Adipocyte is closely related to energy homeostasis. While white adipocyte stores energy as a form of lipids, brown adipocyte dissipates energy by producing heat. In mice and humans exposed to chronic cold temperature, white adipocyte transdifferentiates into brown adipocyte-like cell called beige (brite) cell. The regulation of brown adipocyte function and beigeing of white adipocyte is considered as a potential therapeutic target for obesity (reviewed in Inagaki et al., 2016).

Genome-wide association studies (GWASs) have identified strong association of obesity and genetic variances within introns of FTO which encodes a demethylase for N6-methyladenosine (m6A) residues in mRNA (reviewed in Tung et al., 2014). Recent studies revealed that association of obesity and genetic variances within introns of IRX3 which locates ~500 kilo bases away from FTO loci by forming long range chromatin loop, acting as an enhancer to induce the expression of IRX3 which encodes a transcription factor involved in multiple developmental processes (Smemo et al., 2014 and reviewed in Gorkin and Ren, 2014). It is also reported that the binding of AT-rich interactive domain 5B (ARID5B) repressor protein to the region in the intron 1 of FTO negatively regulates IRX3 expression (Claussnitzer et al., 2015).

In this issue of EBioMedicine, Zou et al. (2017) investigated the expression profile of IRX3 and function of IRX3 protein in adipocytes. They obtained tissues and stromal vascular fractions (SVFs) from different types of adipose tissues (i.e. brown adipose tissue (BAT), subcutaneous white adipose tissue (scWAT), and visceral WAT (vWAT)) of different types of adipose tissue (i.e. brown adipose tissue (BAT), subcutaneous white adipose tissue (scWAT), and visceral WAT (vWAT)) of mouse and human to examine mRNA and protein expressions of IRX3. They revealed increased Irx3 expression in scWAT and BAT in response to β-adrenergic stimulation and also during the time course of beige and brown adipogenesis of mouse SVFs. The profiles of mRNA expression and histological localization of UCP1 and IRX3 were positively correlated. These results indicated a potential role of IRX3 in the regulation of genes related to adipose cell fate.

Cell autonomous study using mouse and human SVFs in which IRX3 expression was knocked down (KD) by infecting lentivirus expressing shRNA against IRX3 showed reduced mRNA expression of thermogenic genes such as UCP1, CIDEA and PPARGC1A and reduced uncoupling heat production, suggesting a positive role of IRX3 for inducing thermogenesis (Zou et al., 2017). However, these results are obviously contrary to the previous reports. It is reported that Irx3 knockout (KO) mice were protected against obesity (Smemo et al., 2014) and human adipocytes overexpressing IRX3 showed decreased thermogenesis (Claussnitzer et al., 2015). In detail, Irx3 KO mice in a previous study showed reduced body weight and protected against diet-induced obesity. Results of transcriptional analysis indicated that Irx3 KO mice showed higher sympathetic tone inducing beigeing of scWAT and higher heat production in BAT increasing energy expenditure (Smemo et al., 2014). In addition, adipose-specific Irx3 dominant-negative (DN) mice and hypothalamus-specific Irx3 DN mice were produced by crossing Rosa26EnR-Irx3 conditional transgenic mice expressing a dominant negative form of Irx3 with pA2-Cre mice and Ins2-Cre mice, respectively (Claussnitzer et al., 2015; Smemo et al., 2014). Intriguingly, both hypothalamus-specific Irx3 DN mice and adipose-specific Irx3 DN mice showed anti-obesity characteristics similar to Irx3 KO mice, while it is not elucidated if these similar phenotypes are mediated by an overlapped mechanism or are derived from inter-organ interaction-associated actions. Adipose-specific Irx3 DN mice are also resistant to weight gain on a high-fat diet and showed an elevated oxygen consumption rate both at room temperature (22 °C) and in the thermoneutral conditions (30 °C) (Claussnitzer et al., 2015).

