Substrates of the m\(^6\)A demethylase FTO: FTO-LINE1 RNA axis regulates chromatin state in mESCs

Pia Sommerkamp

In a recent paper published in *Science*, Wei et al. report that long-interspersed element-1 (LINE1) RNA is a key target of the N\(^6\)-methyladenosine (m\(^6\)A) ‘eraser’ fat mass and obesity-associated protein (FTO). The FTO-LINE1 RNA axis is involved in chromatin regulation and is essential for mammalian development.\(^1\)

m\(^6\)A is the most common RNA modification in mammalian cells and controls, among others, translation, decay and even chromatin state.\(^2,3\) The m\(^6\)A landscape is shaped by m\(^6\)A ‘writers’ and ‘erasers’, while the effect of the m\(^6\)A modification is determined by ‘readers’. FTO is an alpha-ketoglutarate (α-KG)-dependent oxygenase, which functions as an m\(^6\)A demethylase (‘eraser’), making m\(^6\)A modifications reversible.\(^4\) In addition to m\(^6\)A, FTO also mediates demethylation of N6,2′-O-dimethyladenosine (m\(^6\)Am\(^2\)A) and N1-methyladenosine (m\(^1\)A).\(^5\) Recent studies have demonstrated an important role for m\(^6\)A in regulating mouse embryonic stem cell (mESC) fate and controlling early mammalian embryonic development.\(^6\) Wei et al. extend these findings by using Fto\(^−/−\) mESCs and mice to identify physiological substrates of FTO and to investigate its role in early mammalian development.\(^1\)

In Fto\(^−/−\) mESCs, Wei et al. observed changes in m\(^6\)A levels of chromatin-associated (carRNA) and soluble nuclear fraction RNA, suggesting nuclear FTO substrates. carRNAs, and especially chromosome-associated-regulatory RNAs (carRNAs), have recently been shown by the He lab to be targets of the m\(^6\)A ‘writer’ METTL3 in mESCs.\(^7\) As m\(^6\)A ‘writers’ and ‘erasers’ work together to create an equilibrium of m\(^6\)A modifications, subsets of carRNAs could also represent FTO substrates. In line, Wei et al. observed hypermethylation of carRNAs in Fto\(^−/−\) mESCs. The carRNA subset ‘repeat RNAs’ exhibited the strongest correlation between m\(^6\)A hypermethylation and transcript downregulation, with the repeat RNA LINE1 being most strongly affected. LINE1 elements belong to the LINE family and represent autonomous, active retrotransposons, and LINE1 RNA has been shown to regulate chromatin.\(^8\) Wei et al. observed LINE1 RNA-chromatin interaction as well as co-localization and binding of LINE1 RNA and FTO in mESCs, supporting LINE1 RNA as a physiological target of FTO.

Interestingly, LINE1 RNA is essential for mESCs and is involved in suppression of the 2-cell (2C) program.\(^9\) 2C-like cells share features of the 2-cell stage embryo and are characterized by expression of retrotransposons from the murine endogenous retrovirus with leucine tRNA primer (MERVL) family and repression of ESC pluripotency genes.\(^3\) Wei et al. observed that Fto\(^−/−\) mESCs partially recapitulate this phenotype and exhibit a genetic and phenotypic 2C-like state.

The m\(^6\)A ‘reader’ YTHDC1 has previously been shown to destabilize m\(^6\)A-containing carRNAs.\(^8\) In line, mechanistic analyses revealed increased binding of LINE1 RNA by YTHDC1 in Fto\(^−/−\) mESCs. LINE1 RNA was upregulated upon loss of Ythdc1, and Fto knockout (KO) led to enhanced LINE1 RNA decay. These results support the notion that an increase in m\(^6\)A modifications of LINE1 RNA could lead to destabilization via YTHDC1 binding. Fto KO reduced levels of LINE1 RNA-DNA association and R-loop formation at LINE1 loci. In addition, Fto\(^−/−\) mESCs showed decreased RNA synthesis and chromatin accessibility. Closed regions were enriched at m\(^6\)A-modified LINE1 RNA loci. LINE1-containing genes exhibited a stronger decrease in expression upon Fto KO, which was accompanied by an increase in intragenic LINE1 RNA m\(^6\)A levels, respectively. Genes containing down-regulated intragenic LINE1 RNA were particularly affected, arguing for a cis-regulatory role of intragenic LINE1. In line, these gene loci, including early development and pluripotency genes, exhibited a more closed chromatin state. In contrast, 2C genes, such as Dub1 and Zscan4, do not contain LINE1 RNA and exhibited a decrease in expression upon LINE1 RNA-targeted delivery of WT FTO in Fto\(^−/−\) mESCs. This indicates that 2C genes are repressed by LINE1 RNA in the WT setting. Overall, Wei et al. show that LINE1 RNA regulates pluripotency genes in mESCs in cis, while 2C genes are regulated in trans. This program seems to be governed by demethylation via FTO and installed by regulating chromatin accessibility. Fto KO, in contrast, induces closed chromatin at pluripotency gene sites and releases the repression at 2C genes, leading to the observed 2C-like state and differentiation defects.

