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Original Article

Changes in the Histone Acetylation Patterns during the Development of the Nervous System

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Epigenetic modification such as DNA methylation and histone acetylation plays essential roles in many aspects of cellular function and development of animals. There is an increasing amounts of evidence for dynamic changes in the histone acetylation of specific gene segments, but little attempt was made to examine global pattern changes in the histone acetylation in developing nervous system. In this study, we found that acetylated histone H3 and H4 immunoreactivities were relatively weak in neuroepithelial cells in the ventricular zone of developing rat cerebral cortex or chick spinal cord, compared to the immature young neurons in the cortical plate of a rat embryo or lateral motor column in chick spinal cord. On the other hand, adult neural stem cells in the dentate gyrus (DG) of rat hippocampal formation did not exhibit such diminished histone acetylation, compared to neuroblasts and mature DG neurons. These results suggest that the level of histone acetylation is highly dynamic and tightly linked to the neuronal types and the differentiation stages.

Key words: histone acetylation, nervous system, development, neuroepithelium

INTRODUCTION

Many biological processes are not only determined by DNA code itself, but also regulated by activity-related chromosomal remodeling (Clapier and Cairns, 2009). Since acetylation of histone modifies the basicity of histone protein which affects histone-DNA association, histone acetylation is essential event for the epigenetic control of gene expression (Allfrey et al., 1964; Hebbes et al., 1988; Riccio, 2010). The amount of histone acetylation is controlled by histone acetyltranferases (HATs) (Brownell et al., 1996) and histone de-acetylases (HDACs) (Ruiz-Carrillo et al., 1975; Annunziato and Seale, 1983). During the development, changes in the histone acetylation greatly affect the differentiation of stem cells (Lee et al.,

2004), neuronal maturation (Hsieh et al., 2004), and programmed cell death (Pelzel et al., 2010) via modulation of specific gene expressions which are responsible for these biological processes. It is believed that gene transcription machineries recruit histone modification enzymes into their transcriptome complex, and in this way specific gene transcription-mediated biological processes would be promoted. For instance, histone acetylation level declines during the differentiation of embryonic stem cells, which affects the gene transcription of stemness genes, such as Oct4 and Brachyury (Lee et al., 2004). Therefore, treatments of HDAC inhibitors, such as trichostatin A (TSA) and valproic acid (VPA), or deficiency of HDAC greatly affect many aspects of brain function, including differentiation of neurons and oligodendrocytes (Marin-Husstege et al., 2002; Hsieh et al., 2004), neuronal apoptosis (Pelzel et al., 2010), motor innervation (Mejat et al., 2005), and memory consolidation (Korzus et al., 2004).

While there have been extensive investigations of the histone acetylation patterns of specific gene promoter regions, little

attempt was made to explore global changes in the histone acetylation during the normal embryonic development. In this study, we found that histone H3 and H4 acetylation levels were stronger in the early differentiating neurons in the rat cortex and chick spinal cord, comparing to the proliferating neuroepithelial cells in the ventricular zone. These results suggest that transient enhancement of histone acetylation may be an important event for neuronal differentiation.

MATERIALS AND METHODS

Animals

Time-pregnant C57Bl/6 mice were obtained from Orient Co. (Korea), and fertilized eggs were obtained from Pulmuone (Korea). Eggs were incubated in humidified incubator at 38°C for 3 days. Stage of chick embryo was identified according to the Hamilton-Hamburger's criteria (Hamburger and Hamilton, 1992). All experiments were carried out in accordance with the ethical guidelines of Korea University, and with the approval of the Animal Care and Use Committee of Korea University.

Immunohistochemistry

Immunohistochemical analyses were performed as previously reported (Sun et al., 2003). Briefly, brain or trunk tissues were isolated from embryos and fixed with 4% paraformaldehyde overnight. Tissues were then cryoprotected in 30% sucrose, sectioned (20 μm) and attached on a gelatin-coated slide glass. Acetylated Histone

H3 (Upstate, #06-599, 1 : 500) and H4 (Upstate, #06-866, 1 : 1,000) antibodies were applied overnight. After several washes with PBS, secondary antibodies were applied for 30 minutes. Subsequently, sections were washed, counter-stained with Hechest33342, mounted and observed with a fluorescence microscope (Zeiss LSM510, Goettingen, Germany).

