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Histone modification mapping in human brain reveals aberrant expression of histone H3 lysine 79 dimethylation in neural tube defects

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ABSTRACT

Neural tube defects (NTDs) are severe, common birth defects that result from failure of neural tube closure, but their pathological mechanisms are not yet fully understood. Histone modifications have an important role in gene regulation during fetal development. We therefore hypothesized that the human NTDs may be partly caused by an imbalance in metabolism, perhaps caused by nutritional deficiencies, that leads to aberrant histone modifications. Here, we report a screen of fetal brain histone modifications using 2D nano-LC strong cation exchange reverse phase (SCX/RP) MS/MS and the identification of 61 unique post-translational modification sites on histones H1, H2a, H2b, H3, and H4. Of these, 38 sites are novel (not already found in the Uniprot database). Furthermore, we compared the histone modification patterns between normal brains and NTD brains special of which maternal folate levels were lower than of normal control. The results showed that histone H3 lysine 79 dimethylation (H3K79me2) and a novel identified site, H2bK5 monomethylation (H2bK5me1), were completely absent in individuals with NTDs. Follow-up Western blotting validated the decreased H3K79me2 expression in brains with NTDs, but the amplified samples experiments displayed that decreased H3K79me2 expression was not suitable for all samples with NTDs. Furthermore, folate-free treated mouse embryonic stem cells induced the decreased H3K79me2 level. Subsequently, our ChIP results in normal fetal brain tissues showed that H3K79me2 binds to SUFU, RARA and ITGA3 which induce NTDs phenotype after knockout in mice, and in NTDs brain tissues the bindings of H3K79me2 to these three genes were significantly altered. Taken together, our study indicated that low folate treatment might attenuate H3K79 dimethylation, further affect its regulate activation on target genes, some of which are NTDs-resulting associated, lastly interrupt early embryo developing. Our study increases the understanding of normal fetal brain histone modifications and provides a platform for investigating histone modifications in neural disease and also has an insight into a potential role of aberrant histone modification in etiology of NTDs.

Introduction

Histone modification is an important epigenetic modification in transcriptional regulation and genome function (Zhou et al., 2011). The N-terminal tails of core histones (H1, H2A, H2B, H3 and H4) can receive post-translational modifications, such as methylation, acetylation, phosphorylation, and ubiquitination (Bonaldi et al., 2004; Jenuwein and Allis, 2001). Histone modifications show tissue-specificity (Garcia et al., 2008), therefore, although histone modifications map have been reported for some tissues, they have not been fully mapped in the nervous system. Considering the importance of histone modification on transcription, it is essential to understanding the pathogenicity of human diseases of the nervous system.

Neural tube defects (NTDs) form a group of severe congenital malformations, including anencephaly, spina bifida, and encephalocele. The incidence of NTDs is approximately 1 in 1000, but in some geographical regions it is estimated reach to 4–10 in 1000 (Gu et al., 2007; van der Put et al., 2001). These diseases are thought to be associated with genetic variations and environmental factors and to be involved in many functional pathways, such as folate metabolism and the planar cell polarity pathway (Kibar et al., 2009, 2011). Low concentrations of three environmental nutritional factors, maternal folate, vitamin B12 and vitamin C, are associated with an increased risk of NTDs

Abbreviations: NTDs, neural tube defects; H3K79me2, histone H3 lysine 79 dimethylation; H2bK5me1, H2bK5 monomethylation; ESC, embryonic stem cells; SCX/RP, strong cation exchange reverse phase; FN, folate-normal; FR, folate-free; ChIP, chromatin immunoprecipitation.

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(Bower et al., 2009; Mulinare et al., 1988; Smithells et al., 1976, 1981). In some countries mandatory fortification of foods with folate significantly decreased the prevalence of NTDs (Chan et al., 2008; Czeizel et al., 2011; Heseker, 2011). These lines of evidence suggest that folate has an important role in the etiology of NTDs, but until now its molecule involving in remains unknown. Recent studies show that folate can serve as the source of epigenetic modifications (Tibbetts and Appling, 2010). They affect the synthesis and methylation of DNA, proteins and lipids through *S*-adenosylmethionine (SAM)-mediated one-carbon transfer reactions (Muskiet, 2005). SAM is the unique methyl donor for many biological methylation reactions, including histone methylation. Furthermore, *S*-adenosylhomocysteine, another component of one-carbon metabolism, is an inhibitor of methyltransferases such as histone methyltransferases (Park et al., 2012). These observations hint us that folate could regulate histone methylation via feedback as part of one-carbon metabolism.

Recently, increasing lines of evidence have indicated that some components of the histone modification machinery are critical for normal brain function and development, and also that aberrant modification contributes to diseases in the nervous system. For example, the histone H3K9-specific histone methyltransferase, SET domain bifurcated 1 (SETDB1), also known as Erg-associated protein with SET domain (ESET), has an increased expression level in striatum in Huntington's disease, resulting in global H3K9 hyper-trimethylation, which probably contributes to neuronal dysfunction (Ryu et al., 2006; Yang et al., 2002). In addition, mutations in the *SMCX* gene (encoding a member of the H3K4me3-specific demethylase family) are associated with mental retardation and autism (Shi, 2007). The H3K4-specific methyltransferase, MLL1, is essential for hippocampus synaptic plasticity and might be involved in cortical dysfunction in some cases of schizophrenia (Akbarian and Huang, 2009). These studies indicate an indirect linkage between histone modifications regulation and brain function and development.