In vivo analysis revealed ovarian defects and impaired fertility in female KO mice, i.e. reduced numbers of immature germinal vesicle (GV) oocytes and impaired maturation to the mature MI oocyte stage. GV and MI oocytes exhibited reduced LINE1 RNA levels, more closed chromatin and downregulation of LINE1 RNA-containing genes. Mating of female and male WT and Fto\(^−/−\) mice led to genotype-specific phenotypes during embryonic development, with Fto\(^−/−\)KO/H1pat KO embryos not being viable. Analysis of the Fto\(^−/−\)H1pat KO vs WT morula stage revealed repressed LINE1 RNA, decreased expression of essential regulators of early embryonic development and upregulation of 2C markers. Of note, Fto\(^−/−\) offspring (F1) of heterozygous Fto\(^−/−\) mice (P) is viable. The studied phenotypes were observed in the F2 generation after intracytoplasmic sperm injection using female and male Fto\(^−/−\) animals.

In the future, it will be important to further explore the mechanistic link between the FTO-LINE1 axis and YTHDC1. Does FTO demethylate LINE1 RNA in mESCs and during embryonic development to specifically prevent YTHDC1 binding and subsequent LINE1 RNA destabilization and chromatin closure? What other factors are involved in this process, and how are the different functions of LINE1 RNA in cis and trans achieved? To answer these
WT mESC

LINE1-containing loci: LINE1 RNA regulates pluripotency genes in cis
- open chromatin; functional mESC

Non-LINE1-containing loci: LINE1 RNA regulates 2C genes in trans
- closed chromatin; 2C genes suppressed

Fto KO mESC

LINE1-containing loci: Fto KO
- closed chromatin; impaired mESC

Non-LINE1-containing loci: Fto KO
- open chromatin; 2C-like state

Histone  m^A  Polymerase II
FTO  H3K4Me3/H3K27Ac  YTHDC1
LINE1 RNA  H3K9Me3

Fig. 1 Schematic representation of the function of LINE1 RNA in mESCs and consequences of Fto KO. LINE1 RNA is the main substrate of FTO in mESCs. In WT mESCs, LINE1 RNA promotes an open chromatin state at LINE1-containing gene loci, for example by recruitment of histone modifiers that install activation marks. Mechanistically, loss of FTO leads to an increase in m^A levels of LINE1 RNA, which causes destabilization and thereby reduced levels of LINE1 RNA, potentially via YTHDC1. The absence of LINE1 RNA and LINE1 RNA-DNA interaction affects the chromatin state and leads to closed chromatin, installation of repressive histone marks and reduced transcription. This phenomenon is especially observed at the site of LINE1-containing gene loci, which also encode pluripotency genes. These genes are regulated by LINE1 RNA in cis, leading to a decrease in expression upon Fto KO. LINE1 has been shown to be important for silencing of 2C genes. Interestingly, a release of repression of non-LINE1-containing 2C genes is observed in Fto KO mESCs, suggesting a potential role for LINE1 RNA in regulating these loci in trans. Functionally, this leads to a more 2C-like state and loss of the mESC state, including reduced expression of several pluripotency genes and impaired differentiation and self-renewal.

In summary, Wei et al. could show that LINE1 RNA is the major target of FTO in mESCs (Fig. 1). Activity of FTO in the nucleus ensures LINE1 RNA demethylation, which is important for the accessibility and transcription of LINE-containing genes, including pluripotency genes. This process is mediated by LINE1 RNA in cis. In contrast, 2C genes are suppressed by FTO, presumably via LINE1 RNA-mediated regulation in trans. This novel FTO-LINE1 axis is also essential for oocyte and embryo development.

FUNDING
P.S. acknowledges funding from the Peter und Traudl Engelhorn Stiftung. Open Access funding enabled and organized by Projekt DEAL.

ADDITIONAL INFORMATION
Competing interests: The author declares no competing interests.

REFERENCES
1. Wei, J. et al. FTO mediates LINE1 m^A demethylation and chromatin regulation in mESCs and mouse development. Science 376, 968–973 (2022).
2. Zhang, M. et al. Roles of N6-methyladenosine (m^A) in stem cell fate decisions and early embryonic development in mammals. Front. Cell Dev. Biol. 8, 782 (2020).
3. He, C. & Lan, F. RNA m^A meets transposable elements and chromatin. Protein Cell 12, 905–910 (2021).
4. Jia, G. et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat. Chem. Biol. 7, 885–887 (2011).
5. Liu, J. et al. N (6)-methyladenosine of chromosome-associated regulatory RNA regulates chromatin state and transcription. Science 367, 580–586 (2020).
6. Jachowicz, J. W. et al. LINE-1 activation after fertilization regulates global chromatin accessibility in the early mouse embryo. Nat. Genet. 49, 1502–1510 (2017).
