Genomics 104 (2014) 347–351

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno

From development to diseases: The role of 5hmC in brain

Wenjia Sun [a,1], Liqun Zang [a,1], Qiang Shu [b], Xuekun Li [a,b,c,*]

[a] Institute of Genetics, College of Life Sciences, Zhejiang University, China
[b] The Children's Hospital, School of Medicine, Zhejiang University, China
[c] Institute of Translational Medicine, School of Medicine, Zhejiang University, China

ARTICLE INFO

Article history:
Received 23 May 2014
Accepted 26 August 2014
Available online 7 September 2014

Keywords:
5-hydroxymethylcytosine
DNA demethylation
Brain
Development
Neurological disorders

ABSTRACT

Epigenetic modulations play essential roles in diverse biological processes. During the past several years, DNA demethylation has been discovered in embryonic and postnatal development. Although some potential functions of DNA methylation have been demonstrated already, many questions remain in terms of unveiling the role of 5hmC; whether it serves either merely as an intermediate of DNA demethylation or as a stable epigenetic marker. 5-hydroxymethylcytosine (5hmC) is proved to be not merely serving as an intermediate of DNA demethylation, but also acts as a stable epigenetic marker. This review summarizes the current knowledge of the function of 5hmC in brain with the focus on the neuronal activity, neurodevelopment, aging, and neurological diseases.

1. Introduction

DNA methylation at the fifth carbon of cytosine (5-methylcytosine, 5mC) is the best studied epigenetic modification, and plays pivotal roles in multiple biological processes including the regulation of chromatin structure, gene imprinting, X chromosome inactivation, and genomic stability, and this process is implicated in development, aging, and diseases through regulating tissue-specific gene expression [1]. The enzymes that establish and maintain the landscape of DNA methylation, namely the DNA methyltransferases (Dnmts), have three family members, Dnmt1 (preserving the methylation), Dnmt3A and 3B (de novo methyltransferase) and a regulatory subunit Dnmt3L [2]. DNA methylation displays a dynamic pattern during embryonic development and Dnmts absence has been shown to induce the lethal effects during these critical developmental periods [3,4]. In the neuronal system, the depletion of Dnmts not only impaired memory, but also led to inhibited neurogenesis in adult brain [5–7]. Methylated DNA can be recognized by a range of specific "readers", such as methyl-CpG binding protein 2 (MeCP2) and methyl-CpG-binding domain proteins 1–4 (Mbd1–4) [8,9]. The deletion of MeCP2 causes the neurodevelopmental disorder Rett syndrome, and Mbd1 absence induces an autism-like phenotype [10,11]. MeCP2 and Mbd1 also regulate adult neurogenesis through interacting with microRNA pathway [12,13]. Collectively, these results indicate the essential roles of DNA methylation in both development and function of brain.

Although DNA methylation has been regarded as a highly stable marker for the long time, the chasing of a candid DNA demethylase has never stopped. In 1953, a novel DNA modification form, 5-hydroxymethylcytosine (5hmC), was identified in the T-even bacteriophage [14]. Later on, 5hmC was further discovered in mammalian genomes [15]. However, the mechanisms and proteins responsible for generating this marker remained elusive until 2009. Rao's and Heintz's groups confirmed the existence of 5hmC in mammalian brain and uncovered the real player for DNA demethylation, the Ten-eleven translocation (Tet) gene; Tet1 [16,17]. They discovered that Tet1 is a 2-oxoglutarate (2OG)- and Fe (II)-dependent enzyme, and can catalyze the conversion of 5mC to 5hmC [16,17]. Later, other studies identified more Tet family members, Tet2 and Tet3, which share a high degree of homology within their C-terminal catalytic domain and all of which can catalyze the conversion of 5mC to 5hmC [18,19]. Furthermore, studies revealed that the Tet protein can further oxidize 5hmC to 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) [18,19], adding a completely new perspective on the uncovering complexity of the potential function of 5hmC mediated epigenetic regulation during development.

