An epigenetic view of developmental diseases: new targets, new therapies

Pei Xie, Li-Qun Zang, Xue-Kun Li, Qiang Shu
Hangzhou,China

Background: **Function of epigenetic modifications is one of the most competitive fields in life science. Over the past several decades, it has been revealed that epigenetic modifications play essential roles in development and diseases including developmental diseases. In the present review, we summarize the recent progress about the function of epigenetic regulation, especially DNA and RNA modifications in developmental diseases.**

Data sources: **Original research articles and literature reviews published in PubMed-indexed journals.**

Results: **DNA modifications including methylation and demethylation can regulate gene expression, and are involved in development and multiple diseases including Rett syndrome, Autism spectrum disorders, congenital heart disease and cancer, etc. RNA methylation and demethylation play important roles in RNA processing, reprogramming, circadian, and neuronal activity, and then modulate development.**

Conclusions: **DNA and RNA modifications play important roles in development and diseases through regulating gene expression. Epigenetic components could serve as novel targets for the treatment of developmental diseases.**

World J Pediatr 2016;12(3):291-297

Key words: **congenital heart disease;**
development;
DNA modification;
5-hydroxymethylcytosine;
neurological disorders;
RNA modification

Author Affiliations: Institute of Genetics, College of Life Sciences, Zhejiang University (Xie P, Zang L); Children's Hospital, Zhejiang University School of Medicine (Li X, Shu Q); Institute of Translational Medicine, School of Medicine, Zhejiang University (Li X)

Corresponding Author: Qiang Shu, Children's Hospital, Zhejiang University School of Medicine, China (Email: shuqiang@zju.edu.cn)

doi: 10.1007/s12519-016-0020-3

Review article

Introduction

Epigenetics is originally referred to the phenotypes induced by the interaction between genes and their surroundings. Nowadays, epigenetics is defined as stably heritable phenotypes resulting from changes in a chromosome without alterations of the DNA sequence. Epigenetic modifications mainly include histone posttranslational modifications, chromatin remodelling, nucleosome positioning, DNA and RNA methylation and demethylation, and non-coding RNAs. Previous studies have indicated that epigenetic pathways play critical function in diverse biological processes including embryonic development, X chromosome inactivation, imprinting, reprogramming, and gene expression.[1,2] The normal establishment of epigenome is critical for the development and functions, whereas the dysfunction of epigenetic landscapes has been revealed in multiple diseases such as cancer, neurological disorders, cardiovascular disease, leukemia, etc.[3-5]

Epigenetic modifications are reversible, making them potentially be targeted by pharmacological and dietary interventions. Currently, epigenetic-based therapies are on the way to be actively developed. For example, acute myeloid leukemia (AML) is the most common type of acute leukemia in adults, and some compounds targeting on DNA modifications are in various stages of preclinical and clinical development, and two DNA methyltransferase inhibitors, Azacitidine and Decitabine, have been approved.[6] Further, multiple small molecule inhibitors targeting on histone modification machinery including histone methyltransferase (HMT) and demethyltransferase (HDM), histone acetyltransferase (HAT) and histone deacetylase (HDAC) have also been identified.[6,7] Although many challenges have to be dealt with and the potential compounds need to be further studied in detail, epigenetic based treatments would hold great promise in combination with the conventional therapeutic methods.

In this concise review, we briefly introduce the landscape of epigenetics, and summarize the function of epigenetic modifications with a focus on DNA and RNA modifications in developmental diseases.

