Research progress on the relationship between m^{6}A methylation of *YTHDF1* gene in the striatum and stereotyped behavior

Xiaohan Wen[1,2], Xiaodong Liu[1,2], Jingrong Wang[1,2], Longsheng Huang[1], Guihua Liu[1], Haijun Wang[3], Ping Ou[1]

[1]Department of Child Healthcare Center, Fujian Provincial Maternal and Children's Hospital, Affiliated Hospital of Fujian Medical University, Fuzhou, Fujian 350000, China;
[2]School of Clinical Medicine, Fujian Medical University, Fuzhou, Fujian 350004, China;
[3]Department of Maternal and Child Health, School of Public Health, Peking University, Beijing 100191, China.

To the Editor: Stereotyped behavior refers to a series of behaviors with a high frequency and no obvious purpose and meaning. It also includes narrow interest and difficulty in accepting changes in things. In addition to autism spectrum disorder (ASD), stereotyped behavior also exists in various neurodevelopmental disorders, such as Tourette syndrome, Fragile X syndrome, Prader-Willi syndrome, obsessive-compulsive disorder, and many other diseases, which are considered to be related to dysfunction of the striatum.

Epigenetics is the study of heritable changes in gene expression when a gene remains unchanged, including DNA methylation, RNA methylation, and histone modification. RNA methylation, which is involved in the regulation of gene expression, is a hot topic in epigenetics. Methylation modifications of mRNA are widespread in eukaryotes, including m^{1}A, m^{5}C, pseudouridine, and m^{6}A methylation modifications, which can affect protein synthesis in eukaryotes. Among them, m^{6}A methylation modification is the most common modification in eukaryotic mRNA and long-chain non-coding RNA (lncRNA). On average, there are 3 to 5 m^{6}A sites in each mRNA molecule. As the binding protein of m^{6}A, *YTHDF1* plays an important role in the protein translation process.

This article reviews the relationship between m^{6}A RNA methylation based on *YTHDF1* gene and stereotyped behavior.

m^{6}A methylation is the most common internal modification in eukaryotic RNA, which occurs preferentially on the consensus sequence RRACH (R stands for purine, A is the m^{6}A methylation modification site, and H is a non-G base). It is highly enriched near codons and in 3′-UTR.

m^{6}A methylation works through three major types of enzymes: methylase, demethylase, and effector protein. The methylatransferase also called writers, include METTL3, METTL4, WTAP, and KIAA1492, and their function is to modify the mRNA base with m^{6}A methylation. The demethylase includes FTO, ALKBH5, and other homologies, which mainly mediates the demethylation of m^{6}A. Effector proteins are mainly responsible for identifying the information of RNA methylation modification and participate in a series of processes such as translation and degradation of downstream RNA. They all contain a common YTH domain. There are five proteins in the human body that contain YTH domains: YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3. These proteins jointly regulate mRNA homeostasis: YTHDC1 is responsible for regulating mRNA splicing; YTHDC2 may be an RNA helicase. YTHDF1 and YTHDF3 can regulate the translation of mRNA; YTHDF2 regulates the stability of mRNA, which is the main molecule of mRNA degradation induced by m^{6}A. However, there are also new ideas that YTHDF1, YTHDF2, and YTHDF3 jointly regulate the degradation of m^{6}A-modified mRNA, their binding sites are the same with m^{6}A, and their functions can replace each other. A study found that the binding sites of YTHDF1 clustered around the stop codon, similar to the distribution sites of m^{6}A. Using photoactivatable ribonucleoside-crosslinking immunoprecipitation (PAR-CLIP) technology, it was found that YTHDF1 binds near the exact GRAC site (R is G or A).

Mammalian brains contain a large amount of m^{6}A. In rodents, the overall level of m^{6}A is regulated by development, and its expression reaches its peak in adulthood. During the development of the human brain, it can be found that the abundance of m^{6}A gradually increases. Compared with other organs, m^{6}A methylation

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001789

Correspondence to: Ping Ou, Fujian Provincial Maternal and Children's Hospital, Affiliated Hospital of Fujian Medical University, No. 18, Daoshan Road, Gulou District, Fuzhou, Fujian 350000, China
E-Mail: eve1019@126.com

Chinese Medical Journal 2022;135(9)
Received: 14-03-2021; **Online:** 09-12-2021 **Edited by:** Jing Ni

is more abundant in the brain. m^{6}A methylation modification affects the differentiation and development of cells. The imbalance of m^{6}A methylation modification level can lead to severe developmental defects in the cerebral cortex and cerebellum. It has been experimentally confirmed[1] that rodents' autistic-like manifestation, such as their pro-social and stereotyped behaviors, were all improved after reducing the level of modification of m^{6}A methylation.

