Cell Research (2015) 25:3-4.

www.nature.com/cr

RESEARCH HIGHLIGHT

FTO: linking m^{6}A demethylation to adipogenesis

Cell Research (2015) **25**:3-4. doi:10.1038/cr.2014.162; published online 5 December 2014

Polymorphism of the *FTO* gene encoding an N^6-methyladenosine (m^{6}A) RNA demethylase was robustly associated with human obesity; however, the mechanism by which FTO affects metabolism, considering its emerging role in RNA modification, is still poorly understood. A new study published in *Cell Research* reports novel functions implicating FTO in the regulation of mRNA alternative splicing in the control of adipogenesis.

Obesity is a major concern to western societies and to the growing number of overweight individuals which deal with serious health problems associated with overweight (e.g., diabetes, fatty liver, cardiovascular disease, stroke, and neurocognitive decline). Recently, a common variation of *FTO* (the *fat-mass and obesity* gene) was found to be robustly associated with increased body mass in humans [1]. *FTO* encodes an RNA demethylase, whose major substrate is N^6-methyladenosine (m^{6}A) [2]. m^{6}A is the most prevalent mRNA internal modification with a highly conserved architecture across human and mouse [3]. This reversible epigenetic modification was shown to participate in regulating important cellular processes, including transport, degradation, and translation [4]. This diversity of functions is mediated by a variety of m^{6}A reader proteins including the RNA-stabilizing protein, ELAVL1, and a number of YTH-family of proteins, YTHDF2 and YTHDF3, several of which have been identified to directly bind to m^{6}A-containing transcripts [3]. A role for m^{6}A in splicing regulation was demonstrated in several studies. Knockdown of components of the methylating complex (METTL3, METTL14 and WTAP) resulted in different isoforms of m^{6}A-containing transcripts [3-5]. In addition, m^{6}A seems to alleviate the constraints imposed by larger exons on the exon-definition machinery and affect splicing efficiency, resulting in exon inclusion [3]. This is further supported by the fact that ALKBH5, another m^{6}A demethylase, affects the rate of splicing [4]. In addition, ALKBH5, FTO, as well as the m^{6}A methylation complex components, all display nuclear speckle localizations, further supporting a role for m^{6}A in RNA processing [2, 4, 5]. Collectively, these suggest that FTO may affect splicing of its target genes.

A causal link between FTO and obesity was recently established in animal studies. Overexpression of *FTO* led to increased food intake and obesity in mice [6], while silencing of *FTO* protected against obesity [7]. A link between FTO-dependent m^{6}A demethylation and gene expression was demonstrated in two independent studies. FTO-knockout mice exhibited changes in m^{6}A levels in midbrain and striata, with higher m^{6}A methylation level of key genes of the dopaminergic pathway and lower expression of these genes [8]. In addition, *FTO* risk allele was correlated with higher levels of the ghrelin intestinal hunger hormone, along with a reduced level of m^{6}A in ghrelin mRNA transcripts after a test meal [9]. FTO is ubiquitously expressed with highest expression level during development as well as in adult brain, adipose and muscle tissues. Yet, the mechanisms by which FTO-dependent m^{6}A demethylation affects key cellular activities operating in these tissues, and its roles in regulation of energy homeostasis as well as body weight remain to be elucidated.

Now, the pioneering study by Zhao *et al*. [10] identified FTO as an important regulator of mRNA splicing and implicated its necessity in adipogenesis. The authors describe a role for FTO in differentiation of pre-adipocytes by demonstrating that its depletion interferes with pre-adipocyte differentiation, in contrast to an opposite effect observed in knockdown of METTL3. A decrease in FTO is observed along the course of adipocyte differentiation, and as expected the levels of m^{6}A in mRNAs concomitantly rise through this process. The authors show that m^{6}A RNA decoration during adipogenesis is inversely correlated with gene expression, in agreement with the recently demonstrated m^{6}A-dependent mRNA decay mediated by YTHDF2 [4]. Furthermore, they identified a subset of genes related to important cellular pathways including lipid synthesis and translation that respond in an opposite manner to FTO or METTL3 knockdown.