Histone modification

In eukaryotic cells, DNA is wrapped with histones and formed a highly organized and condensed structure, so called "nucleosomal core particle". Each nucleosome contains four core histones (H2A, H2B, H3, H4) with 145–147 base pairs (bp) of DNA wound around it, and one linker histone (H1). Histones can carry many post-translational modifications, which influence chromatin compaction and accessibility in many different ways. Histone modifications include methylation, acetylation and sumoylation, phosphorylation, ubiquitinylation, poly(ADP)-ribosylation and deamination as well as arginine methylation and citrullination, hydroxylation and several others. Diverse histone modifications are established by multiple "writers" and "erasers".[8] Histone acetyltransferases (HATs) include HAT1, CBP/P300, etc. SirtT2 is one of the histone deacetylases (HDACs). Many histone methyltransferases (HMTs), especially lysine methyltransferases have also been identified, including G9a, MLL1-5, SET1A and 1B, EZH2, etc. LSD1, JHDM and JMJD protein families are identified as histone demethylases (HDMs).[8]

Of note, distinct histone modifications can influence each other and regulate DNA transcription. Among these modifications, the most extensively investigated are histone acetylation/deacetylation and histone methylation/demethylation of lysines and arginines, though other regions of the histone can also be modified. Different histone modifications mark at distinct genomic regions such as enhancer, promoter, and gene body, and exert differential function in regulating gene expression. Lysine acetylation always correlates with gene transcription, but the effects of lysine methylation depend on which residue is modified. For example, histone 3 lysine 4 (H3K4) and lysine 36 (H3K36) trimethylation is typically associated with active transcription. In contrast, methylation of H3K27, H3K9, H4K20 generally represses gene expression.[9]

DNA modification

DNA methylation, mainly on the fifth carbon of cytosine (5-methylcytosine, 5mC) in mammalian, is established by DNA methyltransferases (DNMTs), DNMT1, DNMT3A and DNMT3B. DNMT1 contributes to the maintenance of DNA methylation, while DNMT3A and DNMT3B orchestrated with DNMT3L catalyze de-novo DNA methylation. The methylation of DNA mainly occurs in the context of CpG dinucleotides of distinct genomic regions in mammalian, though the methylation in non-CpG context is also identified. Of note, CpG Island, which is characterized by high (G+C) and CpG content, predominantly exists at promoter region and generally remains unmethylated. The methylated CpG can be read by methyl-CpG-binding domain proteins (MBDs) including MBD1-4, MeCP2 and KAISO, which recruit various inhibitory proteins and induce the repressive effects.[2,4]

DNA methylation is associated with diverse cellular functions and also dynamic in development and diseases.[4] The proper establishment of DNA methylation is essential for normal development. In early embryonic development, the paternal genome is actively demethylated followed by the demethylation of maternal genome. In the blastocyst, genomewide methylation levels increase rapidly and cell-specific methylation landscape is established. In embryonic and postnatal neuronal development, the deficiency of DNMTs affects embryonic viability, and cell survival, synaptic development, learning and memory; meanwhile, neuronal activity could influence DNA methylation, suggesting DNA methylation is important for normal neuronal function.[10-12]

Recently, Ten-eleven translocation proteins (Tet) have been revealed to convert 5mC to 5-hydroxymethylcytosine (5hmC), an active process of DNA demethylation.[13,14] 5hmC can be further catalyzed to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) by Tet, and 5caC can be efficiently removed by TDG (Fig. A).[14-16] The distribution of 5hmC displays tissue/cell-specific pattern, and enriches at distinct genomic regions.[17,18] Further, in cell fate determination and postnatal neuronal development, 5hmC exhibit dynamic features.[17,19] Compelling evidences indicate that 5hmC-mediate epigenetic regulation play crucial roles in stem cell modulation, development, and diverse diseases, etc.[19]

RNA modification

More than 100 types of RNA modification have been identified in messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), transfer-messenger RNA (tmRNA), and small nuclear RNA (snRNA), which exist across all regions of the transcripts including 5' end and 3' end.[20] The biological significance of RNA modifications are under extensive study. The methylation of RNA as m6A is the most prevalent internal modification of mRNAs and long non-coding RNA, and each mRNA contains on average from three to five m6A modifications.[20,21] Antibody based next-generation sequencing studies reveal over 12 000 m6A sites in more than 7000 mRNAs and 300 non-coding RNAs in human cells, of which predominantly enrich around stop codons, 3' UTR and long internal exons, and distributed in a consensus