Many human diseases are currently known to be related to methylation. m^{6}A methylation has a huge impact on neurological diseases such as ASD, depression, and attention deficit hyperactivity disorder (ADHD). It has been found that, compared with normal people, the methylation modification including m^{6}A methylation in the brain of ASD patients is abnormal.[2] In a study, in a Chinese Han population suffering from major depression, the gene of the m^{6}A demethylase ALKBH5 may play a role in severe depression in the Chinese population. Studies have been shown that the m^{6}A demethylase FTO is involved in the regulation of the ADHD phenotype, which may be the result of regulating a variety of neural signaling pathways, including the DA signaling pathway.

Stereotyped behavior is related to the striatum, and the cortical-striatum circuit is impaired in children with ASD. The striatum is the main input channel of the basal ganglia, receiving projections from sensory, motor, and related cortical areas and projecting to the corresponding nuclei through the direct or indirect pathways of the basal ganglia. It is now believed that the imbalance between the direct and indirect pathway interactions in the striatum, especially the inhibition of indirect pathway activation, can lead to stereotyped behavior. Stimulating the subthalamic nucleus, that is, stimulating the indirect pathway, can improve stereotyped behavior to a certain extent.

The maintenance of the normal physiological function of the striatum requires normal synaptic structure and the normal function of the synapses. Abnormal synaptic protein synthesis leads to changes in synaptic structure, abnormal synaptic function, and striatal dysfunction. In a study on the *Shank3* mutant mouse model, researchers found that *Shank3* mutant mice exhibited stereotyped behavior like ASD and damaged social interactions. When *Shank3* gene was re-expressed, the protein synthesis returned to normal, the synaptic structure was restored, the synaptic function was improved, and stereotyped behavior like ASD were also corrected.

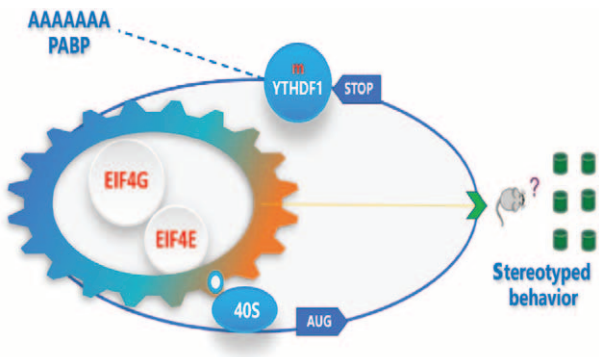

Figure 1: YTHDF1 interacts with eIF to regulate mRNA translation.[8] eIF: Eukaryotic Initiation factor; PABP: Poly(A) binding protein;40s: 40s ribosomal subunit.

As a binding protein of m^{6}A methylation modification, YTHDF1 plays an important role in synaptic formation and function properly.

YTHDF1 is closely related to the synaptic formation. Robo3.1 is an axon-guiding protein that plays an important role in axon generation. YTHDF1 can promote the translation of Robo3.1 and affect the occurrence of axons and then affect the synaptic plasticity. When the m^{6}A site was mutated, the positive regulation of YTHDF1 on Robo3.1 translation also disappeared, and the protein level of YTHDF1 and the protein expression level of Robo3.1 in Ythdf1-knockout mice decreased significantly, while the mRNA level of Robo3.1 was not affected.

YTHDF1 is also closely related to the synaptic function. In the Morris water maze and the probe test, the *Ythdf1*-knockout mice showed learning and memory disorders. The YTHDF1 protein was not detected in the dentate gyrus and CA2/3 region of the hippocampus, and the synaptic base transmission and long-term potentiation (LTP) in the hippocampus were impaired. After the re-expression of *Ythdf1* gene, YTHDF1 protein was detected in the hippocampus again. The learning and memory impairment and LTP of the model mice were reversed, and the synaptic function was restored.

m^{6}A methylation may be an important link in regulating the function of the striatum and stereotyped behavior by regulating the level of eukaryotic initiation factor (eIF). The initiation of protein translation in eukaryotes is a complex process that requires the participation of a series of proteins, which are called eIF. In the initial stage of translation, the formation of eIF-4F complex is critical.

The eIF-4F complex can ensure the correct identification of the initiation codon and ensure smooth translation. The eIF-4F complex is composed of eIF-4G, eIF-4E, and eIF-4A. Among them, eIF-4G is a multi-domain adaptor protein, responsible for binding eIF-4E, eIF-3, and poly-A tail binding protein PAB; eIF-4E is responsible for binding the 5′-end cap structure of mRNA; eIF-4A has activity of RNA helicase, which can remove the hairpin structure at the 5′-end of the mRNA and bind it to the small ribosomal subunit. Among them, the assembly of the eIF-4E/eIF-4G complex plays a central role in the regulation of gene expression at the translation initiation level.

Existing experiments have shown that the binding protein YTHDF1 of m^{6}A can regulate mRNA translation through interaction with eIF, thereby affecting protein synthesis. YTHDF1 can recognize the m^{6}A methylation modification on the mRNA in the cell, promote the connection of the target mRNA with the ribosome, make the target mRNA form a closed loop, and improve its translation efficiency. This process requires the participation of eIF.