Genome-wide studies revealed that 5hmC could enrich in gene bodies, promoters, and distal regulatory regions [20–23]. The enrichment of 5hmC is positively correlated with transcript level, which might be achieved through the interaction with histone modification. Meanwhile, cell- and tissue-specific features of 5hmC were also revealed [17,20,24–27]. Upon ES cell differentiation, global 5hmC decreases; however, overall 5hmC increases during neuronal development. 5hmC is approximately 10-fold more abundant in neurons than other tissues or ESCs [17,20], suggesting that 5hmC might have a significant role in brain, as an important and stable epigenetic marker. Here, we reviewed

* Corresponding author at: Institute of Genetics, College of Life Sciences, Zhejiang University, 866 Yuhangtang Rd, Hangzhou, Zhejiang 310058, China.
E-mail address: xuekun_li@zju.edu.cn (X. Li).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.ygeno.2014.08.021
0888-7543/

the recent progress regarding 5hmC in brain focusing particularly on the function of 5hmC in neuronal activity, neurodevelopment, aging, and neurodevelopmental and neurodegenerative diseases. See also reviews by the Preifer, Marques and Tang labs in this Special Issue.

2. DNA demethylation in neuronal activity and memory

Neuronal activation could induce the alteration of the neuronal methylome including demethylation and de novo methylation, which consequentially modulated gene expression [28,29]. Activity-modified CpGs, usually low-density CpGs, are enriched in neuronal genes and preferentially associated with alternative splicing variants [28]. Active genes in neurons display the enrichment of 5hmC in gene bodies [30]. Even in different types of neuronal cells, such as Purkinje cells (PCs), granule cells (GCs) and the terminally differentiated Bergmann glial cells (BGs), 5hmC displays cell type-specific distribution in genome [30], indicating potential roles of 5hmC in the process of cell identity determination [20,27,30].

Two groups found that over-expression of Tet1 resulted in the demethylation of the promoter IX of Bdnf (brain-derived neurotrophic factor) (Bdnf IX), and a brain-specific promoter of Fgf1 (fibroblast growth factor 1) (Fgf1B), two loci exhibiting neuronal activity-induced active DNA demethylation in the adult dentate granule cells [31,32]. The inhibition of Tet1 could repress neuronal activity induced promoter demethylation of Bdnf IX and Fgf1B, and the expression of these two genes [31]. An earlier study found that Tet1 depletion led to impaired adult neurogenesis and poor learning and memory [33]. Later, another group found that although Tet1 KO mice display normal memory formation, these mice showed specific impairments in extinction learning and abnormally enhanced long-term depression [34]. The over-expression of both Tet1 and its catalytic inactivation form impaired long-term memory formation while no effect on short-term memory [32]. Of note, the expression of Tet1, but not Tet2 and Tet3, is also regulated by neuronal activity [32]. These results indicated that Tet-mediated 5hmC pathway is normally required for specific type memory formation and extinction. Meanwhile, these discoveries raise the possibility that Tet members play differential functions in brain, and their function could be catalytic activity independent.

3. DNA demethylation in neurodevelopment

The studies from the developing embryo and embryonic stem cells have indicated the dynamic features of 5hmC during development [35,36]. Hahn et al. found an acquirement of 5hmC during embryonic brain development [27]. Further, 5hmC also increased globally in different brain regions during postnatal neuronal development [20]. The manipulation of the Tet enzymes could lead to the alteration of 5hmC, which resulted in specific phenotypes [27]. During postnatal brain development, DNA methylation in non-CG contexts (CH) accumulates during brain development and becomes the dominant form of methylation in adult brain [37]. Non-CG methylation was usually depleted in expressed genes, which is opposite to that observed in embryonic stem cells [37]. Furthermore, the rapid accumulation of CH methylation in neurons during postnatal development is in parallel with synaptogenesis [37]. In Tet2 mutant mice, some regions become hypermethylated and CG methylation was significantly increased [37]. Considering all three Tet genes are expressed in brain, it is possible that individual Tet genes could have different functions in different regions of the brain and at different ages.

During neuronal development, 5hmC highly enriched in active genomic regions, and gene bodies of highly expressed genes while the lowly expressed genes showed intragenic depletion of 5hmC [37–40]. Meanwhile, developmentally down-regulated genes were accompanied by a loss of 5hmC enrichment. The enhancers of the regions becoming hypomethylated gained active histone modifications including H3K4me1 and H3K9ac; consistently, the regions acquiring methylation lost these active histone modifications [37] (Fig. 1). All these studies indicate intragenic 5hmC levels which might be associated with transcriptional activity.