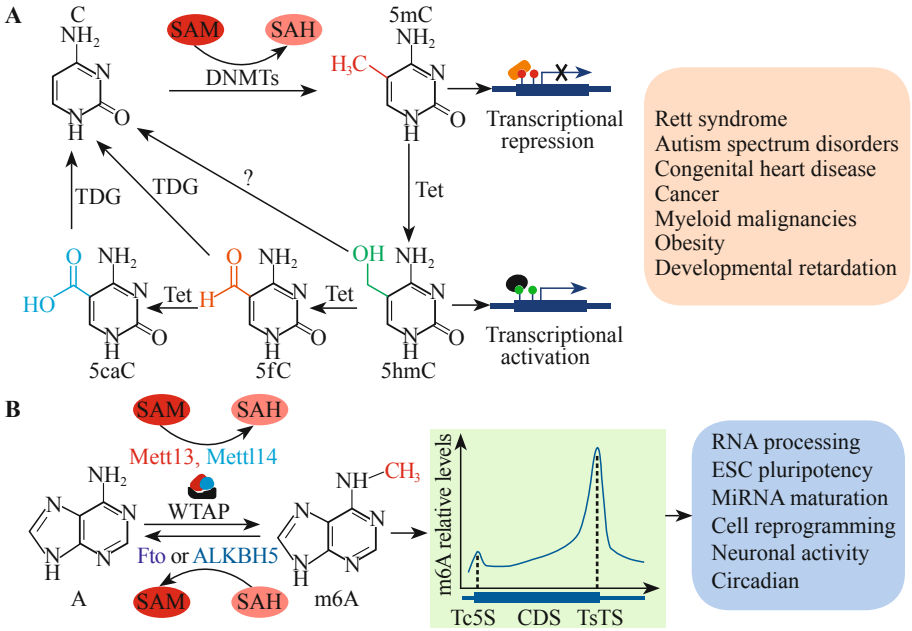

Fig. Schematic diagram showing the methylation and demethylation of DNA and RNA, and the biological functions. **A**: DNA methylation is established by DNA methyltransferases, DNMT1, DNMT3a and 3b. Methylated DNA (5mC) can be actively demethylated by TET, and generates 5hmC, 5fC, and 5caC. DNA modifications can regulate gene expression, and involve in diverse biological functions and diseases. **B:** RNA methylation is catalyzed by Methyltransferase-like 3 METTL3, METTL14, which forms a complex with Wilms tumour 1associated protein (WTAP). The methylated adenine (m6A) highly enriches at 5'- and 3'-UTR in transcripts, and can be demethylated by fat mass and obesity-associated protein (FTO) and α-ketoglutarate-dependent dioxygenase alkB homologue 5 (ALKBH5). DNMT: DNA methyltransferase; 5hmC: 5-hydroxymethylcytosine; 5fC: 5-formylcytosine; 5caC: 5-carboxylcytosine; TDG: thymine-DNA glycosylase; m6A: N6-methyladenosine; SAM: S-adenosyl-L-methionine; TcSS: transcription start site; TcTS: transcription terminal site; ESC: embryonic stem cell.

sequence of (Pu[G>A]m6AC[A/C/U]).[22] Importantly, a strong conservation of m6A sites is observed between humans and mice, suggesting m6A play critical regulatory roles (Fig. B).[22,23]

M6A modification is catalyzed by Mettl3 in mammalian.[24] Later on, it was found that Mettl3 could form a stable complex with Mettl14 and WTAP to catalyze m6A methylation.[25] M6A can be erased by the fat mass and obesity associated protein (FTO) and alkylated DNA repair protein alkB homolog 5 (ALKBH5).[26,27] Up to date, two YTH (YT521-B homology)-domain containing proteins, YTHDF2 and YTHDC1, have been demonstrated as m6A binding proteins.[28]

