FTO, RNA epigenetics and epilepsy

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Several recent landmark papers describing N[6]-methyladenosine (m6A) RNA modifications have provided valuable new insights as to the importance of m6A in the RNA transcriptome and in furthering the understanding of RNA epigenetics. One endogenous enzyme responsible for demethylating RNA m6A, *FTO*, is highly expressed in the CNS and is likely involved in mRNA metabolism, splicing or other nuclear RNA processing events. microRNAs (miRNAs), a family of small, non-coding transcripts that bind to target mRNAs and inhibit subsequent translation, are highly expressed in the CNS and are associated with several neurological disorders, including epilepsy. miRNAs frequently bind to recognition sequences in the 3'UTR, a region that is also enriched for m6A. Certain specific miRNAs are upregulated by neuronal activity and are coupled to epileptogenesis; these miRNAs contain a consensus m6A site that if methylated could possibly regulate miRNA processing or function. This Point-of-View highlights aspects from recent papers to propose a functional association between *FTO*, RNA epigenetics and epilepsy.

FTO is Important in the CNS

The gene fat mass and obesity-associated (*FTO*) was first identified in mice as one of the genes encoded by the 1.6 Mb deletion that produced a phenotype with partial syndactyly of forelimbs and extensive thymic hyperplasia.[1] Subsequently, a common variant in the *FTO* gene was identified as a risk allele for type 2 diabetes and increased body mass,[2] and many studies of *FTO* have focused on the association with diabetes, obesity and metabolism.[3] In the course of conducting these large scale genotyping studies, the role of *FTO* in human disorders was expanded to include the central nervous system (CNS). Variants have been found to be associated with neurological disease conditions including depression[4] and Alzheimer disease.[5] *FTO* is highly expressed in brain tissue[6] and is essential for normal development of the CNS in human.[7] The generation of mice that were specifically deleted only for neuronal *FTO* had a similar phenotype of growth retardation as the whole body *FTO* deletion, suggesting that a major function of *FTO* occurs in the brain.[8] The obesity-associated risk allele has been shown to have a potential pathological effect on brain volume: healthy elderly subjects with the risk allele had brain volume deficits (average differences of 8% in frontal lobes and 12% in occipital lobes) compared with non-carriers.[9] Recently, brain derived neurotrophic factor (BDNF) was identified as a candidate gene for functional coupling to *FTO*, leading the authors to speculate on a role of *FTO* in neuronal plasticity possibly via interaction with CCAAT/enhancer binding protein β.[10] These data provide strong evidence that *FTO* has a functional role in the CNS and, by implication, to CNS disorders.

Fto Demethylates m6A RNA

Most studies involving *FTO* have focused at the genome level and on correlation of variants with phenotypes. Evidence for the molecular action of the expressed protein (Fto) is more limited. Fto has been shown to localize to the nucleus and to catalyze
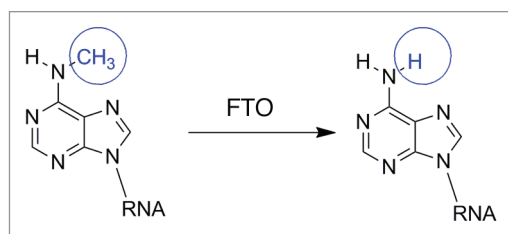
Figure 1. *FTO* catalyzes the conversion of N⁶-methyladenosine in RNA to adenosine.

the Fe(II) and 2-oxoglutarate-dependent demethylation of 3-methylthymine in ssDNA.[11] One year later it was shown to catalyze the demethylation of 3-methyluracil in ssRNA with slightly higher efficiency over that of 3-methylthymine in ssDNA.[12] However, a recent article provides the strongest evidence to date on the enzymatic activity of Fto. Jia et al.[13] provide evidence that Fto strongly prefers to demethylate N⁶-methyladenosine (m⁶A) in ssRNA (**Fig. 1**). By direct comparison with other substrates these authors conclude that m⁶A in ssRNA is the best substrate discovered so far for Fto, having a greater than 50-fold preference for m⁶A over 3-methyluracil.[13] Expected changes in levels of m⁶A in mRNA were found when human cells were manipulated to either overexpress Fto (which caused a decreased level of m⁶A) or underexpress Fto (which caused an increased level of m⁶A). These authors further showed that Fto partially co-localizes with nuclear splicing speckle factors (SART1 and SC35) and with RNA polymerase II phosphorylated at Ser2, but not with markers for other nuclear subregions such as telomeres, replication site, Cajal body, cleavage body or P-body.[13] These authors conclude that m⁶A in nuclear RNA is the physiological substrate of Fto, and that the function of Fto likely affects the processing of pre-mRNA and/or other nuclear RNAs. This report provides strong evidence that Fto is involved in the processing of nuclear RNAs and in RNA epigenetic modifications. As such, Fto is the first m⁶A RNA demethylase that exhibits significant regulatory functions.