Previous studies had noted that modified cytosine changed at the exon–intron boundary of genes with the intensity being higher on the exonic side, which did not distinguish 5hmC and 5mC due to technical limitations [41,42]. Khare et al. found 5hmC uniquely marked the exon-intron boundaries in mammalian brain but not in non-neural organs [40]. Exonic 5hmC in multi-exon genes was higher than that of intronless or single-exon genes; furthermore, 5hmC densities were lower in alternatively spliced exons relative to constitutive exons [40], indicating the potential function of 5hmC in splicing regulation.

4. DNA demethylation in aging

The brain aging process is characterized by increased DNA damage, distinct structural alternations, synaptic dysfunction and cognitive decline, with some regions including neocortical and hippocampal networks showing age-related pathology. Region-specific alterations in gene expression, occurring in the aging brain, including the down regulation of genes related to synaptic plasticity and neurotrophic support, are likely to be susceptible to epigenetic modulation.

The alteration of DNA methylation has been indicated during brain aging process, which involves in the decline of cognitive function [43]. Our previous study found that 5hmC globally increased during aging process (6-week to 1-year) in cerebellum regions, but displayed slight decrease in the hippocampus [20]. 5hmC also exhibited dynamic features during this process, suggesting that 5hmC modification at specific loci could play roles in the aging process [20]. A further two studies found that global 5hmC also increased in the more aged hippocampus such as at two years of age [44,45]. More detailed studies should be performed to uncover 5hmC changes at later stages of aging.

Oxidative stress is one of the essential factors contributing to brain aging, and mitochondria are the primary source of free radicals and neurodegeneration. One isoform of Dnmt1, mtDnmt1, is the only member of Dnmt family which exists in mitochondria and is essential for mtDNA methylation. The expression of mtDnmt1 is regulated by factors that respond to oxidative stress stimuli [46]. Besides 5mC, 5hmC was also found in mtDNA, suggesting that 5hmC could modify mitochondria genome and regulate its function [46]. These discoveries link 5-hmC to oxidative stress, and point out to a potential function in aging. Further studies need to be carried out to unravel the details how 5hmC in mitochondria is converted.

5. DNA demethylation in neurodevelopment disorders

The dynamic and unique features of 5hmC in differential tissues and at different ages prompt the investigation of its potential roles in diseases. Several studies strongly indicate the dysregulation of 5hmC could be involved in multiple diseases including cancer and neurological disorders including neurodevelopmental disorders such as Rett syndrome and Autism, and neurodegenerative diseases including Huntington's disease and Alzheimer's disease (Table 1).

MeCP2, the gene commonly dysregulated in Rett syndrome, binds to methylated DNA and represses gene expression. Surprisingly, it was found that MeCP2 also served as a reader for 5hmC in brain [47]. The overlapping of readers for 5hmC and 5mC makes the story complicated but more intriguing. Our previous study revealed a reverse correlation between Rett syndrome gene MeCP2 and 5hmC level, suggesting that MeCP2 bind to 5mC blocking the conversion of 5mC to 5hmC [20]. We identified stable and dynamic differential hydroxymethylated regions (DhMRs) between adult and aged stages. Interestingly, MeCP2 absence specifically decreased 5hmC signal of those dynamic DhMRs without effecting on stable DhMRs [20]. Another detailed study unexpectedly revealed that MeCP2 could bind to 5hmC modified loci in the neuronal genome with similar affinity as 5mC [30]. One mutated form of MeCP2,