The reasons for the discrepancy between the findings of above previous studies and the observation by Zou et al. (2017) are totally unknown. However, several possibilities could be considered. Firstly, it could be due to the difference of the genetic background or the age of mice as discussed by Zou et al. (2017). Secondly, it is possible that the different study models to manipulate the Irx3 function, such that either the overexpression of dominant negative form or the knocking down using shRNA, resulted in irrelevant formation of the transcriptional regulatory complex. In this context, future studies using adipocyte specific Irx3 knockout mice and transgenic mice will provide additional evidence. It should be clarified the detailed molecular mechanisms how IRX3 regulates target gene transcription. Related to this notion, Zou et al. (2017) performed ChIP assay and presented the recruitment of Irx3 to its response element locates ~3.5 kb upstream of Ucp1. Reporter assay using the sequence containing this element revealed that...
overexpression of Irx3 induces reporter activity in HEK293T cells. The replacement mutation of the response element did not reduce the reporter activity, while the effect of deletion of the element is even stronger compared to the WT reporter. These results suggest the possibility of competitive binding by an inhibitory factor, so that deletion of binding site abolishes inhibitory effect of such an endogenous inhibitory factor. Because a lot of new factors are reported as transcriptional regulators during beige adipogenesis in addition to well-known core factors such as PRDM16, C/EBPβ, PPARγ and PGC1α (Inagaki et al., 2016), it is expected that IRX3 forms complex with other factors and binds to the regulatory regions of thermogenic genes. Thus, there is a possibility that IRX3 is a component of protein complexes which either positively or negatively regulate thermogenic genes. Therefore, the phenotypes of the different study models (i.e. Irx3 KD cells, Irx3 KO mice, or Irx3 DN mice) could depend on what complex is dominantly formed in each model. It is also speculated that dominant negative form of Irx3 maintains the complex formation, while knockdown of Irx3 expression disrupts the complex formation. Considering different regulation of the binding site by IRX3 in different cell types, it would be better to employ adipocyte model for reporter assay instead of using HEK293T cell. The possibility of different expression patterns of multiple splicing variants of IRX3 in different cell types is also an open question. RNA-seq study may help to clarify this issue.

Notably, Zou et al. (2017) also examined the association of IRX3 with human obesity. Whole-exon sequencing of IRX3 in obese and lean subjects presented that IRX3 rare/low-frequency variants were enriched in obese individuals. Furthermore, they identified rare heterozygous missense/frameshift IRX3 variants which relate to transcriptional activity of UCP1. These findings indicate that disruption of IRX3 expression could be one of the multiple factors related to human obesity. Although it is too preliminary to consider that IRX3 can be a potential target for the therapy for obesity, it is reasonable to continue further investigation to elucidate if heterozygous missense/frameshift IRX3 variants are associated with lower energy-expenditure in beige and brown adipose tissue and would be a risk factor for obesity.

Unrevealed mechanisms in the studies of IRX3 are (1) how the cold induced β-adrenergic signal is sensed during the beiging mediated by IRX3, (2) which regulatory proteins form a transcriptional regulatory complex(es) with IRX3, (3) where do genome-wide binding regions locate, and (4) how chromatin structures are modified by IRX3 in the induction of thermogenic genes. For example, a recent study on the molecular mechanism of heat production in brown adipocytes presented that cold-induced β-adrenergic signal is sensed by the phosphorylation of histone demethylase JMJD1A, which in turn forms a complex with SWI/SNF chromatin remodeler and PPARγ causing a chromatin conformational change to induce the enhancer-promoter proximity of thermogenic genes (Abe et al., 2015). Similar regulatory mechanism could be adapted in the regulation of thermogenic gene expression by IRX3. Elucidating the precise molecular mechanism of IRX3 regulation of adipose cell fate will provide insight into novel therapeutic approaches for the people with high risk factors for obesity such as SNPs around IRX3 as well as FTO.

Disclosure

The author declared no conflicts of interest.

References

Abe, Y., Rozzio, R., Matsumura, Y., Kawamura, T., Nakaki, R., Tsurutani, Y., Tanimura-Inagaki, K., Shiono, A., Magoori, K., Nakamura, K., et al., 2015. JMJD1A is a signal-sensing scaffold that regulates acute chromatin dynamics via SWI/SNF association for thermogenesis. Nat. Commun. 6, 7052.

Claussnitzer, M., Dankel, S.N., Kim, K.H., Quon, G., Meuleman, W., Haugen, C., Glunk, V., Sousa, I.S., Beaudry, J.L., Puvion-Dutilleul, V., et al., 2015. FTO obesity variant circuitry and adipocyte browning in humans. N. Engl. J. Med. 373, 895–907.

Gorkin, D.U., Ren, B., 2014. Genetics: closing the distance on obesity culprits. Nature 507, 309–310.

Inagaki, T., Sakai, J., Kajimura, S., 2016. Transcriptional and epigenetic control of brown and beige adipocyte cell fate and function. Nat. Rev. Mol. Cell Biol. 17, 480–495.

Smeño, S., Tena, J.J., Kim, K.H., Gamazon, E.R., Sakabe, N.J., Gomez-Marin, C., Aneas, I., Credidio, F.L., Sobreira, D.R., Wasserman, N.F., et al., 2014. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature 507, 371–375.

Tung, Y.C., Yeo, G.S., O’Rahilly, S., Coll, A.P., 2014. Obesity and FTO: changing focus at a complex locus. Cell Metab. 20, 710–718.

Zou, Y., Lu, P., Shi, J., Liu, W., Yang, M., Zhao, S., Chen, N., Chen, M., Sun, Y., Gao, A., et al., 2017. IRX3 promotes the browning of white adipocytes and its rare variants are associated with obesity risk. EBioMedicine 24, 64–75.