All the above-mentioned evidence indicates that histone modifications can be regulated by folate, and that further aberrant modifications might result in diseases of the nervous system. Therefore, we hypothesized that histone modifications is abnormal in folate-related NTDs and they could be causative in NTD formation. In the present study, we used a MS-based proteomic screen (2D nano-LC strong cation exchange reverse phase (SCX/RP) MS/MS) to examine histone modification mapping in the brains of four normal fetuses and four fetuses with NTDs and compared the mapping between them. The results indicated that two sites of histone modification differed in NTD brains, and this was validated by western blot. Furthermore, normal fetal brain tissue ChIP results showed that H3K79me2 bind to SUFU, RARA and ITGA3 which induce NTDs phenotype after knockout in mice, but in NTDs brain tissue, the binding abilities were altered. These results indicated that abnormal modification of H3K79me2 is a potential pathogenic mechanism in NTDs. Our results provide an overview of histone modifications in the fetal human brain and suggest that abnormal modifications might be involved in the etiology of folate-related NTDs.

Materials and methods

Subjects

The NTD (spina bifida) and normal control samples were from the Lvliang area of Shanxi Province in northern China. Brain tissue from four fetuses with spina bifida and four normal control fetuses with a gestational age of approximately 20 weeks was analyzed. NTD fetuses were from medical abortions and had been diagnosed with spina bifida by B-mode ultrasound in the early stages of pregnancy; the sex, gestational age, and general development were also recorded in details. Pathological diagnosis of NTD was completed by experienced pathologists according to the International Classification of Disease. Fetuses whose mothers had been taking folic acid or in which the NTD was accompanied by other congenital malformations were excluded. Control fetuses that had been aborted for non-medical reasons were enrolled from the same region during the same pregnancy period, and were matched in age and sex with the NTD fetuses (details in our previous papers (Wang et al., 2010b; Zhang et al., 2008)). Any fetuses displaying pathological malformations or intrauterine growth retardation were excluded from the control group.

Histone extraction

Core histone proteins were extracted from the brain samples using acid extraction (Hake et al., 2007). Briefly, the brain tissue was first homogenized in lysis buffer (10 ml solution containing 10 mM Tris–Cl pH 8.0, 1 mM KCl, 1.5 mM $MgCl_2$ and 1 mM dithiothreitol (DTT)) and chilled on ice. Protease and phosphatase inhibitors as described below were added immediately before lysis of cells, and nuclei were isolated by centrifugation (1500 *g* for 10 min). For the preparation of histones, nuclei were incubated with four volumes of 0.2 N sulfuric acid (H_2SO_4) for overnight at 4 °C. The supernatant was precipitated with 33% trichloroacetic acid (final concentration) and followed by centrifugation (12,000 *g* for 20 min). The obtained pellet was washed with cold acetone and subsequently dissolved in distilled water. The samples were stored at −80 °C before analysis.

In-solution protein digestion

As described in our previous paper (Hou et al., 2010), 40 μg of core histone protein mixture extracted from brain was digested as follows. Disulfide bonds were reduced with 10 mM (final concentration) DTT for 60 min at 37 °C, then alkylation was carried out by adding 40 mM (final concentration) iodoacetamide for 60 min at room temperature in the dark. The alkylation reaction was quenched by treating with 40 mM DTT for 15 min. After diluting the urea to less than 1 M with 25 mM NH_4HCO_3, sequence-grade trypsin was added at a ratio of 1:40 (enzyme:total protein) and proteins were then digested at 37 °C for 4 h. The tryptic digestion was quenched by adding 1.0% trifluoroacetic acid, and the solution was then centrifuged at 13000 *g* for 10 min to remove insoluble material. The supernatant was collected for subsequent experiments.

2D nano-LC-MS/MS analysis

The 2D nano-LC-SCX/RP MS/MS protocol we used here was the same as in our previous paper (Hou et al., 2010). Core histone samples were analyzed using a high-throughput LTQ ion trap tandem mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a nanoelectrospray device constructed in house with modifications. HPLC was performed using a Surveyor pump (Thermo Fisher Scientific). For each analysis, the sample was dissolved in 50 μl 0.1% formic acid (FA), then pressure-loaded onto a biphasic silica capillary column (200 μm i.d.) packed with a 3 cm bed volume of reversed phase C18 resin (3-μm particles, 120-Å pore size, SunChrom, Friedrichsdorf, Germany) and a 3 cm bed volume of strong cation exchange resin (Luna 5-μm particles, SCX 100-Å pore size, Phenomenex, Torrance, CA). The buffers used were 0.1% FA (buffer A), 80% ACN/0.1% FA (buffer B), and 700 mM ammonium acetate/5% ACN/0.1% FA (buffer C). The biphasic column was first desalted with buffer A and then eluted using an 8-step salt gradient ranging from 0 to 700 mM ammonium acetate. The peptide effluents of the biphasic column in each step were directed onto a 12 cm C18 analytical column (75 μm i.d.) with a 3–5 μm spray tip. Step 1 consisted of a 100 min gradient from 0% to 100% buffer B. Steps 2–7 had the following profile: 3 min of 100% buffer A, 5 min of X% buffer C, 5 min gradient from 0 to 10% buffer B, 77 min linear gradient from 10% to 45% buffer B, 10 min gradient from 45% to 100% buffer B, 10 min of 100% buffer B, and a final 10 min of 100% buffer B to equilibrate the column. The percentages of buffer C (X) were 20, 30, 40, 50, 60 and 80% for the 6-step analysis. In the last step, the gradient applied was

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