m⁶A and RNA Epigenetics

m⁶A is the most common mRNA modification in eukaryotes and also in the RNA of viruses that replicate in eukaryotes. The

modification is catalyzed by the methyltransferase like 3 (METTL3) enzyme, which is thought to be one component of a multi-component complex.[14] A degenerate methylation consensus sequence, purine-purine-m⁶A-C-[A/C/U], has been known for many years.[15] The presence of this consensus sequence does not guarantee methylation, suggesting that this process is regulated. There is also a report of another m⁶A methylase activity (toward U6 snRNA) that does not utilize this consensus sequence and appears to be a separate enzyme.[16] Two recent independent studies utilizing m⁶A-specific antibodies and next generation sequencing provide a transcriptome-wide assessment of mRNA m⁶A methylation, substantially increasing knowledge of this modification.[17,18] These papers clearly demonstrated that m⁶A methylation is a very prominent mRNA modification, identifying more than 7,000 genes that contain m⁶A. They were in general agreement on a recognition consensus sequence for the adenosine that is methylated, the overall distribution of m⁶A sites along the length of the transcripts and in the high conservation between human and mouse of major elements of this common RNA modification. These papers provide valuable new insights of m⁶A in the RNA transcriptome and further the understanding of RNA epigenetics.[19] One of these studies identified potential m⁶A-specific binding proteins, which may have functional significance.[17] Evidence was also presented that m⁶A affects RNA splicing. Using conditions that focused on differentially expressed isoforms, a positive relationship was seen between m⁶A and isoform switching. Further, differentially spliced exons and introns were significantly enriched with m⁶A.[17] These data indicate a role for m⁶A in splicing, further supporting the role of the m⁶A demethylase, Fto, in this process.

miRNA and Epilepsy

Recently, a connection has been made between RNA processing and epilepsy. MicroRNA (miRNA) is a major RNA regulatory gene family in eukaryotes of which hundreds have been identified. Mature miRNA forms base pairs with mRNA, often in the 3'UTR, to induce mRNA degradation, translational repression, or both. Since a single miRNA can target multiple mRNAs, and multiple miRNAs can act on a single mRNA, miRNAs are thought to operate highly complex regulatory networks that silence targeted genes.[20] A recent review highlights the functions of miRNAs in CNS development and provides multiple examples in which misregulation of CNS miRNAs is associated with neurological disorders, including epilepsy.[21] Specific miRNAs are also being investigated in the context of epilepsy. miRNA-146a has been shown to be upregulated in an animal model of temporal lobe epilepsy (TLE), as well as in hippocampal tissue from patients with TLE and hippocampal sclerosis.[22] Similarly, miRNA-134 has been shown to be upregulated in an experimental model of epilepsy involving status epilepticus and also in temporal neocortex tissue from patients with TLE.[23] These reports suggest that these miRNAs are neuronally activated and are coupled to epileptogenesis. Upregulation of miRNA-134 had a suppressive effect on a known target, LIM kinase 1 (Limk1). Antagomir silencing of miRNA-134 after status epilepticus caused a substantial reduction in the number of subsequent seizures, and the rescue of Lim1k was implicated in this protection.[23] While the mechanism of this dramatic seizure suppression could not be absolutely defined, an antiepileptogenic effect was a considered possibility. This paper clearly shows that silencing miRNA-134 has a neuroprotective and seizure-suppressive effect and provides another avenue in which to study epileptogenesis.