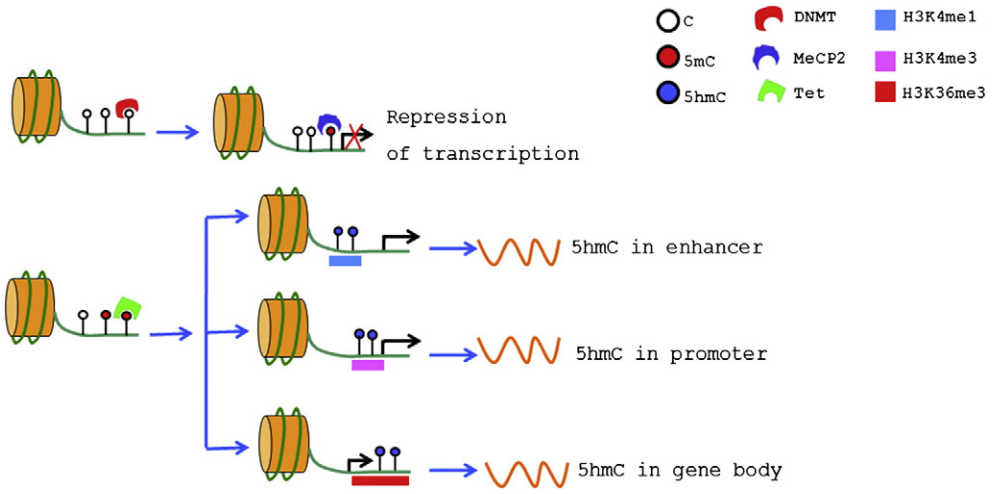

Fig. 1. The schematic illustration of the relationship of DNA methylation and demethylation and gene expression. A. DNA methylation in promoter region leads to the repression of gene expression. B. Tet gene members convert 5mC to 5hmC in distinct genomic regions, which potentially involves in the regulation of gene expression.

R133C, which occurs in Rett syndrome preferentially disrupts MeCP2 binding to 5hmC [30]. These results indicate that 5hmC-mediated DNA demethylation pathway could be involved in the pathogenesis of Rett syndrome.

Autism is a developmental neurological disorder, and is characterized by deficits in social interactions, communication skills, and unusual repetitive behaviors. The etiology of autism is very complicated and the exact mechanism remains unclear. Past studies have revealed that epigenetic factors such as DNA methylation could contribute to autism. Fragile X syndrome is the most common monogenic causes of autism spectrum disorders. 5hmC highly correlated with neurodevelopment genes and identified DhMRs in fetus and adult human cerebellum showed a significant overlapping with genes, including some autism candidate genes such as Shank3, Nlgn3 and Tsc2 [39]. Genome-wide profiling of 5hmC also reveals that 5hmC is highly enriched in synapse-related, fragile X mental retardation protein (Fmrp) targeted, and autism-related genes [39,40], suggesting a potential role of 5hmC in autism.

Specifically, the increased 5hmC and decreased 5mC were detected in the promoters of autism spectrum disorders (ASD) candidate genes including glutamic acid decarboxylase-67 (Gad1), -65 (Gad2) and Reelin (Reln) in the cerebella of ASD patients [48]. It is of interesting to note that in ASD cases Tet1 transcript level and its binding to Gad1 and Reln promoters both increased [48]. Consistently, 5hmC enriched at the promoters of Gad1 and Reln. Meanwhile, MeCP2 also displayed higher binding to the promoters of Gad1 and Reln in ASD cerebella tissues [48]. Therefore, these results supported a hypothesis that the enrichment of 5hmC/5mC ratios at some specific gene domains such as promoters, by Tet1, could enhance the binding of MeCP2 to distinct genomic regions, and then inhibited the expression of target genes in ASD cerebella.

Table 1
The differential alteration of 5hmC in neurological disorders.

Diseases	The alteration of 5hmC	Reference
Autism (Autism spectrum disorders)	Enrichment on autism related genes.	[39,40,48]
Rett syndrome	Global decrease in the genome.	[20]
Angelman syndrome	Global increase in the genome.	[20]
Fragile X syndrome (FXS)	Enriching in FXS related genes.	[39]
Alzheimer's disease (AD)	Decrease or increase in the genome.	[50–54]
Huntington's disease (HD)	Global decrease.	[49]

6. DNA demethylation in neurodegenerative diseases

Huntington disease (HD) is a serious neurodegenerative disorder, found to be an autosomal dominant genetic disease, characterized by chorea, cognitive decline and psychiatric abnormalities. It is caused by the abnormal expression of CAG repeat, which has 36 or more repeats in HTT (Huntington protein) of HD patients. In transgenic HD mice, the global level of 5hmC significantly decreased in both striatum and cortex relative to age-matched wild-type mice [49]. High-throughput sequencing data showed that 5hmC was enriched in gene bodies and depleted in intergenic regions [49]. Although the overall distribution patterns were not affected, 747 DhMRs (49 up- and 698 down-regulated) correlating to 30 and 406 genes, and 362 DhMRs (38 up- and 324 down-regulated) corresponding to 28 and 171 genes, were identified in transgenic striatum and cortex, respectively [49]. These DhMR-associated genes displayed a good correlation with glutamate receptor/calcium signaling/dopamine signaling/neuronal Creb signaling pathways, which have been previously implicated in HD pathogenesis. These results suggested that abnormal 5hmC could be involved in the pathogenesis and progression of HD through dysregulating gene expression. Adora2a (Adenosine A2A receptor, A2AR) is a G-protein coupled receptor, and its expression is severely reduced HD patients and mice. The Adora2a protein level was abnormally reduced in putamen and caudate nucleus from an early stage of the HD patient's brain. Interestingly, the 5' UTR of Adora2a displayed an increased 5mC level but decreased 5hmC [50]. Together these studies highlight a role of 5hmC in HD.