RESULTS

### Acetylation of histone H3 and H4 in developing rat cerebral cortex

Embryonic rat cerebral cortex (E17) was immunolabeled with anti-acetylated histone H3 or H4 antibodies (Fig. 1). Interestingly, both acetylated histone H3 and H4 immunoreactivities were strong in the cortical plate (CP) where post-mitotic neurons localize, compared to the ventricular zone (VZ) and intermediate zone (IZ) enriched for mitotic/migrating neuroblasts. The concentration of histone proteins was proportional to the total DNA density, which may be affected by the size of nuclei. Therefore, we counterstained the nuclei DNA by Hoechst33342, and the relative densities of acetylated histones and DNA were measured. This analysis further confirmed the preferential histone acetylation in the CP.

Acetylation of histone H3 and H4 in developing chick spinal cord

To determine whether similar difference in histone acetylation levels in developing nervous system in other species appear, we also examined the developing chick (E7) spinal cord (Fig. 2). Similar to the rat cerebral cortex, the level of histone acetylations were strong in the lateral motor column (LMC) where post-mitotic motor neurons localize, whereas histone acetylation
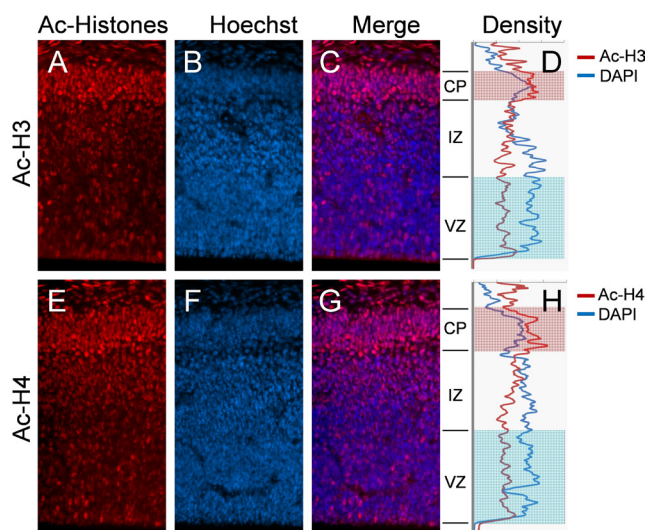
Fig. 1. Acetylation of histone H3 (A~D) and H4 (E~H) in E17 mouse cerebral cortex. Acetylated Histones were labeled with red (A, E), and nuclei were labeled with blue (B, F). Merged images were shown in C and G. Densities of each signal were measured by ImageJ (NIH), and shown in D and H. VZ: ventricular zone, IZ: intermediate zone, CP: cortical plate.
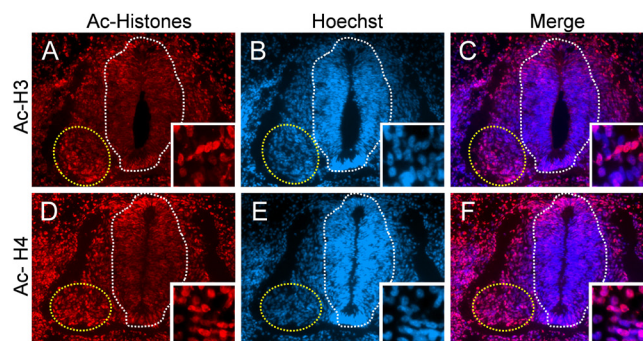

Fig. 2. Acetylation of histone H3 (A~C) and H4 (D~F) in E7 chick spinal cord. Acetylated Histones were labeled with red (A, D), and nuclei were labeled with blue (B, E). Merged images were shown in C and F. White lines mark proliferating zone near the central canal, and yellow lines mark lateral motor column (LMC). Insets show large magnification view of LMC.

were relatively weak in the periventricular regions enriched for proliferating neuroepithelial cells. Another important aspect of histone acetylation pattern is the heterogeneity within the same population. For instance, large magnification images of LMC clearly show that the intensities of acetylated histones were different among motor neurons.

