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Commentary

Regulations of Adipocyte Phenotype and Obesity by IRX3. Positive or Negative?

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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 N^6-methyladenosine (m6A) residues in mRNA (reviewed in Tung et al., 2014). Recent studies revealed that this association is mediated by the mechanism independent of enzyme activity of FTO (Claussnitzer et al., 2015; Smemo et al., 2014). A region in the intron 1 of *FTO* physically interacts with the promoter sequence of *IRX3* which locates ~500 kilo bases away from *FTO* locus 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 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 $Rosa26^{EnR\text{-}Irx3}$ conditional transgenic mice expressing a dominant negative form of *Irx3* with aP2-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 evidences. 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

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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 beigeing 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.

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