Alzheimer's disease (AD) is the most common neurodegenerative disease which is characterized by a progressive decline in cognitive functions and the loss of neuronal cells, and the presence of neurofibrillary tangles (NFTs) and amyloid beta (Aβ) plaques in the cortex. The existence of 5hmC, 5fC and 5caC was revealed in brain, and a significant decrease of 5hmC levels was observed in all three hippocampal subregions, entorhinal cortex and cerebellum of AD cases [51,52]. Moreover, a negative relationship between 5hmC level and amyloid plaque loading was also identified in the hippocampus [51]. However, other studies found that in the hippocampus/parahippocampal gyrus of preclinical AD and late-stage AD, 5hmC levels showed significantly increase in the middle frontal gyrus and the middle temporal gyrus of AD patients [53,54]. Interestingly, the levels of 5fC and 5caC decreased [54]. The discrepancy between studies could be due to multiple factors. One possibility is brain sampling, and another possibility could be the stage of AD. Furthermore, all these studies only used immunostaining method and

studied global level of 5hmC. Other technologies such as liquid chromatography–mass spectrometry and next-generation sequencing are required to clarify the change of 5hmC in AD and its relationship with Aβ deposition.

7. Conclusion and perspective

Past studies have indicated that 5hmC does not merely serve as a DNA demethylation intermediate but also functions as a stable epigenetic mark. 5hmC enriches in gene bodies, promoters, and transcription factor binding sites and mounting evidence suggest roles of 5hmC in regulating gene expression and controlling cell identity. The dysregulation of 5hmC levels may lead to neurological diseases including neurodevelopment and neurodegenerative diseases. These discoveries collectively provide new insights in understanding the function of this epigenetic modification including DNA demethylation in brain. Despite these progresses, our understanding about the function of 5hmC in brain is very limited. Future studies need to address several important questions regarding the function of 5hmC (in brain). The current evidences mainly indicate a correlative relationship between 5hmC enrichment and gene expression. In order to allow better understanding the function of 5hmC, it is necessary to provide more direct proof showing the function of 5hmC in gene expression. Pertinent questions remain regarding the exact functions of 5hmC in the brain, how the DNA demethylation pathway interacts with particular signaling pathways, and indeed which pathways these may be in order to further investigate the potential role of this epigenetic modification in disease pathogenesis in the brain. As a long-term goal, could we target specific 5hmC modified loci to treat neurological disorders and then identify novel therapeutic strategy? Collectively these and future studies will advance our understanding about the function of 5hmC.

Acknowledgments

Xuekun Li is supported by National Natural Science Foundation of China (31371309), the grant from National Key Basic Research Program of China (No. 2014CB943001), and the Fundamental Research Funds for the Central Universities (2014QNA6002).

