Existence and possible roles of independent non-CpG methylation in mammalian brain

CURRENT STATUS: POSTED

Jong-Hun Lee
The University of Tokyo

Yutaka Saito
National Institute of Advanced Industrial Science and Technology

Sung-Joon Park
The University of Tokyo

Kenta Nakai
the University of Tokyo

✉ knakai@ims.u-tokyo.ac.jp *Corresponding Author*
ORCID: <https://orcid.org/0000-0002-8721-8883>

DOI:

10.21203/rs.2.22195/v1

SUBJECT AREAS

Epigenetics & Genomics

KEYWORDS

Non-CpG methylation, Hidden Markov model, Neuro-epigenetics

Abstract

Background DNA methylations at non-CpG sites (mCpHs) yield weak enrichment signals and co-localize with methylated CpGs (mCpGs). Accordingly, they are thought to represent byproducts of hyperactive methyltransferases. However, recent studies revealed that mCpHs are highly cell type-specific and associated with cellular functions in mammal, although their distributional and functional dependency to mCpGs remains to be elucidated.

Results Through computational analysis of a large publicly available DNA methylation dataset from human and mouse cells, we revealed that mCpHs tend to co-localize with mCpGs in pluripotent stem cells but not in brain cells. A systematic investigation using an empirical hidden Markov model confirmed that there are genomic regions where CpGs and CpHs are differentially methylated in the brain cells (~25%). Remarkably, the regions were frequently located in putative enhancers, and mCpHs within the enhancers were greatly increased as brain aged. We detected 1669 hyper-mCpH enhancers functionally enriched to immune system development, aberration of which can lead to neuro-inflammation and neuro-degeneration.

Conclusions In mammalian brain cells, there are genomic regions where CpH alone is methylated (or de-methylated). The mCpHs are accumulated in enhancers as brain aged, potentially associated to neuro-inflammation and degeneration. Altogether, this study provides insight into the roles of non-CpG methylation as an epigenetic code in the genomes of mammalian brain cells.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed.

However, the manuscript can be downloaded and accessed as a PDF.

Figures

Figure 1

(a) Distribution of mCpH levels around mCpGs in human PSCs. (b) Distribution of mCpH levels around mCpGs in brain and control tissues. Numbers following underscores identify biological replicates. (c) Difference in mCpH levels at mCpG-proximal ($< \pm 100$ bp) and CpG-distal ($> \pm 100$ bp) CpH sites. Beta values were used as average methylation levels, and P-value was calculated by the Wilcoxon signed-rank test. (d) Distribution of methylation levels around mCpGs and unmethylated CpGs in DNMT1-knockout mouse ESCs. mCpG, methylated CpG; mCpH, methylated non-CpG; PSC, pluripotent stem cell; Br, brain; y, year; d, day.

Figure 2

(a) Schematic representation of the three states of the HMM. In the P-state, either both

CpGs and CpHs are methylated or neither is methylated, resulting in a positive correlation between the mCpG and mCpH levels. In the N-state, only CpGs or CpHs are methylated, resulting in a negative correlation between the levels. In the U-state, the levels are not correlated. The bin size was set to 180 bp because DNMT3a and DNMT3b methylate DNA strands with a ~180 bp periodicity. N-state bins are considered as CpG-CpH differentially methylated regions (CpG-CpH DMRs). (b) Schematic diagram of the state transition in the HMM. The transition probability was estimated using the EM algorithm. (c) Distribution of methylation levels of each bin assigned to one of the three states, using the highest emission probability. (d) Distribution of methylation levels of each bin assigned to one of the three states, using the Viterbi algorithm. (c) and (d) used 180 bp bins in chromosome 19 of sample Br-81y_1.

Figure 3

(a) Fraction of the P-, N-, and U-state regions detected by the highest emission probability or by the Viterbi algorithm. EP, emission probability. (b) Fraction of whole genome and of the known genomic elements overlapped with DMRs. The P-value of Student's t-test between N-state regions in the whole genome, and in each genomic element within the brain samples, was marked as follows: *, <0.01; **, <0.001. (c) Distribution of mCpH levels in P- and N-state regions of brain cells. (d) Fraction of whole genome and of the known genomic

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

[Supplementary_Figures.pdf](#)

[Supplementary_Tables.xlsx](#)