Variations associated with the *eIF4E* gene have been found in ASD patients.[3,4] These suggest that *eIF4E* gene may play a role in the pathogenesis of ASD. In the study of eIF-4E transgenic mice, researchers found that eIF-4E levels in eIF-4E transgenic mice increased, the eIF-4E/eIF-4G interaction level was significantly enhanced, the control of protein translation is dysfunctional, and the mice exhibit abnormal behavior consistent with ASD.[5] In the marble burying experiment, compared with wild-type litter pups, the eIF-4E transgenic mice showed more severe repetitive excavation behavior, and the number of self-modifications increased and the time was prolonged. Compared with wild-type litter pups, eIF-4E transgenic mice showed enhanced long-term depression, showing changes in synaptic function and plasticity in the striatum, and synaptic function was damaged. When eIF-4E transgenic mice were injected with 4EGI-1 (a kind of eIF-4E/eIF-4G interaction inhibitor), the synaptic function of the striatum was restored, and 4EGI-1 reversed the autistic-like phenotype caused by elevated eIF-4E levels[5,6]; the stereotyped behavior of eIF-4E transgenic mice in the marble burial experiment is greatly reduced. Dysregulation of translation and abnormal levels of synaptic proteins, resulting in changes in synaptic structure, may be one of the mechanisms leading to repetitive stereotyped behavioral phenotypes.[7]

At the same time, it has also been pointed out that YTHDF protein plays a role in mRNA degradation, but there is no evidence that it can promote translation. When YTHDF1, YTHDF2, and YTHDF3 were present at the same time, the degradation rate of m^6A-modified mRNA was the highest. When YTHDF1 protein was knocked out with YTHDF2 or YTHDF3 protein, the expression of m^6A-modified mRNA was increased.

Stereotyped behavior is not only one of the core symptoms of ASD but also a manifestation of a variety of neurodevelopmental disorders, which is thought to be related to striatal dysfunction. Epigenetic modification plays an important role in the normal functioning of synapses by participating in the regulation of gene expression. Animal experiments have pointed out that changes in eIF levels in the striatum can affect the phenotype of stereotyped behavior. As a binding protein modified by m^6A methylation, YTHDF1 plays an important role in the process of synapse formation and synapse function. However, further research is needed to determine whether YTHDF1 can regulate repetitive and rigid behavior by regulating mRNA translation. This will provide a theoretical basis for the pathogenesis of stereotyped behavior of ASD and also will provide a new idea and approach for the intervention of core symptoms of ASD.

Conflicts of interest

None.

References

1. Edupuganti RR, Geiger S, Lindeboom RGH, Shi H, Hsu PJ, Lu Z, *et al*. N6-methyladenosine (m6A) recruits and repels proteins to regulate mRNA homeostasis. Nat Struct Mol Biol 2017;24:870–878. doi: 10.1038/nsmb.3462.
2. Yoon KJ, Ringeling FR, Vissers C, Jacob F, Pokrass M, Jimenez-Cyrus D, *et al*. Temporal control of mammalian cortical neurogenesis by m6A methylation. Cell 2017;171:877–889e17. doi: 10.1016/j. cell.2017.09.003.
3. Waltes R, Gfesser J, Haslinger D, Schneider-Momm K, Biscaldi M, Voran A, *et al*. Common EIF4E variants modulate risk for autism spectrum disorders in the high-functioning range. J Neural Transm (Vienna) 2014;121:1107–1116. doi: 10.1007/s00702-014-1230-2.
4. Lombardo MV, Moon HM, Su J, Palmer TD, Courchesne E, Pramparo T. Maternal immune activation dysregulation of the fetal brain transcriptome and relevance to the pathophysiology of autism spectrum disorder. Mol Psychiatry 2018;23:1001–1013. doi: 10.1038/mp.2017.15.
5. Santini E, Huynh TN, MacAskill AF, Carter AG, Pierre P, Ruggero D, *et al*. Exaggerated translation causes synaptic and behavioural aberrations associated with autism. Nature 2013;493:411–415. doi: 10.1038/nature11782.
6. Gkogkas CG, Khoutorsky A, Ran I, Rampakakis E, Nevarko T, Weatherill DB, *et al*. Autism-related deficits via dysregulated eIF4E-dependent translational control. Nature 2013;493:371–377. doi: 10.1038/nature11628.
7. Auerbach BD, Osterweil EK, Bear MF. Mutations causing syndromic autism define an axis of synaptic pathophysiology. Nature 2011;480:63–68. doi: 10.1038/nature10658.
8. Wang X, Zhao BS, Roundtree IA, Lu Z, Han D, Ma H, *et al*. N6-methyladenosine modulates messenger RNA translation efficiency. Cell 2015;161 6:1388–1399. doi: 10.1016/j. cell.2015.05.014.

How to cite this article: Wen X, Liu X, Wang J, Huang L, Liu G, Wang H, Ou P. Research progress on the relationship between m^6A methylation of *YTHDF1* gene in the striatum and stereotyped behavior. Chin Med J 2022;135:1120–1122. doi: 10.1097/CM9.0000000000001789