Epigenetic modifications and developmental diseases

Rett syndrome

Rett syndrome (RTT) is a postnatal neurodevelopmental disorder, and affects around 1 in 10 000 live female births and is rarely found in males due to the mutation of only one copy of MeCP2 induced severe lethal effect. RTT is caused by the mutation of methyl-CpG binding protein 2 (MeCP2), which is highly expressed in neuronal system and becomes more enriched along with the neuronal maturation.[29] RTT patients develop normally during the first 6-18 months of life, after that period, they progressively exhibit a serious of developmental defects including the loss of language abilities, a slowing growth of head and brain, stereotyped behavior, ataxia and autism, etc. It is worthy to note the duplication of MeCP2 also leads to neurological abnormality, and the patients suffer from mental retardation, progressive spasticity, seizures, recurrent respiratory infections, and premature death.[30]

The dosage of MeCP2 has the reverse effect on the global level of 5hmC in the genome: the global level of 5hmC increased in MeCP2 knock-out mice and decreased in MeCP2 over-expression mice.[17] MeCP2 absence significantly increases the enrichment of 5hmC on gene bodies, and does not affect the distribution of stable 5hmC peaks, but significantly decreases the dynamic loci during the postnatal development.[17] Interestingly, the deletion of MeCP2 also alters the dynamics of 5hmC during the postnatal neurodevelopment.[17] Very recently, it was found that in addition to binding to 5mC, MeCP2 can also bind to hydroxymethylated DNA in the brain.[31] R133C mutation of MeCP2 presenting in some RTT patients could abolish its capability of binding to 5mC.[31]

Further, compared to 5mC, 5hmC enriched-DNA can be easily released from chromatin by enzyme digestion, which can be delayed at the condition of MeCP2 depletion.[31] These studies suggest an interaction between MeCP2, 5hmC and chromatin, and underscore a complicated mechanism of gene regulation in Rett syndrome.

Autism spectrum disorders

Autism spectrum disorder (ASD) is a broad group of neurodevelopmental disorders, characterized by stereotyped or repetitive behavior, as well as the deficits in social skills, cognition, attention and communication. ASD has a prevalence of approximately 1 in 100 children and strong male bias (male:female ratio approximately 4:1). To date, hundreds of genes have been linked to ASD. However, only around 10% of ASD cases are caused by known gene mutations, and approximately 90% of autism cases are idiopathic, suggesting a complex etiology of ASD.

Large scale exome sequencing studies indicates the function of histone modifications in ASD. Some of genes implicated in ASD are involved in histone remodelling, such as SET lysine methyltransferases and jumonji lysine demethylases.[32,33] Interestingly, these two studies also identified the mutations of *CHD8* in ASD patients. CHD8 is an ATP-dependent chromatin remodeller and a specific reader for H3K4me2. During neuronal development, CHD8 targets many ASD risk genes, and its depletion causes the dysfunction of ASD risk genes, indicating the roles of histone modifications in ASD.[34] Increased histone H3K4 trimethylation and decreased histone H3K27 trimethylation are observed in the engrailed-2(EN-2) homeobox gene in the autism samples, which is a gene relevant to cerebellar abnormalities and autism.[35]

Recent studies have found that many ASD associated genes, for example, oxytocin receptor gene (OXTR), Glutamate decarboxylase 1 (GAD1), and Engrailed-2 (EN2), exhibited altered DNA methylation and demethylation status.[35-37] During neuronal development of human, DhMRs (differentially hydroxymethylated regions) well overlap with FMR1 regulated genes, and autism related genes.[38] Interestingly, Tet1 expression increases in autistic brain of human and increased enrichment of 5hmC at the promoters of RELN and GAD1 is associated with autism.[37] These findings are further validated in autism mouse model, in which DhMRs showed a high overlap with autism associated genes including Nrxn1 and Reln.[39] Together, these studies indicate 5hmC-mediated epigenetic regulation could play important roles in autism.

Congenital heart disease

The development of vertebrate heart is a very complicated process and precisely regulated by the interplay of a group of core pathways, transcription factors (Mesp1, Isl1, Nkx2.5, Mef2c, Tbx1, Gata4, Foxa2/c1/c2/h1 and Hand2), growth factors (Fgf8/10, Wnt3a/5a/11, Bmp2/4/7, Shh and others), and epigenetic modifications.[5,9] From the specification of pluripotent cells to mesodermal and cardiac precursors prior to terminal differentiation, epigenetic modifications are involved in the coordinated gene expression.

