myogenic transcriptional signatures such as Myf5 and Pax7 [2–4]. PRD1-BF-1-RIZ1 homologous domain 16 (*PRDM16*) and CCAAT/enhancer-binding protein-β (*C/EBPβ*) complexes that induce the expression of peroxisome proliferator-activated receptor γ (*PPARγ*) and peroxisome proliferator-activated receptor-γ coactivator-1 (*PGC-1*), key regulators of the brown fat programming, are responsible for the differentiation of brown adipocyte from myoblast [2, 5]. As a third type of adipocyte, brown-in-white (brite)/beige cells are recruited in WAT by cold exposure or β_3-adrenoceptor agonist treatment and they express uncoupling protein-1 (*UCP1*), one of the specific markers of brown adipocyte [6]. Brite/beige cell derives from $PDGFR\alpha^+CD34^+Sca1^+$ precursor cell rather than *Myf5*-positive myoblast [3, 7], but its gene signatures have a similarity with those of classical BAT [8]. Recently, zinc finger in the cerebellum 1 (*Zic1*) is reported as one of the brown adipocyte markers [9], since its expression is restricted specifically in BAT, not in WAT or brite/beige cell [10]. In addition, *Zic1* overexpression induces upregulation of *Myf5*, the myogenic master regulator, in $C_3H_{10}T_{1/2}$ cells with Gli-dependent manner [11]. The function of *Zic1* in brown adipocytes, however, has not been well studied. Given that BAT derives from *Myf5*-positive progenitors and expresses *Zic1* which control *Myf5* expression, we hypothesized that *Zic1* may regulate brown adipogenesis. To investigate the functional role of *Zic1* in brown adipocyte, we overexpress *Zic1* in $C_3H_{10}T_{1/2}$ mesenchymal stem cells and then induce differentiation in vitro.

To compare the expression level of *Zic1* in adipose tissues, we first analyzed the *Zic1* mRNA expression in two different WATs, epididymal fat and inguinal fat, and BAT from

8 weeks of age C57BL/6J mice fed with normal diet. All mice would be sacrificed by cervical dislocation before sampling and every experiment about animal were performed in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and have received approval from the Ethical Review Board (Institute of Zoology, Beijing, China). The level of *Zic1* was highly elevated in BAT compared with that in WATs (Fig. 1a). This result supported the previous report that *Zic1* expression is restricted in BAT than in WAT [10], which suggests a potential role of *Zic1* in brown adipogenesis. To test this hypothesis, $C_3H_{10}T_{1/2}$ cells underwent brown adipocyte differentiation after infection with a lentiviral system expressing *Zic1* or control vector. *Zic1* expression was upregulated by 8 folds compared with the control group at day 3 post-viral transduction (Fig. 1b). Interestingly, Oil Red O assay demonstrated a markedly reduced lipid accumulation in fully differentiated brown adipocytes with overexpressed *Zic1* (Fig 1c, d), suggesting that *Zic1* may negatively regulate brown adipogenesis. We then evaluated the effects of *Zic1* on the expression of genes that control adipogenesis. As shown in Fig. 2a, *Zic1* overexpression dramatically decreased the expressions of *PPARγ2* and *C/EBPα*, the two key adipogenic factors. By contrast, the early adipogenic marker, *C/EBPβ*, was modest but significantly upregulated. In addition, *Zic1* overexpression markedly suppressed the expression of genes that regulates fatty acid oxidation, such as *PPARα*, carnitine palmitoyltransferase 1α (*CPT1α*), *CPT1β* and *COX7α1* (Fig. 2b). These results indicate that *Zic1* might suppress brown adipogenesis by inhibiting the expression of genes regulating adipogenesis and fatty acid oxidation. Additionally, BAT-specific thermogenic gene, *UCP1*, was decreased by *Zic1* overexpression (Fig. 2c). The high number of mitochondria is a key feature of BAT. It is well known that *PGC-1α* and *PRDM16* are key molecules that regulate mitochondria biogenesis [12]. The inhibitive effect of *Zic1* on mitochondria biogenesis (Fig. 2c) led us to further investigate whether *Zic1* has an impact on mitochondrial oxidative phosphorylation (OXPHOS). Consistent with mRNA levels, the protein level of Zic1 was increased 7.1-fold than that of control (Fig. 2d). Along with the significant reduction in protein levels of PPARγ2 and PGC-1α (Fig 2d, e), *Zic1* overexpression also significantly decreased the levels of four mitochondrial OXPHOS proteins (ATP5α, UQCRC2, SDHB and NDUFB5) (Fig 2d, f) (Supporting information Materials and methods).