One of the most significant observations of the study by Zhao *et al*. is the identification of FTO as an important regulator of mRNA alternative splicing. As previously reported, significant differences in the proportion of single- and multi-isoform genes exists between m^{6}A-methylated and non-methylated genes [3], suggesting that m^{6}A may pose an important mark for recruiting components of the splicing machinery. In accordance, the authors show that the splicing regulatory (SR) proteins Srsf1 and Srsf2 are recruited in an m^{6}A-dependent manner. They show that m^{6}A

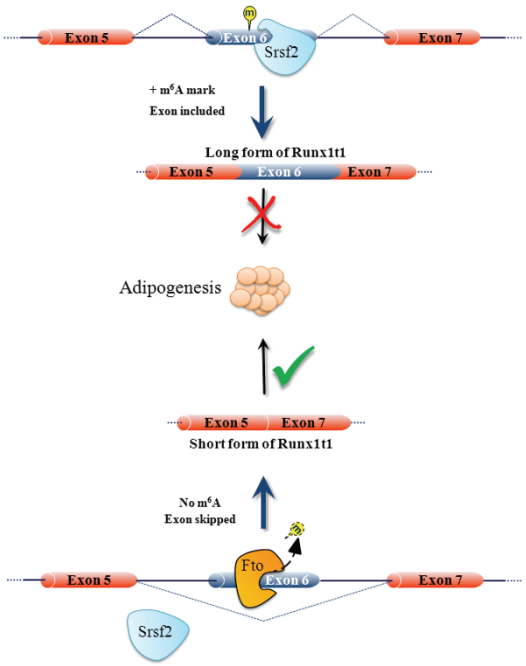

Figure 1 An illustration of alternative splicing of Runx1t1 mediated by FTO-dependent m^{6}A demethylation and Srsf2. The typical removal of m^{6}A by FTO (lower panel) minimizes the recruitment of Srsf2, which in its absence prompts the skipping of exon 6, resulting in a short product of Runx1t1. In turn, the short isoform of Runx1t1 induces pre-adipocyte differentiation. Conversely, m^{6}A-containing transcripts (enhanced by FTO knockdown) are more likely to recruit Srsf2 and induce exon 6 inclusion, thereby inhibiting pre-adipocyte differentiation.

sites overlap with binding sites of Srsf1 and Srsf2, and that m^{6}A is overrepresented in 5′ and 3′ exonic sequences flanking the splice sites. Consequentially, knocking down FTO enhances the binding affinity of Srsf2 to several of its targets, which coincides with high methylation levels of these targets, promoting exon inclusion.

During the course of differentiation of pre-adipocytes to adipocytes, the authors observed over 17 000 differentially expressed isoforms. Gene ontology (GO) analysis encompassing differentially expressed isoforms with opposed expression patterns in FTO vs METTL3 knockdown, revealed enrichment in pathways associated with sterol metabolism and organelle organization. Remarkably, the authors demonstrate that knockdown of FTO affects alternative splicing patterns of several genes, including two splice variants of the adipogenesis-related gene Runt-related transcription factor 1 (Runx1t1). Knocking down FTO dramatically leads to higher inclusion rate of exon 6 and generation of long Runx1t1 transcripts, with nearly complete elimination of the short splice variant (Figure 1). Notably, during the progression of adipocyte differentiation the expression level of the short splice isoform of Runx1t1 decreases in a manner similar to the one observed with FTO expression level. In addition, fat-deposits staining and triglyceride assays further support a role for the long Runx1t1 isoform in inhibiting differentiation, while the short isoform appears to promote it.

Collectively, the current study provides valuable evidence for the role of FTO, via m^{6}A demethylation, in modulating pre-adipocyte differentiation. The present study makes an important advancement in our understanding of the link between FTO and metabolism, by unraveling a novel mechanism of splicing regulation of its methylated transcript targets. Interestingly, it identified the adipogenesis-related gene Runx1t1 as a prime example of an FTO-regulated alternatively spliced target. Further studies will be required in order to identify whether Srsf1 and Srsf2 are direct readers of m^{6}A or are recruited through mediation of other m^{6}A reader proteins. Similarly, *in vivo* examination of the effects of FTO on gene splicing and influence on adipose and other metabolic tissues in a whole animal model would prove valuable.

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