During the cardiac differentiation, histone modifications display preactivation pattern at the promoters of a set of genes related with heart development and cardiac function, and enrich at stage-specific enhancers.[40] DNA methylome is very dynamic during the postnatal heart development, and significantly alters in neonatal, adult healthy and adult failing cardiomyocytes, and DNA methylome in failing cardiomyocytes partially returns to the neonatal.[41] Demethylated regions enriches at the cell type-specific enhancer regions and gene bodies of cardiomyocyte genes.[41] These studies indicate that both histone modifications and DNA methylations play essential roles in orchestrating gene expression during cardiac development.

Congenital heart disease (CHD) is the most common type of birth malformation and affects 1.35 million newborns each year, but the etiology of CHD remains largely unknown. GATA-4, NKX2-5 and HAND1 are key regulators of heart development. In sporadic tetralogy of Fallot (TOF), GATA-4, NKX2-5 and HAND1displayed aberrant DNA methylation status at the promoter region and gene body.[42] The global DNA hypermethylation has also been found in CHD children and their mothers, suggesting a role of DNA methylation in CHD.[43,44] The inactivation of Ezh2, a subunit of Polycomb repressive complex 2 (PRC2), causes lethal congenital heart malformations.[45] *De novo* mutations of the "writers", "erasers" and "readers" of H3K4 methylation have been identified in CHD patients.[46] SMAD2 signaling in the embryonic left-right organizer induces demethylation of H3K27me. The mutations of SMAD2 were also identified in CHD patients.[46] These studies collectively indicate that DNA methylation and histone modifications could be involved in CHD.

Function of RNA modifications in stem cells and diseases

Although its existence was discovered several decades ago, the function of m6A methylation in mRNA is just to be unveiled. The mounting evidence indicates m6A modification can regulate RNA stability, splicing,

translation, transport and localization. In murine embryonic stem cells (mESCs), the knockdown of either Mettl3 or Mettl14 leads to similar phenotype: the reduced m6A methylation and the lost self-renewal capability.[28] Recently, two groups reported some conflicting results. In epiblast and mESCs, the complete deletion of Mettl3 abolishes m6A methylation in mRNA, and the KO cells fail to exit from self-renewal and to undergo regular differentiation, suggesting the proper establishment of m6A methylation landscape involve in the fate determination of stem cells and is required for differentiation (Fig.).[47,48]

One mechanism that m6A methylation plays function is through interacting with miRNA pathway. It is found that Mettl3 could methylate primary miRNA, and the depletion of Mettl3 leads to a global reduction of mature miRNAs, and the accumulation of unprocessed primary miRNAs.[49] Another interesting study has found that m6A modification enriched at miRNA target sites, and the formation of m6A depends on Dicer, a processor of miRNA maturation.[50] The modulation of miRNAs could affect the binding of Mettl3 to mRNA, and thus affect the abundance of m6A.[50] This study also reveals that m6A modification is required for the reprogramming, and can increase the reprogramming efficiency.[50]

Several genome wide association studies indicated an association between *FTO* gene and childhood and adult obesity.[51,52] The Over-expression of Fto increases food intake and results in obesity in mice; the mutation of Fto leads to postnatal growth retardation, significant reduction in adipose tissue, lean body mass, and protects mice from obesity.[53,54] Fto deletion also impairs the proliferation and advances the senescence of cultured skin fibroblast, and loss-of-function of FTO gene is responsible for a severe syndrome in human.[55] In neuronal system, dopamine neuron specific mutation of Fto impaires neuronal activity and resultes in the behavioral deficits in locomotion.[56] M6A sites are also found in the transcripts of many clock genes, and the inhibition of m6A methylation by the deletion of Mettl3 induces circadian period elongation and RNA processing delay, suggesting m6A methylation could serve as a circadian pacesetter.[57] Several studies also find the decreased expression of mRNA demethylases Fto and ALKBH5, and the increased expression of Mettl3 in some cancers.[20] These findings suggest the essential roles of m6A methylation and m6A dynamics for development and diseases (Fig.).