Although *Zic1* was reported previously as one of the BAT-specific markers [9, 10], little is known about its function in brown adipogenesis. We showed that *Zic1* overexpression unexpectedly prevented lipid accumulation in $C_3H_{10}T_{1/2}$ cells after differentiation (Fig. 1). *PPARγ* and *C/EBPα*, two major transcriptional factors involved in adipogenesis, interact each other to commit adipocyte differentiation [13]. In addition to

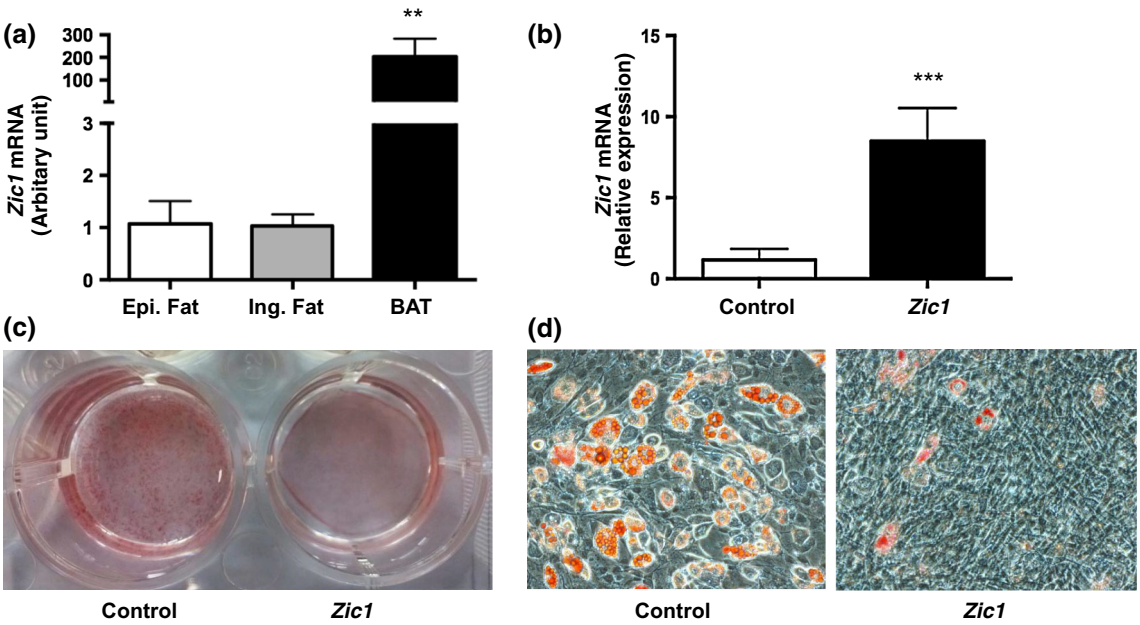

Fig. 1 **a** The relative expression of *Zic1* mRNA in epididymal fat (Epi. Fat), inguinal fat (Ing. fat) and BAT from 8 weeks of age C57BL/6J mice is analyzed. The relative ratio of Epi. fat is arbitrarily presented as 1. Bars represent the means ± SD ($n = 3$). Zic1 overexpression suppresses lipid accumulation in $C_3H_{10}T_{1/2}$ cells, **b** *Zic1* mRNA expression at 3 days after viral infection. The relative ratio of control is arbitrarily presented as 1. Bars represent the means ± SD ($n = 4$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus the relative ratio of control, **c** a representative image of fully differentiated $C_3H_{10}T_{1/2}$ cells in 6-well dish after Oil Red O staining, **d** A representative image of Oil Red O stained $C_3H_{10}T_{1/2}$ cells under microscope with ×20