Acetylation of histone H3 and H4 in in adult mouse dentate gyrus

These results suggest that histone acetylation is closely associated with the mitosis and differentiation of neural stem/progenitor cells. Neural stem cells remain in the specific areas of adult brain, and they continue to produce neurons. Therefore, we next examined whether adult neural stem cells and their progenitors also showed different levels of histone H3 acetylation (Fig. 3). To identify different cell types, three specific markers were used; glial fibrary acidic protein (GFAP), a marker for astrocytes and neural stem cells, doublecortin (DCX), a marker for neuroblasts, and neuronal nuclei (NeuN), a marker for differentiated neurons. Although a considerable heterogeneity of histone H3 acetylation levels was observed among the same cell populations, there was no significant difference among cell types.
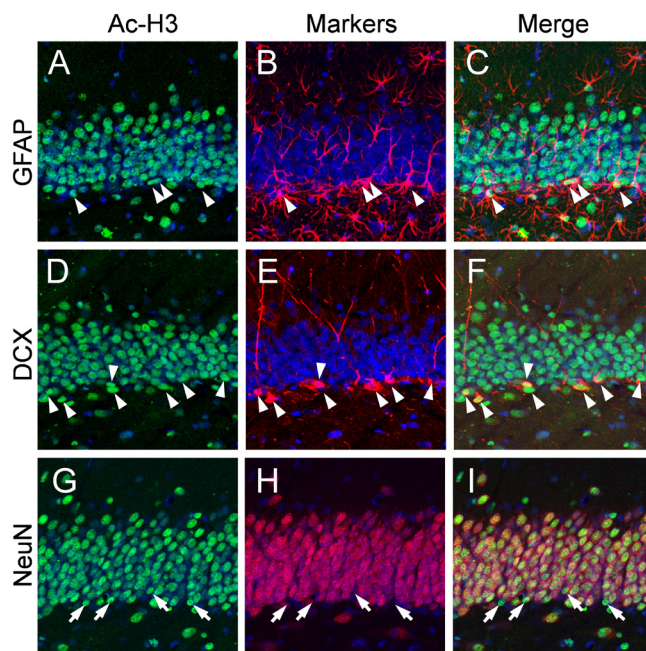
Fig. 3. Acetylation of histone H3 in adult mouse dentate gyrus. Markers for neural stem cells (GFAP, B), early neuroblasts (DCX, E), and mature neurons (NeuN, H) were co-labeled with acetylated histone H3 (A, D, G). Nuclei were counter- stained in blue with Hoechst33342. Merged images were shown in C, F, and I. Arrowheads in A~F indicate double-labeled cells, and arrows in G~I indicate NeuN-negative nuclei in the subgranular zone.

DISCUSSION

Increased acetylation of histones may promote the overall gene transcription efficiency. In this study, we found that developing neurons in the mouse cerebral cortex and chick spinal cord exhibit considerably high level of histone H3 and H4 acetylation, compared to the proliferating stem/progenitor populations. The level of histone acetylation was reported to increase during the postnatal development of rat brain (Serra et al., 1986), consistent with our current observation. Although there was little attempt to compare histone acetylation or RNA synthesis rates between stem cells and progenitor neurons, neurons appear to have higher histone acetylation level than glial cells (Hsieh et al., 2004; Shen et al., 2005; Humphrey et al., 2008). Considering that the size of neurons greatly expands during the differentiation, it appears that neurons have high demands for synthesis of many different RNA species during neuronal differentiation. However, it is noted that histone acetylation does not directly indicate the rate of RNA synthesis., Histone acetylation do not control the RNA polymerase activity, but it allows RNA polymerases to bind to the DNA. In this respect, increased histone acetylation may be linked to other biological processes.

In contrast to our *in vivo* data, it has been reported that cultured neural stem cells *in vitro* exhibit high level of global histone acetylation compared to their progenitor neurons or glial cells (Hsieh et al., 2004; Shen et al., 2005; Humphrey et al., 2008). Although it is unclear why these *in vitro* results are opposite to our current *in vivo* observations, it is important to note that epigenetic modification mainly depends on the extracellular signals, rather than cell autonomous events (Turner, 2009; Riccio, 2010). In this respect, it is no wonder that *in vitro* situation and *in vivo* environment resulted in different histone acetylation profiles. Considering that histone acetylation allows the "active" status of stem cells, and thus many stemness gene promoter regions are less acetylated, it does not mean that the global histone acetylation level should be high in less differentiated stem/progenitor cells.

Another important aspects about global histone acetylation levels identified in this study, is the heterogeneity among the same cell population. For instance, we found that only a subset of LMC chick motoneurons exhibited very strong level of histone acetylation. We failed to correlate the levels with known MN subtypes (data not shown), suggesting that these are differences among the same type of cells. This heterogeneity may be caused by highly dynamic nature of histone acetylation. In fact, histone acetylation is mainly regulated by two enzyme systems, histone acetyltransferases (HAT) and histone deacetylases (HDAC) (Riccio, 2010). Since these enzyme activities are dynamically

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regulated by multiple mechanisms, it is believed that the level of histone acetylation in cells are flexible and modulated by multiple factors, which may produce the diversity of histone acetylation even in the same population of cells.

In contrast to the developmental nervous system, we failed to detect any significant difference in the global histone acetylation levels among neural stem cells, neuroblasts, and mature neurons in the adult dentate gyrus. Compared to the embryonic development, neural stem cells and their progenitor neurons are similar in size and space, thus they may experience less dramatic changes in cell-autonomous gene transcription and extracellular environment. Additional studies would clarify how these differences occur, and the mechanism/significance underlying it.

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