Conclusions

Over the past decades, the rapid advances of epigenetic field together with the sequencing technology have revealed the essential roles of epigenetic modifications in human diseases. The abnormality of epigenetic modification identified in diseases suggested it could be served as novel markers for early diagnosis and treatment of diseases, termed as "epigenetic therapies". The significant progress has been made in the development of drugs targeting on histone and DNA methylation, and some have been approved for the clinical application. For example, DNMT inhibitors, 5-aza-2′-deoxycytidine reverses the aberrant hypermethylation, and has been used for the treatment of cancer patients.

Although the advance in epigenetic field has shed light on the diagnosis and treatment of diseases.[58-60] It is still an open question that the deregulated DNA and RNA demethylation is a cause or a consequence of diseases. Future studies are needed to uncover the function of epigenetic modifications especially DNA demethylation, RNA methylation and demethylation in developmental diseases, which would greatly contribute to the development of personal and precise medicine.

Funding: This work was supported by the International Collaboration Program of Science Technology Department of Zhejiang Province (2015C34001; 2016C34004) to Shu Q and Li X; Zhejiang Provincial Program for the Cultivation of High-level Innovative Health talents to Shu Q; the National Key Basic Research Program of China (No. 2014CB943001) and National Natural Science Foundation of China (No. 31371309) to Li X.
Ethical approval: This article does not include any ethical matters to be approved.
Competing interest: The authors declare that they have no competing financial interests.
Contributors: Xie P and Zang L contributed equally to this work. All authors contributed to the intellectual content and approved the final version.

References

1 Li X, Zhao X. Epigenetic regulation of mammalian stem cells. Stem Cells Dev 2008;17:1043-1052.
2 Bird A. DNA methylation patterns and epigenetic memory. Genes Dev 2002;16:6-21.
3 Sun W, Zang L, Shu Q, Li X. From development to diseases: the role of 5hmC in brain. Genomics 2014;104:347-351.
4 Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet 2003;33 Suppl:245-254.
5 Chang CP, Bruneau BG. Epigenetics and cardiovascular development. Annu Rev Physiol 2012;74:41-68.
6 Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. Blood 2016;127:42-52.
7 Zhang C, Zhong JF, Stucky A, Chen XL, Press MF, Zhang X. Histone acetylation: novel target for the treatment of acute lymphoblastic leukemia. Clin Epigenetics 2015;7:117.
8 Kouzarides T. Chromatin modifications and their function. Cell 2007;128:693-705.