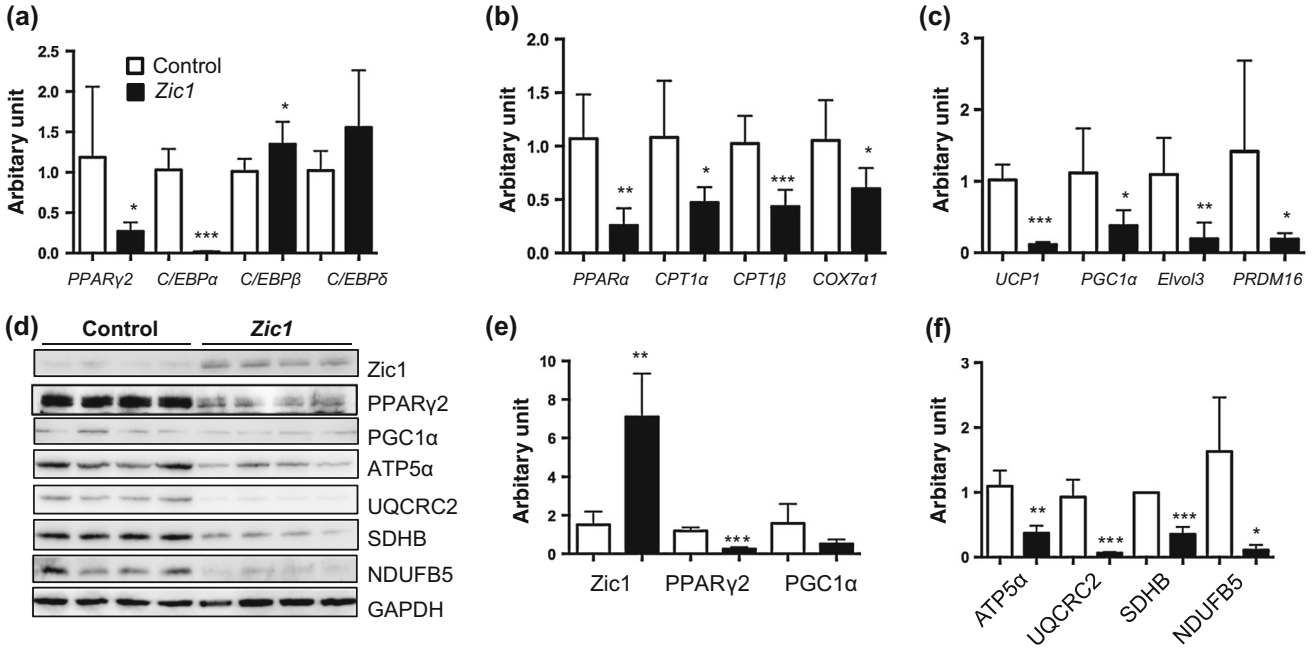

Fig. 2 **a** The relative expression of adipogenesis regulatory genes, **b** fatty acid oxidation regulatory genes and **c** BAT-specific genes after *Zic1* overexpression were shown, **d** *Zic1* overexpression decreases protein levels of PPARγ2, PGC-1α and OXPHOS, and **e**, **f** densitometric analyses are presented as the relative ratios of each protein to total GAPDH. The relative ratio of control is arbitrarily presented as 1. Bars represent the means ± SD ($n = 4$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the relative ratio of control

PPARγ2 and *C/EBPβ*, *PRDM16* determines brown adipocyte phenotype and regulates adaptive thermogenesis and fatty acid oxidation by coactivating *PGC-1α*, *UCP1* and *PPARα* [3, 14]. Interestingly, our current study demonstrated that both *PRDM16* and *PGC-1α* were downregulated by *Zic1* overexpression in fully differentiated brown adipocytes (Fig. 2c). In parallel, the expression of fatty acid oxidation related genes such as *PPARα*, *CPT1α*, *CPT1β*, *COX7α1* (Fig. 2b) and OXPHOS-related proteins were significantly decreased by *Zic1* overexpression (Fig 2d). Collectively, these results suggest that the downregulations of *PGC-1α* and its downstream molecules, *UCP1* and *PPARα*, are possibly mediated by *PRDM16* and/or *PPARγ2* rather than *C/EBPβ* after *Zic1* overexpression, resulting in the impairments of fatty acid oxidation, mitochondria biogenesis and eventually thermogenesis in brown adipocytes.

Acknowledgments This work was supported by the Strategic Priority Research Program (XDB13030000), Key Research Program (KJZD-EW-L01-3), the One *Hundred Talents Program* of the Chinese Academy of Sciences and the Ministry of Science and Technology of China (2012CBA01301 and 2012CB944701, respectively), as well as by the National Natural Science Foundation of China (31171131, 81370951).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Tseng YH, Kokkotou E, Schulz TJ et al (2008) Nature 454:1000–1004
2. Timmons JA, Wennmalm K, Larsson O et al (2007) Proc Natl Acad Sci USA 104:4401–4406
3. Seale P, Bjork B, Yang W et al (2008) Nature 454:961–967
4. Lepper C, Fan CM (2010) Genesis 48:424–436
5. Kajimura S, Seale P, Kubota K et al (2009) Nature 460:1154–1158
6. Ohno H, Shinoda K, Spiegelman BM et al (2012) Cell Metab 15:395–404
7. Lee YH, Petkova AP, Mottillo EP et al (2012) Cell Metab 15:480–491
8. Jespersen NZ, Larsen TJ, Peijs L et al (2013) Cell Metab 17:798–805
9. Lidell ME, Betz MJ, Dahlqvist Leinhard O et al (2013) Nat Med 19:631–634
10. Walden TB, Hansen IR, Timmons JA et al (2012) Am J Physiol Endocrinol Metab 302:E19–E31
11. Pan H, Gustafsson MK, Aruga J et al (2011) Dev Biol 351:120–127
12. Lin J, Handschin C, Spiegelman BM (2005) Cell Metab 1:361–370
13. Rosen ED, Hsu CH, Wang X et al (2002) Genes Dev 16:22–26
14. Seale P, Kajimura S, Yang W et al (2007) Cell Metab 6:38–54