References

[1] E. Li, Chromatin modification and epigenetic reprogramming in mammalian development, Nat. Rev. Genet. 3 (2002) 662–673.
[2] M.M. Suzuki, A. Bird, DNA methylation landscapes: provocative insights from epigenomics, Nat. Rev. Genet. 9 (2008) 465–476.
[3] E. Li, T.H. Bestor, R. Jaenisch, Targeted mutation of the DNA methyltransferase gene results in embryonic lethality, Cell 69 (1992) 915–926.
[4] W. Reik, Stability and flexibility of epigenetic gene regulation in mammalian development, Nature 447 (2007) 425–432.
[5] H. Wu, V. Coskun, J. Tao, W. Xie, W. Ge, K. Yoshikawa, E. Li, Y. Zhang, Y.E. Sun, Dnmt3a-dependent nonpromoter DNA methylation facilitates transcription of neurogenic genes, Science 329 (2010) 444–448.
[6] Z. Wu, K. Huang, J. Yu, T. Le, M. Namihira, Y. Liu, J. Zhang, Z. Xue, L. Cheng, G. Fan, Dnmt3a regulates both proliferation and differentiation of mouse neural stem cells, J. Neurosci. Res. 90 (2012) 1883–1891.
[7] C.A. Miller, J.D. Sweatt, Covalent modification of DNA regulates memory formation, Neuron 53 (2007) 857–869.
[8] B. Hendrich, A. Bird, Identification and characterization of a family of mammalian methyl-CpG binding proteins, Mol. Cell. Biol. 18 (1998) 6538–6547.
[9] O. Bogdanovic, G.J. Veenstra, DNA methylation and methyl-CpG binding proteins: developmental requirements and function, Chromosoma 118 (2009) 549–565.
[10] R.E. Amir, I.B. Van den Veyver, M. Wan, C.Q. Tran, U. Francke, H.Y. Zoghbi, Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2, Nat. Genet. 23 (1999) 185–188.
[11] A.M. Allan, X. Liang, Y. Luo, C. Pak, X. Li, K.E. Szulwach, D. Chen, P. Jin, X. Zhao, The loss of methyl-CpG binding protein 1 leads to autism-like behavioral deficits, Hum. Mol. Genet. 17 (2008) 2047–2057.
[12] C. Liu, Z.Q. Teng, N.J. Santistevan, K.E. Szulwach, W. Guo, P. Jin, X. Zhao, Epigenetic regulation of miR-184 by MBD1 governs neural stem cell proliferation and differentiation, Cell Stem Cell 6 (2010) 433–444.
[13] K.E. Szulwach, X. Li, R.D. Smrt, Y. Li, Y. Luo, L. Lin, N.J. Santistevan, W. Li, X. Zhao, P. Jin, Cross talk between microRNA and epigenetic regulation in adult neurogenesis, J. Cell Biol. 189 (2010) 127–141.
[14] G.R. Wyatt, S.S. Cohen, The bases of the nucleic acids of some bacterial and animal viruses: the occurrence of 5-hydroxymethylcytosine, Biochem. J. 55 (1953) 774–782.
[15] N.W. Penn, R. Suwalski, C. O'Riley, K. Bojanowski, R. Yura, The presence of 5-hydroxymethylcytosine in animal deoxyribonucleic acid, Biochem. J. 126 (1972) 781–790.
[16] M. Tahiliani, K.P. Koh, Y. Shen, W.A. Pastor, H. Bandukwala, Y. Brudno, S. Agarwal, L.M. Iyer, D.R. Liu, L. Aravind, A. Rao, Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1, Science 324 (2009) 930–935.
[17] S. Kriaucionis, N. Heintz, The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain, Science 324 (2009) 929–930.
[18] Y.F. He, B.Z. Li, Z. Li, P. Liu, Y. Wang, Q. Tang, J. Ding, Y. Jia, Z. Chen, L. Li, et al., Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA, Science 333 (2011) 1303–1307.
[19] S. Ito, L. Shen, Q. Dai, S.C. Wu, L.B. Collins, J.A. Swenberg, C. He, Y. Zhang, Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine, Science 333 (2011) 1300–1303.
[20] K.E. Szulwach, X. Li, Y. Li, C.X. Song, H. Wu, Q. Dai, H. Irier, A.K. Upadhyay, M. Gearing, A.I. Levey, et al., 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging, Nat. Neurosci. 14 (2011) 1607–1616.
[21] C.X. Song, K.E. Szulwach, Q. Dai, Y. Fu, S.Q. Mao, L. Lin, C. Street, Y. Li, M. Poidevin, H. Wu, et al., Genome-wide profiling of 5-formylcytosine reveals its roles in epigenetic priming, Cell 153 (2013) 678–691.
[22] M. Yu, G.C. Hon, K.E. Szulwach, C.X. Song, L. Zhang, A. Kim, X. Li, Q. Dai, Y. Shen, B. Park, et al., Base-resolution analysis of 5-hydroxymethylcytosine in the mammalian genome, Cell 149 (2012) 1368–1380.
[23] L. Shen, H. Wu, D. Diep, S. Yamaguchi, A.C. D'Alessio, H.L. Fung, K. Zhang, Y. Zhang, Genome-wide analysis reveals TET- and TDG-dependent 5-methylcytosine oxidation dynamics, Cell 153 (2013) 692–706.
