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Profiling post-translational modifications of histones in neural differentiation of embryonic stem cells using liquid chromatography–mass spectrometry

Shuzhen Zheng[a], Ming Sun[c], Kai Zhang[a,b,**], Junjie Gu[c], Zhenchang Guo[b], Shanshan Tian[b], Guijin Zhai[b], Xiwen He[a], Ying Jin[c,*], Yukui Zhang[a,d,*]

[a] Department of Chemistry, Nankai University, Tianjin 300071, China
[b] 2011Collaborative Innovation Center of Tianjin for Medical Epigenetics, Tianjin Key Laboratory of Medical Epigenetics, Department of Biochemistry and Molecular Biology, Tianjin Medical University, 300070 Tianjin, China
[c] Key Laboratory of Stem Cell Biology, Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences/Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China
[d] National Chromatographic Research and Analysis Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China

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ABSTRACT

The neural differentiation of embryonic stem cells (ESCs) is of great significance for understanding of the mechanism of diseases. Histone post-translational modifications (HPTMs) play a key role in the regulation of ESCs differentiation. Here, we combined the stable isotope chemical derivatization with nano-HPLC-mass spectrometry (MS) for comprehensive analysis and quantification of histone post-translational modifications (HPTMs) in mouse embryonic stem cells (mESCs) and neural progenitor cells (mNPCs) that was derived from ESCs. We identified 85 core HPTM sites in ESCs and 78HPTM sites in NPCs including some novel lysine modifications. Our quantitative analysis results further revealed the changes of HPTMs from ESCs to NPCs and suggested effect of combinational HPTMs in the differentiation. This study demonstrates that HPLC–MS-based quantitative proteomics has a considerable advantage on quantification of combinational PTMs and expands our understanding of HPTMs in the differentiation.

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1. Introduction

The fundamental packaging unit of eukaryotic DNA is nucleosome, which is composed of a segment of DNA wrapped around a histone octamer (two copies of four core histone proteins (H2A, H2B, H3 and H4). The histone proteins, particularly their *N*-terminal tails, are frequently decorated by a large number of post-translational modifications (PTMs) including differential methylation of arginine and lysine, lysine acetylation, ubiquitylation, and crotonylation [1–3]. The histone PTMs (HPTMs) have been considered to be a group of epigenetic code that might change the histone charges thus exerting histone-DNA interaction. Histone PTMs play a crucial role in cell differentiation and development [4]. Embryonic stem cells (ESCs) possess an unlimited potential to self-renew and the capacity to differentiate into multiple types of cells [5]. The status of HPTMs has been observed to be dynamic changes during cells differentiation [6]. Meanwhile the differentiation of ESCs is mediated by the regulation of HPTMs. For example, appropriately timed methylation of H3K9 may be involved in the control switch for exiting the cell cycle and entering differentiation [7]. Neural progenitor cells (NPCs) are multipotent, self-renewing precursor cells which can be further induced into neurons for cell-replacement therapies [8]. The neural differentiation of human ESCs is of great significance to elucidate the key mechanisms involved in human neurogenesis [9]. It has been considered that HPTMs play a key role in regulation of gene expression associated with differentiation from ESCs to NPCs. However, little has been known about how HPTMs undergo global changes during the differentiation. In order to fill in the knowledge gap, it is necessary to comprehensively analyze the difference of HPTMs between ESCs and NPCs.

High performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) has been developed a fundamental tool for the identification and quantification of proteins and protein PTMs [10–12]. Separation and analysis of histone PTMs have paid

* Corresponding authors.
** Corresponding author at: Department of Biochemistry and Molecular Biology, Tianjin Medical University, 300070 Tianjin, China.
E-mail address: kzhang@tmu.edu.cn (K. Zhang).

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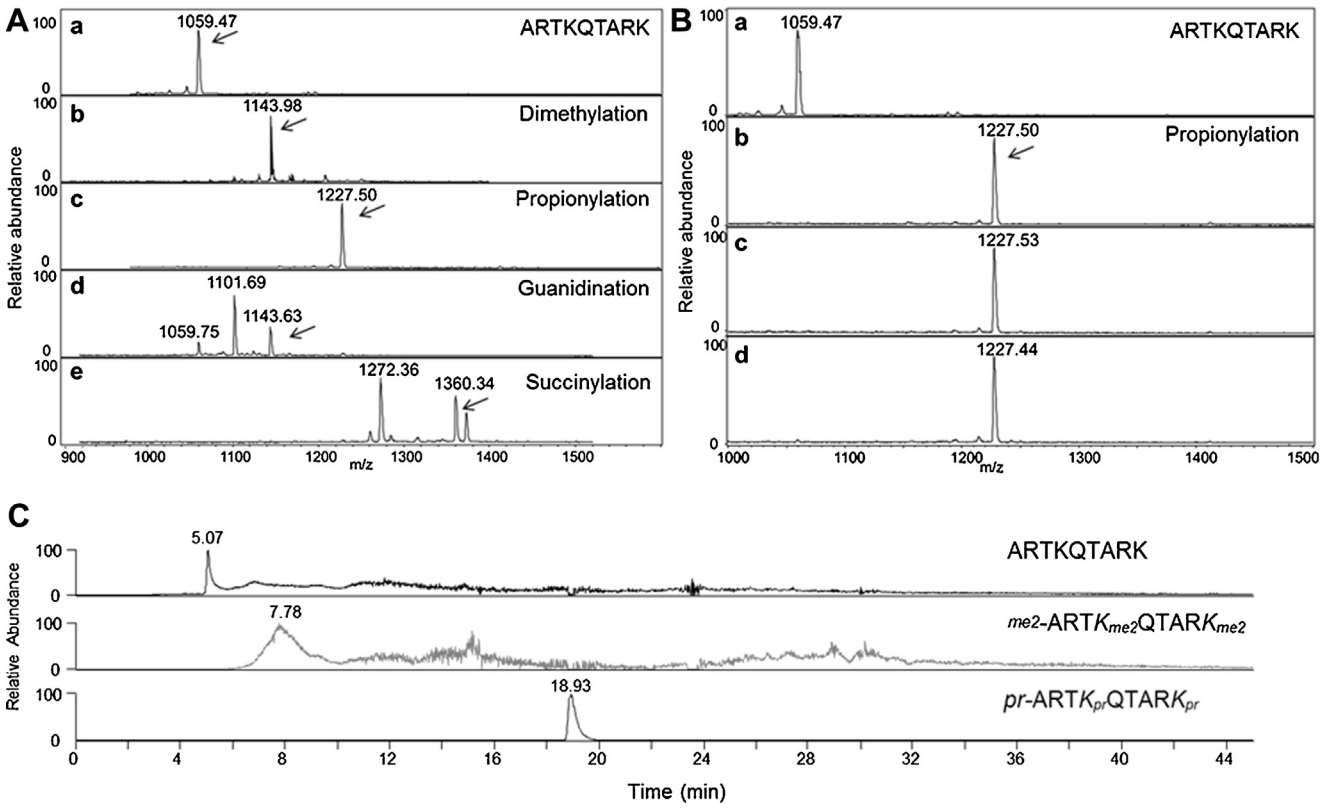