9 Zhang QJ, Liu ZP. Histone methylations in heart development, congenital and adult heart diseases. Epigenomics 2015;7:321-330.
10 LaPlant Q, Vialou V, Covington HE, 3rd, Dumitriu D, Feng J, Warren BL, et al. Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. Nat Neurosci 2010;13:1137-1143.
11 Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, et al. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 2010;13:423-430.
12 Guo JU, Ma DK, Mo H, Ball MP, Jang MH, Bonaguidi MA, et al. Neuronal activity modifies the DNA methylation landscape in the adult brain. Nat Neurosci 2011;14:1345-1351.
13 Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science 2009;324:929-930.
14 Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science 2009;324:930-935.
15 He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science 2011;333:1303-1307.
16 Gu TP, Guo F, Yang H, Wu HP, Xu GF, Liu W, et al. The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature 2011;477:606-610.
17 Szulwach KE, Li X, Li Y, Song CX, Wu H, Dai Q, et al. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat Neurosci 2011;14:1607-1616.
18 Nestor CE, Ottaviano R, Reddington J, Sproul D, Reinhardt D, Dunican D, et al. Tissue type is a major modifier of the 5-hydroxymethylcytosine content of human genes. Genome Res 2012;22:467-477.
19 Cimmino L, Abdel-Wahab O, Levine RL, Aifantis I. TET family proteins and their role in stem cell differentiation and transformation. Cell Stem Cell 2011;9:193-204.
20 Li S, Mason CE. The pivotal regulatory landscape of RNA modifications. Annu Rev Genomics Hum Genet 2014;15:127-150.
21 Yue Y, Liu J, He C. RNA N6-methyladenosine methylation in post-transcriptional gene expression regulation. Genes Dev 2015;29:1343-1355.
22 Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR. Comprehensive analysis of mRNA methylation reveals enrichment in 3' UTRs and near stop codons. Cell 2012;149:1635-1646.
23 Dominissini D, Moshitch-Moshkovitz S, Schwartz S, Salmon-Divon M, Ungar L, Osenberg S, et al. Topology of the human and mouse m6A RNA methylomes revealed by m6A-seq. Nature 2012;485:201-206.
24 Bokar JA, Shambaugh ME, Polayes D, Matera AG, Rottman FM. Purification and cDNA cloning of the AdoMet-binding subunit of the human mRNA (N6-adenosine)-methyltransferase. RNA 1997;3:1233-1247.
25 Liu X, McEachron TA, Schwartzentruber J, Wu G. Histone H3 mutations in pediatric brain tumors. Cold Spring Harb Perspect Biol 2014;6:a018689.
26 Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, et al. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011;7:885-887.
27 Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, et al. ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 2013;49:18-29.
28 Wang Y, Li Y, Toth JI, Petroski MD, Zhang Z, Zhao JC. N6-methyladenosine modification destabilizes developmental regulators in embryonic stem cells. Nat Cell Biol 2014;16:191-198.
29 Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 1999;23:185-188.
30 Chahrour M, Zoghbi HY. The story of Rett syndrome: from clinic to neurobiology. Neuron 2007;56:422-437.
31 Mellen M, Ayata P, Dewell S, Kriaucionis S, Heintz N. MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. Cell 2012;151:1417-1430.
32 De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Cicek AE, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. Nature 2014;515:209-215.
33 Iossifov I, O'Roak BJ, Sanders SJ, Ronemus M, Krumm N, Levy D, et al. The contribution of de novo coding mutations to autism spectrum disorder. Nature 2014;515:216-221.
34 Cotney J, Muhle RA, Sanders SJ, Liu L, Willsey AJ, Niu W, et al. The autism-associated chromatin modifier CHD8 regulates other autism risk genes during human neurodevelopment. Nat Commun 2015;6:6404.
35 James SJ, Shpyleva S, Melnyk S, Pavliv O, Pogribny IP. Complex epigenetic regulation of engrailed-2 (EN-2) homeobox gene in the autism cerebellum. Transl Psychiatry 2013;3:e232.
36 James SJ, Shpyleva S, Melnyk S, Pavliv O, Pogribny IP. Elevated 5-hydroxymethylcytosine in the Engrailed-2 (EN-2) promoter is associated with increased gene expression and decreased MeCP2 binding in autism cerebellum. Transl Psychiatry 2014;4:e460.
37 Zhubi A, Chen Y, Dong E, Cook EH, Guidotti A, Grayson DR. Increased binding of MeCP2 to the GAD1 and RELN promoters may be mediated by an enrichment of 5-hmC in autism spectrum disorder (ASD) cerebellum. Transl Psychiatry 2014;4:e349.
38 Wang T, Pan Q, Lin L, Szulwach KE, Song CX, He C, et al. Genome-wide DNA hydroxymethylation changes are associated with neurodevelopmental genes in the developing human cerebellum. Hum Mol Genet 2012;21:5500-5510.
39 Papale LA, Zhang Q, Li S, Chen K, Keles S, Alisch RS. Genome-wide disruption of 5-hydroxymethylcytosine in a mouse model of autism. Hum Mol Genet 2015;24:7121-7131.
40 Wamstad JA, Alexander JM, Truty RM, Shrikumar A, Li F, Eilertson KE, et al. Dynamic and coordinated epigenetic regulation of developmental transitions in the cardiac lineage. Cell 2012;151:206-220.
41 Gilsbach R, Preissl S, Gruning BA, Schnick T, Burger L, Benes V, et al. Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. Nat Commun 2014;5:5288.
42 Sheng W, Qian Y, Wang H, Ma X, Zhang P, Diao L, et al. DNA methylation status of NKX2-5, GATA4 and HAND1 in patients with tetralogy of fallot. BMC Med Genomics 2013;6:46.
43 van Driel LM, de Jonge R, Helbing WA, van Zelst BD, Ottenkamp J, Steegers EA, et al. Maternal global methylation status and risk of congenital heart diseases. Obstet Gynecol 2008;112:277-283.
44 Obermann-Borst SA, van Driel LM, Helbing WA, de Jonge R, Wildhagen MF, Steegers EA, et al. Congenital heart defects and biomarkers of methylation in children: a case-control study. Eur J Clin Invest 2011;41:143-150.
45 He A, Ma Q, Cao J, von Gise A, Zhou P, Xie H, et al. Polycomb