[24] C.E. Nestor, R. Ottaviano, J. Reddington, D. Sproul, D. Reinhardt, D. Dunican, E. Katz, J.M. Dixon, D.J. Harrison, R.R. Meehan, Tissue type is a major modifier of the 5-hydroxymethylcytosine content of human genes, Genome Res. 22 (2012) 467–477.
[25] S. Ito, A.C. D'Alessio, O.V. Taranova, K. Hong, L.C. Sowers, Y. Zhang, Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification, Nature 466 (2010) 1129–1133.
[26] G. Ficz, M.R. Branco, S. Seisenberger, F. Santos, F. Krueger, T.A. Hore, C.J. Marques, S. Andrews, W. Reik, Dynamic regulation of 5-hydroxymethylcytosine in mouse ES cells and during differentiation, Nature 473 (2011) 398–402.
[27] M.A. Hahn, R. Qiu, X. Wu, A.X. Li, H. Zhang, J. Wang, J. Jui, S.G. Jin, Y. Jiang, G.P. Pfeifer, Q. Lu, Dynamics of 5-hydroxymethylcytosine and chromatin marks in mammalian neurogenesis, Cell Rep. 3 (2013) 291–300.
[28] J.U. Guo, D.K. Ma, H. Mo, M.P. Ball, M.H. Jang, M.A. Bonaguidi, J.A. Balazer, H.L. Eaves, B. Xie, E. Ford, et al., Neuronal activity modifies the DNA methylation landscape in the adult brain, Nat. Neurosci. 14 (2011) 1345–1351.
[29] D.K. Ma, M.H. Jang, J.U. Guo, Y. Kitabatake, M.L. Chang, N. Pow-Anpongkul, R.A. Flavell, B. Lu, G.L. Ming, H. Song, Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis, Science 323 (2009) 1074–1077.
[30] M. Mellen, P. Ayata, S. Dewell, S. Kriaucionis, N. Heintz, MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system, Cell 151 (2012) 1417–1430.
[31] J.U. Guo, Y. Su, C. Zhong, G.L. Ming, H. Song, Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain, Cell 145 (2011) 423–434.
[32] G.A. Kaas, C. Zhong, D.E. Eason, D.L. Ross, R.V. Vachhani, G.L. Ming, J.R. King, H. Song, J.D. Sweatt, TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation, Neuron 79 (2013) 1086–1093.
[33] R.R. Zhang, Q.Y. Cui, K. Murai, Y.C. Lim, Z.D. Smith, S. Jin, P. Ye, L. Rosa, Y.K. Lee, H.P. Wu, et al., Tet1 regulates adult hippocampal neurogenesis and cognition, Cell Stem Cell 13 (2013) 237–245.
[34] A. Rudenko, M.M. Dawlaty, J. Seo, A.W. Cheng, J. Meng, T. Le, K.F. Faull, R. Jaenisch, L.H. Tsai, Tet1 is critical for neuronal activity-regulated gene expression and memory extinction, Neuron 79 (2013) 1109–1122.
[35] W. Sun, M. Guan, X. Li, 5-hydroxymethylcytosine-mediated DNA demethylation in stem cells and development, Stem Cells Dev. 23 (2014) 923–930.
[36] B. Yao, P. Jin, Cytosine modifications in neurodevelopment and diseases, Cell. Mol. Life Sci. 71 (2014) 405–418.
[37] R. Lister, E.A. Mukamel, J.R. Nery, M. Urich, C.A. Puddifoot, N.D. Johnson, J. Lucero, Y. Huang, A.J. Dwork, M.D. Schultz, et al., Global epigenomic reconfiguration during mammalian brain development, Science 341 (2013) 1237905.
[38] K.E. Szulwach, X. Li, Y. Li, C.X. Song, J.W. Han, S. Kim, S. Namburi, K. Hermetz, J.J. Kim, M.K. Rudd, et al., Integrating 5-hydroxymethylcytosine into the epigenomic landscape of human embryonic stem cells, PLoS Genet. 7 (2011) e1002154.
[39] T. Wang, Q. Pan, L. Lin, K.E. Szulwach, C.X. Song, C. He, H. Wu, S.T. Warren, P. Jin, R. Duan, X. Li, Genome-wide DNA hydroxymethylation changes are associated with neurodevelopmental genes in the developing human cerebellum, Hum. Mol. Genet. 21 (2012) 5500–5510.
[40] T. Khare, S. Pai, K. Koncevicius, M. Pal, E. Kriukiene, Z. Liutkeviciute, M. Irimia, P. Jia, C. Ptak, M. Xia, et al., 5-hmC in the brain is abundant in synaptic genes and shows differences at the exon-intron boundary, Nat. Struct. Mol. Biol. 19 (2012) 1037–1043.
[41] R.K. Chodavarapu, S. Feng, Y.V. Bernatavichute, P.Y. Chen, H. Stroud, Y. Yu, J.A. Hetzel, F. Kuo, J. Kim, S.J. Cokus, et al., Relationship between nucleosome positioning and DNA methylation, Nature 466 (2010) 388–392.
[42] L. Laurent, E. Wong, G. Li, T. Huynh, A. Tsirigos, C.T. Ong, H.M. Low, K.W. Kin Sung, I. Rigoutsos, J. Loring, C.L. Wei, Dynamic changes in the human methylome during differentiation, Genome Res. 20 (2010) 320–331.

