Chinese Medical Journal®

Histone crotonylation in neurobiology: to be or not to be?

Cechuan Deng[1,2], Jia-Hua Qu[3], InKyeom Kim[4,5,6], Xiaoqiang Tang[1]

[1]Key Laboratory of Birth Defects and Related Diseases of Women and Children of MOE, State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[2]Department of Medical Genetics, Prenatal Diagnostic Center, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, China;
[3]Laboratory of Cardiovascular Science, Intramural Research Program, National Institute on Aging, National Institutes of Health, Baltimore, Maryland 21224, USA;
[4]Department of Pharmacology, School of Medicine, Kyungpook National University, Daegu, Republic of Korea;
[5]Cardiovascular Research Institute, School of Medicine, Kyungpook National University, Daegu, Republic of Korea;
[6]Department of Biomedical Science, BK21 Plus KNU Biomedical Convergence Program, School of Medicine, Kyungpook National University, Daegu, Republic of Korea.

Epigenetic regulation is a pivotal mechanism that controls gene transcription and cell fate. During the past decades, it has been observed that histone, DNA, and RNA modifications participate in determining the fate of neural stem cells (NSCs). These modifications include histone acetylation and methylation, as well as DNA and RNA methylation. Of note, non-coding RNAs also participate in neural differentiation.[1] In addition to acetylation, many other types of acylations to histone lysines, including crotonylation, propionylation, succinylation, and malonylation, have been identified.[2] The roles of these histone acylations in neuroscience remain elusive.

A decade ago, Zhao's lab characterized >60 types of histone modifications and identified histone crotonylation as a hallmark of active transcription.[2] Crotonylation is a type of short-chain lysine acylation that is reversely regulated by acetyltransferases and deacetylases. P300 and GCN5 are the typical writers of histone crotonylation, whereas class I histone deacetylases and Sirtuins 1–3 act as erasers. Short-chain enoyl-CoA hydratase (ECHS1) and chromodomain-Y-like (CDYL) protein act as crotonyl-CoA hydratases to "control the intracellular concentration of crotonyl-CoA and the extent of histone crotonylation".[2] Subsequent studies identified some key histone lysine crotonylation (Kcr) sites involved in transcriptional regulation, such as H3K18cr, H2BK12cr, H3K9cr, and H3K27cr.[2,3] Interestingly, the histone lysine crotonylation and acetylation in chromatins have temporal and spatial differences,[2] revealing the distinct roles of these modifications, despite that they share many writers, readers, and erasers.

Recent studies from our lab and those of others have uncovered the critical roles of histone crotonylation in cardiac dysfunction, spermatogenesis, tumor biology, infection, and embryonic development.[2,3] For instance, we identified the contribution of histone crotonylation (H3K18cr and H2BK12cr) in cardiac hypertrophy in humans and rodents.[3] Furthermore, another study identified a histone crotonylation-mediated mechanism promoting endodermal commitment by pluripotent stem cells in humans and mice.[2] Such studies indicate the potential roles of histone crotonylation in development and neurobiology. However, the genome-wide distribution, dynamic changes, and gene expression associations of histone crotonylation during developmental processes, especially in the development of the central nervous system, are largely unknown. This study searched PubMed and Google Scholar with the keywords "crotonylation," "crotonate," "neuron," and "brain" and found several relevant publications on the role of crotonylation in neurobiology.

Notably, Liu's lab at the Institute of Zoology (Chinese Academy of Sciences) performed genome-wide multiple omics analyses and identified the critical role of histone crotonylation in regulating NSC biology.[4,5] The researchers applied multi-omics profiling (bulk RNA-seq, chromatin immunoprecipitation followed by sequencing [ChIP-seq], and assay for transposase-accessible chromatin with high-throughput sequencing [ATAC-seq]) to analyze H3K9cr in the embryonic forebrain, and their bioinformatics analysis revealed that H3K9cr-targeted

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000001945

Correspondence to: Xiaoqiang Tang, Key Laboratory of Birth Defects and Related Diseases of Women and Children of MOE, State Key Laboratory of Biotherapy, West China Second University Hospital, Sichuan University, Chengdu, Sichuan 610041, China
E-Mail: tangxiaoqiang@scu.edu.cn; txiaoqiang@yeah.net

Copyright © 2022 The Chinese Medical Association, produced by Wolters Kluwer, Inc. under the CC-BY-NC-ND license. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Chinese Medical Journal 2022;135(9)

Received: 18-08-2021; **Online:** 20-01-2022 **Edited by:** Ningning Wang and Peifang Wei