repressive complex 2 regulates normal development of the mouse heart. Circ Res 2012;110:406-415.
46 Zaidi S, Choi M, Wakimoto H, Ma L, Jiang J, Overton JD, et al. De novo mutations in histone-modifying genes in congenital heart disease. Nature 2013;498:220-223.
47 Batista PJ, Molinie B, Wang J, Qu K, Zhang J, Li L, et al. m(6) A RNA modification controls cell fate transition in mammalian embryonic stem cells. Cell Stem Cell 2014;15:707-719.
48 Geula S, Moshitch-Moshkovitz S, Dominissini D, Mansour AA, Kol N, Salmon-Divon M, et al. Stem cells. m6A mRNA methylation facilitates resolution of naive pluripotency toward differentiation. Science 2015;347:1002-1006.
49 Alarcon CR, Lee H, Goodarzi H, Halberg N, Tavazoie SF. N6-methyladenosine marks primary microRNAs for processing. Nature 2015;519:482-485.
50 Chen T, Hao YJ, Zhang Y, Li MM, Wang M, Han W, et al. m(6) A RNA methylation is regulated by microRNAs and promotes reprogramming to pluripotency. Cell Stem Cell 2015;16:289-301.
51 Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, et al. Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 2007;39:724-726.
52 Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316:889-894.
53 Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Bruning JC, et al. Inactivation of the Fto gene protects from obesity. Nature 2009;458:894-898.
54 Church C, Moir L, McMurray F, Girard C, Banks GT, Teboul L, et al. Overexpression of Fto leads to increased food intake and results in obesity. Nat Genet 2010;42:1086-1092.
55 Boissel S, Reish O, Proulx K, Kawagoe-Takaki H, Sedgwick B, Yeo GS, et al. Loss-of-function mutation in the dioxygenase-encoding *FTO* gene causes severe growth retardation and multiple malformations. Am J Hum Genet 2009;85:106-111.
56 Hess ME, Hess S, Meyer KD, Verhagen LA, Koch L, Bronneke HS, et al. The fat mass and obesity associated gene (Fto) regulates activity of the dopaminergic midbrain circuitry. Nat Neurosci 2013;16:1042-1048.
57 Fustin JM, Doi M, Yamaguchi Y, Hida H, Nishimura S, Yoshida M, et al. RNA-methylation-dependent RNA processing controls the speed of the circadian clock. Cell 2013;155:793-806.
58 Maze I, Shen L, Zhang B, Garcia BA, Shao N, Mitchell A, et al. Analytical tools and current challenges in the modern era of neuroepigenomics. Nat Neurosci 2014;17:1476-1490.
59 Sweatt JD. The emerging field of neuroepigenetics. Neuron 2013;80:624-632.
60 Shin J, Ming GL, Song H. Seeking a roadmap toward neuroepigenetics. Neuron 2015;86:12-15.

Received August 11, 2015
Accepted after revision May 21, 2016