miRNA and m⁶A

The methylation status of miRNA has been shown to affect stability and turnover.[24] As mentioned above, the location
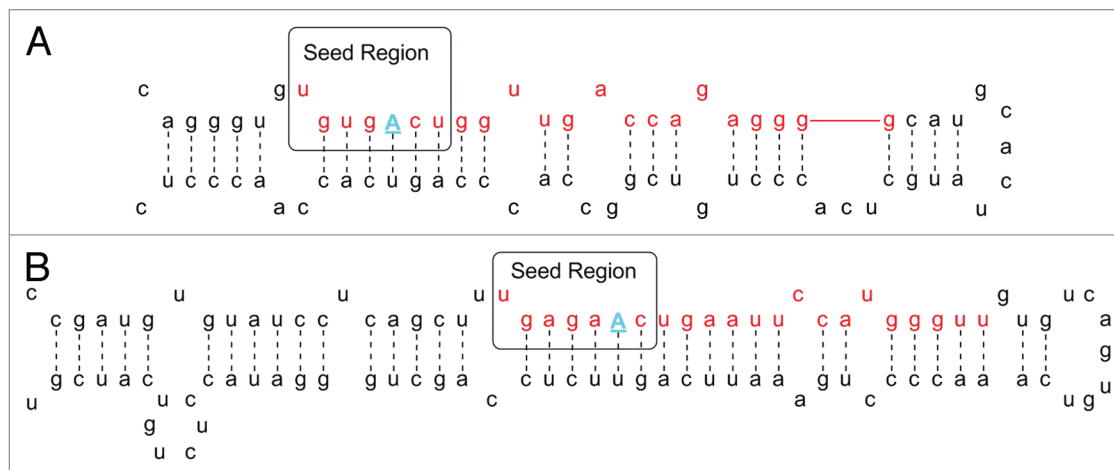
Figure 2. (**A**) Pre-miRNA-134. Mature miRNA-134 is in red, the potential m⁶A methylation residue is in cyan (UGAC, from ref. 18), and the seed region of the mature miRNA is in the box. (**B**) Pre-miRNA-146a. Mature miRNA-146a is in red, the potential m⁶A methylation residue is in cyan [Pu-Pu-AC(A/C/U)], and the seed region of the mature miRNA is in the box. Sequences were obtained from the microRNA database, www.mirbase.org/index.shtml

of m⁶A residues in the 3'UTR of the transcriptome has been linked with miRNA binding sites.[18] These authors found that 67% of 3'UTRs that contain m⁶A peaks also contained at least one predicted miRNA binding site, significantly greater than expected by chance alone. They also showed that highly expressed brain miRNAs had a significantly greater percentage of target transcripts that contain m⁶A than those of lowly expressed miRNAs, further supporting a link between the CNS, miRNAs, and m⁶A. Based on the evidence that miRNA-134 and miRNA-146a are involved in the pathogenesis of epilepsy, we searched the sequence of these miRNAs for a m⁶A consensus site. We found that both miRNA-134 and miRNA-146a contain a potential m⁶A site in the seed region, which is thought to play an important role in miRNA recognition of target mRNA (**Fig. 2**). This suggests that m⁶A methylation of miRNAs could interfere with binding to target mRNAs, in a manner analogous to that which is known for m⁶A DNA methylation to inhibit the binding of certain restriction endonucleases. The occurrence of m⁶A in dsRNA is also known to lower melting temperature and destabilize base-paired duplex structures: a single m⁶A modification of an octamer is estimated to increase (destabilize) ΔG by about +1 to 1.2 kcal/mol.[25] m⁶A modification of miRNAs may therefore affect the processing of primary miRNAs to mature miRNAs, which involves duplex formation,[26] and/or the binding of miRNA to mRNA, which also involves a dsRNA component. The confirmation of the m⁶A modification on these miRNAs remains to be determined, although m⁶A is known to occur in 3'UTRs of target mRNA. The presence of m⁶A in either the miRNA or in the 3'UTR in mRNA would affect the subsequent interactions, likely via destabilization of duplexes. Furthermore, the presence of m⁶A in miRNA or mRNA may affect the interaction with RNA-binding proteins.[17,27] These effects suggest an important additional layer of control over miRNA actions to impact neurological development and disease states.

Summary

Based on recent evidence, we have provided a possible link between Fto, RNA epigenetics and epilepsy. Fto is highly expressed in the CNS and demethylates m⁶A in ssRNA. Two recent, independent studies provide ample evidence that m⁶A is a prevalent and important RNA epigenetic mark, thus implicating Fto in the regulation of these RNA epigenetic events. Reversible RNA methylation involving Fto suggests that dynamic methylation states of mRNA or miRNA may modulate protein expression and cell status, analogous to reversible DNA and histone methylations. miRNAs play important roles in CNS physiology and pathology, including epilepsy, and several have been implicated in epileptogenesis. Certain m⁶A sites may play a role in miRNA stability, processing or regulation. Thus, the recently discovered m⁶A RNA demethylation activity of Fto places it in the heart of RNA epigenetics with subsequent regulatory roles on critical CNS processes.

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