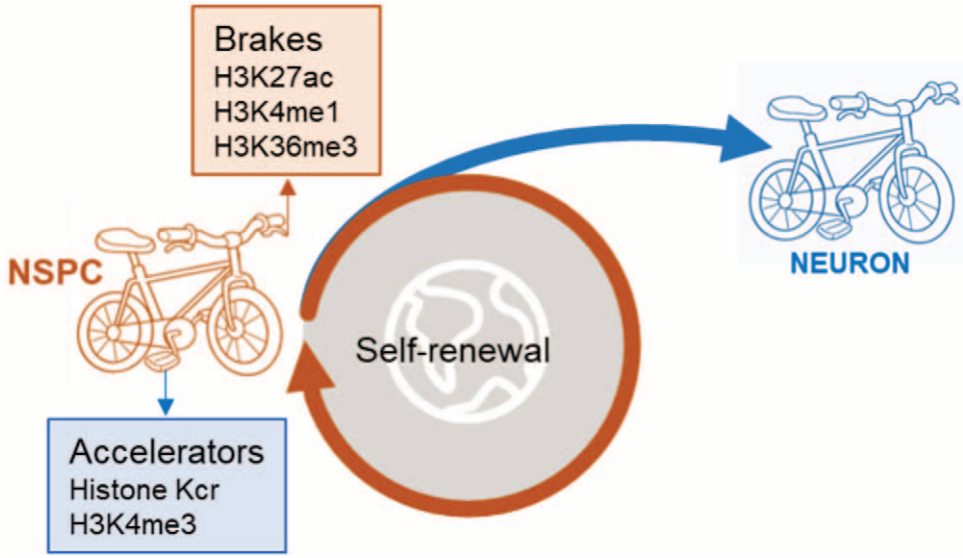

Figure 1: Historie modifications control NSC fate. Under normal conditions, histone hypo crotonylatIon, H3K27ac, H3K4me1, and H3K36me3 promote the expression of genes related to metabolism and proliferation and serve as brakes to slow neural differentiation and to maintain the stemness of NSPCs. By contrast, histone hypercrotonylation, H3K9ac, and H3K4me3 facilitate chromatin openness and act as accelerators to promote neuronal differentiation of NSPCs by activating bivalent promoters. NSC: Neural stem cell; NSPCs: Neural stem/progenitor cells.

genes are associated with stemness maintenance and neural differentiation. The authors demonstrated that histone crotonylation regulates the expression of genes involved in the metabolism and proliferation of neural progenitor/stem cells (NPSCs). Significantly, enrichment of histone crotonylation activates bivalent promoters to drive gene expression in NSPCs by facilitating chromatin openness and recruiting RNA polymerase II, which reprograms the transcriptome and promotes neuronal differentiation. In a follow-up study by the same group, the authors described the dynamic profiling and functional interpretation of histone lysine crotonylation and lactylation during neural development.[5] These novel findings sketched epigenetic maps for histone acetylation, methylation, crotonylation, lactylation, and DNA methylation, modifications that regulate NPSC differentiation. These studies expanded our understanding of the epigenetic mechanisms underlying NPSC fate decision and the clinical implications of such mechanisms [Figure 1].

However, some open questions remain unanswered. For example, the authors demonstrated, *via* ChIP-seq with pan-crotonylation antibodies, that pan-crotonylation of histone-regulated genes is involved in the maintenance of neural stemness. Although the authors showed the enrichment of H3K9cr in the promoter regions of target genes, further studies are required to explore which histone lysine crotonylation sites play pivotal roles in determining NPSC fate *in vitro* and *in vivo*. Further studies are also needed to explore the mechanisms underlying histone crotonylation-mediated functions in neurobiology, although the involvement of the histone Kcr-miR-203-Bmi1 regulatory axis has been suggested. In other words, it remains unknown which genes are regulated by which Kcr sites and how they are regulated (by promoters, enhancers, or other regulatory elements) to reshape the transcriptome and to determine NPSC fate. Researchers have applied multi-omics to investigate chromatin modification and openness and identified the participation of histone acetylation (H3K9ac and H3K27ac), methylation (H3K4me1/2/3, H3K9me3, H3K27me3, and H3K36me3), lactylation (H3K18la), and DNA methylation in NPSC maintenance and differentiation. It is interesting, although not easy, to integrate these multi-omics data with single-cell RNA sequencing data to identify the potential principles governing the interactions of these epigenetic modifications in modulating the stemness of NPSCs.

Notably, the clinical implications of histone crotonylation in brain development and diseases are yet to be addressed. The contributions of histone crotonylation in neural development *in vivo* and its involvement in neuropathy remain inconclusive, but some experimental and genetic findings may provide clues. For instance, elevated Kcr levels were observed in the brains of BTBR T+Itpr3tf/J mice that have developmental disorders in the central nervous system and many aberrant neuroanatomical structures.[6] In addition, ECHS1, a regulator of histone crotonylation,[3] is critical for human brain development.[7] Mutations in *ECHS1* cause developmental defects, such as Leigh syndrome, a devastating neurodegenerative disease, in children.[7] Germline knockout of *ECHS1* in mice leads to embryonic death,[3] which may be related to developmental defects in the neuronal system. These findings imply that dysregulation of histone crotonylation may lead to developmental defects in the neuronal system and may result in neuropathy. CDYL is another enzyme that controls histone crotonylation and methylation. In mice, CDYL suppresses epileptogenesis by repressing axonal Nav1.6 sodium channel expression,[8] whereas CDYL deficiency disrupts neuronal migration and increases susceptibility to epilepsy.[9] In addition, a genome-wide association study explored the genetic basis for responsiveness to ketogenic dietary therapies for drug-resistant epilepsy in humans, and the results revealed an association locus at 6p25.1, 61 kb upstream of *CDYL*