[43] J.J. Day, J.D. Sweatt, Epigenetic mechanisms in cognition, Neuron 70 (2011) 813–829.
[44] L. Chouliaras, D.L. van den Hove, G. Kenis, S. Keitel, P.R. Hof, J. van Os, H.W. Steinbusch, C. Schmitz, B.P. Rutten, Age-related increase in levels of 5-hydroxymethylcytosine in mouse hippocampus is prevented by caloric restriction, Curr. Alzheimer Res. 9 (2012) 536–544.
[45] H. Chen, S. Dzitoyeva, H. Manev, Effect of aging on 5-hydroxymethylcytosine in the mouse hippocampus, Restor. Neurol. Neurosci. 30 (2012) 237–245.
[46] L.S. Shock, P.V. Thakkar, E.J. Peterson, R.G. Moran, S.M. Taylor, DNA methyltransferase 1, cytosine methylation, and cytosine hydroxymethylation in mammalian mitochondria, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 3630–3635.
[47] C.G. Spruijt, F. Gnerlich, A.H. Smits, T. Pfaffeneder, P.W. Jansen, C. Bauer, M. Munzel, M. Wagner, M. Muller, F. Khan, et al., Dynamic readers for 5-(hydroxy)methylcytosine and its oxidized derivatives, Cell 152 (2013) 1146–1159.
[48] A. Zhubi, Y. Chen, E. Dong, E.H. Cook, A. Guidotti, D.R. Grayson, 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 4 (2014) e349.
[49] F. Wang, Y. Yang, X. Lin, J.Q. Wang, Y.S. Wu, W. Xie, D. Wang, S. Zhu, Y.Q. Liao, Q. Sun, et al., Genome-wide loss of 5-hmC is a novel epigenetic feature of Huntington's disease, Hum. Mol. Genet. 22 (2013) 3641–3653.
[50] I. Villar-Menendez, M. Blanch, S. Tyebji, T. Pereira-Veiga, J.L. Albasanz, M. Martin, I. Ferrer, E. Perez-Navarro, M. Barrachina, Increased 5-methylcytosine and decreased 5-hydroxymethylcytosine levels are associated with reduced striatal A2AR levels in Huntington's disease, Neuromolecular Med. 15 (2013) 295–309.
[51] L. Chouliaras, D. Mastroeni, E. Delvaux, A. Grover, G. Kenis, P.R. Hof, H.W. Steinbusch, P.D. Coleman, B.P. Rutten, D.L. van den Hove, Consistent decrease in global DNA methylation and hydroxymethylation in the hippocampus of Alzheimer's disease patients, Neurobiol. Aging 34 (2013) 2091–2099.
[52] D. Condliffe, A. Wong, C. Troakes, P. Proitsi, Y. Patel, L. Chouliaras, C. Fernandes, J. Cooper, S. Lovestone, L. Schalkwyk, et al., Cross-region reduction in 5-hydroxymethylcytosine in Alzheimer's disease brain, Neurobiol. Aging 35 (2014) 1850–1854.
[53] N. Coppieters, B.V. Dieriks, C. Lill, R.L. Faull, M.A. Curtis, M. Dragunow, Global changes in DNA methylation and hydroxymethylation in Alzheimer's disease human brain, Neurobiol. Aging 35 (2014) 1334–1344.
[54] M.A. Bradley-Whitman, M.A. Lovell, Epigenetic changes in the progression of Alzheimer's disease, Mech. Ageing Dev. 134 (2013) 486–495.