Fig. 1. (A) MALDI mass spectra of the peptide ARTKQTARK (1058.63 Da) labeled with chemical modification reagents in molar ratio of amino/reagent (1:50): (a) Direct analysis; (b) Dimethylation; (c) Propionylation; (d) Guanidination; (e) Succinylation. (B) MALDI mass spectra of the peptide ARTKQTARK (1058.63 Da) labeled propionic anhydride in different molar ratio of amino/propionic anhydride: (a) 1:5; (b) 1:10; (c) 1:50. (C) Extracted ion chromatograms of unmodified peptide, dimethylated peptide and propinoylated peptide.

much attention due to their biological significance [1–3,13–15]. The dense distribution of lysine residues makes the tryptic peptides of histone proteins highly hydrophilic, thus difficult to be retained in a traditional reversed-phase HPLC column [16,17]. So histone tryptic peptides are subjected to poor resolution, which further lead to unsatisfactory MS analysis. To improve the HPLC separation and MS identification, chemical derivatization of ε-amine group of lysine residues has been applied [16]. Here, we investigated the derivatization f ε-amine group of lysine residues using several chemical reagents and evaluated HPLC retention of derivative peptides. Further we combined the stable isotope labeling with HPLC–MS/MS analysis for comprehensive analysis of HPTMs in ESCs and NPCs. The differential expression of HPTMs between the two cell lines was further analyzed and discussed. Our results showed that HPLC–MS/MS based approach could provide highly precise quantification data to understand the differential expression of HPTMs in epigenetic events.

2. Experimental

2.1. Reagents and materials

Propionic anhydride (^{12}C6, 99%) and propionic anhydride (^{13}C6, 99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Water, acetonitrile (ACN) and methanol (MeOH) were HPLC grade from Fisher Scientific (Pittsburgh, PA, USA). Trifluoroacetic acid (TFA) was from Sigma Aldrich (St. Louis, MO, USA). Colloidal Blue Staining Kit was from Invitrogen (Carlsbad, CA, USA). Sequencing-grade trypsin was from Promega (Madison, WI). A typical histone H3 peptide ARTKQTARK (1058.63 Da, histone H3, residues 1–9) was synthesized by Abgent (Wuxi, China). Peptides bearing potential PTMs of interest were synthesized by GL Biochem (Shanghai, China). *O*-methyl isourea hemisulfate (95%), formaldehyde (37%), sodium cyanoborohydride ($NaBH_3CN$) and succinic anhydride (99%) were form J&K Scientific (Beijing, China). C18 ZipTips, immobilon transfer membranes (PVDF), Western Lightning Chemiluminescence Reagent Plus and antibodies were from Millipore (Bedford, MA, USA), Horseradish peroxidase-conjugated anti-mouse IgG was from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals including ammonium hydroxide (NH_4OH), sodium acetate (NaOAc), trichloroacetic acid (TCA), sodium bicarbonate ($NaHCO_3$) and ammonium bicarbonate (NH_4HCO_3) were analytic grade from Sangon Biotech (Shanghai, China).

2.2. Derivatization reaction of standard peptide

We investigated the derivatization reaction of ε-amine group of lysine residues using formaldehyde, O-methyl isourea hemisulfate, propionic anhydride and succinic anhydride, respectively. Take propionylation for example, the synthetic peptide ARTKQTARK (1058.63 Da, histone H3, residues 1–9) (0.1 mg) was dissolved in 0.5 mL of 100 mM NH_4HCO_3. 25 uL of propionic anhydride was dissolved in 75 ul MeOH. And then equal volumes of the derivatization reagent and the synthetic peptide (20 ug) were mixed. A pH of 8 was adjusted by ammonium hydroxide. The reaction was performed at 51 °C for 30 min as described previously [16], finally it was terminated by adding about 5 ul 50% (v/v) formic acid. The solution was dried in vacuum concentrator and desalted by C18 ZipTip prior to MS analysis. All details are shown in Supporting information.

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