(rs12204701; $P = 3.83 \times 10^{-8}$).[10] However, further exploration with a larger replication cohort is warranted to clarify whether CDYL and dysregulated histone crotonylation are the causal factors underlying human epilepsy and the associated drug resistance. Additionally, CDYL-mediated histone crotonylation also plays a critical role in regulating stress-induced depression,[11] although the underlying mechanism and clinical implications remain unknown. Finally, crotonate is a metabolite of the gut microbe and contributes to histone crotonylation[12]; it would be interesting to investigate the relevance of crotonate and histone crotonylation to the gut-brain axis and gut-mediated brain health and diseases.

In conclusion, recent studies on the role of histone crotonylation in neurobiology will certainly stimulate future research and open new avenues for therapeutic advancement in neurobiology. It is important to investigate the complex roles of histone crotonylation in neural development and diseases and to explore crotonylation-targeted strategies for treating human neural diseases.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (Nos. 81970426 and 81800273), the Young Elite Scientists Sponsorship Program of China Association for Science and Technology (No.2018QNRC001), and the Scientific and Technological Innovation Talents Program of Sichuan Province (No. 2020JDRC0017).

Conflicts of interest

None.

References

1. Yao B, Christian KM, He C, Jin P, Ming GL, Song H. Epigenetic mechanisms in neurogenesis. Nat Rev Neurosci 2016;17:537–549. doi: 10.1038/nrn.2016.70.
2. Ntorla A, Burgoyne JR. The regulation and function of histone crotonylation. Front Cell Dev Biol 2021;9:624914. doi: 10.3389/fcell.2021.624914.
3. Tang X, Chen XF, Sun X, Xu P, Zhao X, Tong Y, *et al*. Short-chain enoyl-CoA hydratase mediates histone crotonylation and contributes to cardiac homeostasis. Circulation 2021;143:1066–1069. doi: 10.1161/CIRCULATIONAHA.120.049438.
4. Dai SK, Liu PP, Du HZ, Liu X, Xu YJ, Liu C, *et al*. Histone crotonylation regulates neural stem cell fate decisions by activating bivalent promoters. EMBO Rep 2021;22:e52023. doi: 10.15252/embr.202052023.
5. Dai SK, Liu PP, Teng ZQ, Liu CM. Dynamic profiling and functional interpretation of histone lysine crotonylation and lactylation during neural development. bioRxiv 2021. doi: 10.1101/2021.05.21.444394.
6. Wang M, Chang Q, Yang H, Liu Y, Wang C, Hu F, *et al*. Elevated lysine crotonylation and succinylation in the brains of BTBR mice. Int J Dev Neurosci 2019;76:61–64. doi: 10.1016/j.ijdevneu.2019.06.011.
7. Sun D, Liu Z, Liu Y, Wu M, Fang F, Deng X, *et al*. Novel ECHS1 mutations in Leigh syndrome identified by whole-exome sequencing in five Chinese families: case report. BMC Med Genet 2020;21:149. doi: 10.1186/s12881-020-01083-1.
8. Liu Y, Lai S, Ma W, Ke W, Zhang C, Liu S, *et al*. CDYL suppresses epileptogenesis in mice through repression of axonal Nav1.6 sodium channel expression. Nat Commun 2017;8:355. doi: 10.1038/s41467-017-00368-z.
9. Qin R, Cao S, Lyu T, Qi C, Zhang W, Wang Y. CDYL deficiency disrupts neuronal migration and increases susceptibility to epilepsy. Cell Rep 2017;18:380–390. doi: 10.1016/j.celrep.2016.12.043.
10. Schoeler NE, Leu C, Balestrini S, Mudge JM, Steward CA, Frankish A, *et al*. Genome-wide association study: exploring the genetic basis for responsiveness to ketogenic dietary therapies for drug-resistant epilepsy. Epilepsia 2018;59:1557–1566. doi: 10.1111/epi.14516.
11. Liu Y, Li M, Fan M, Song Y, Yu H, Zhi X, *et al*. Chromodomain Y-like protein-mediated histone crotonylation regulates stress-induced depressive behaviors. Biol Psychiatry 2018;85:635–649. doi: 10.1016/j.biopsych.2018.11.025.
12. Chen XF, Ren SC, Tang G, Wu C, Chen X, Tang XQ. Short-chain fatty acids in blood pressure, friend or foe. Chin Med J 2021;134:2393–2394. doi: 10.1097/CM9.0000000000001578.

How to cite this article: Deng C, Qu JH, Kim I, Tang X. Histone crotonylation in neurobiology: To be or not to be? Chin Med J 2022;135:1036–1038. doi: 10.1097/CM9.0000000000001945