An effective mass spectrometry-based strategy for characterizing histone H3K9 modifications

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Mass spectrometry (MS)-based analytical approaches to examine histone post-translational modifications (PTMs) are useful tools to understand epigenetic function. Histone H3K9 methylation plays an important role in chromatin remodeling, which is important in stem cell self-renewal and differentiation. With a simple, rapid, and accurate top-down strategy using matrix assisted laser desorption/ionization (MALDI)-in source decay (ISD), we have recently identified K9 PTMs on H3 variants (H3.1, H3.2, H3.3, and H3t) in the mouse testis. Mono-, di-, and tri-methylated K9 sites were identified on H3 variants separated using liquid chromatography and an ion-pairing reagent. These modifications were also observed in the testis-specific histone H3 using MALDI-ISD. Our findings demonstrate a novel top-down approach for characterizing PTMs on histone tails that will have applications in future research.

Keywords: MALDI-ISD; Testis-specific H3 histone; K9 modifications


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Nuclear DNA is normally organized into compact chromatin structures by histones. There are several core histones encoded in the mammalian genome, with different variants expressed at various developmental stages [1–3]. Several post-translational modifications (PTMs) are applied to these histone variants, including methylation, acetylation, and phosphorylation. PTMs are associated with changes to chromatin dynamics, including whether a region is the more active euchromatin or condensed heterochromatin [2, 4–6]. Histone acetylation, for example, is a typical modification observed in transcriptionally active chromatin complexes. Several other PTMs, such as methylation and ubiquitination, have also been reported to be linked to repressed or active chromatin states [4, 6]. Importantly, the regulation of chromatin structure is a mediator of transcriptional activity and gene expression [4–6]. Among the various PTMs on different histone variants, H3 PTMs have been a focus of research due to a relationship with several biological processes, including transcription and chromatin remodeling [5, 7, 8]. Histone H3 variants are modified by several PTMs, including methylation, acetylation, phosphorylation, O-GlcNAcylation, and ubiquitination [5, 7, 8]. In particular, lysine methylations have been reported to play an important role in transcriptional heterochromatin associated with stem cell self-renewal and multipotency [9–11].

Approaches to characterize histone PTMs have focused on understanding novel or specific patterns that are linked to
biological processes. Techniques to analyze histone PTMs are traditionally immunoassays that use antibodies to interrogate structure [2, 4, 6]. These antibody-based methods have proven sensitive enough to detect PTMs on histones. Unfortunately classification of histone variants with this method is difficult and there are certain other shortcomings, such as cross-reactivity and ambiguous specificity, that limit its potential [2, 4, 6]. Mass spectrometry (MS) is an alternate and powerful tool for the characterization of histone PTMs. The technology is sensitive, rapid, accurate, and can be adapted for high-throughput assays [6, 12]. MS-based proteomic approaches to characterize histone PTMs typically rely on “bottom-up” strategies, requiring enzyme-digestion step [12]. Despite the high sensitivity of mass fingerprinting digested peptides, there is a loss of whole protein information due to very short peptides. Such information includes complete histone sequences and PTMs [6, 12]. Alternate MS-based “top-down” approaches are more suited for characterizing proteins with low abundance, such as histones. The technique has been successfully used to comprehensively characterize whole histones, including conserved PTM information [6, 12]. Top-down strategies are clearly useful to distinguish different histone variants and to detect PTMs without the loss of proteomic and epigenetic information from intact proteins [6, 12]. Top-down approaches using MS to identify intact H3 variants with their PTMs have been increasingly used in functional research [7, 13–17].

Spermatogenesis is a biological process in which sperm are produced in the testes. The expression of several histone variants, including testis-specific histones, have been reported to be linked with cellular activity during the three stages of spermatogenesis; mitosis (renewal and differentiation of stem cells), meiosis, and spermiogenesis [18, 19]. Phosphorylation, acetylation, methylation, and ubiquitination of histones have all been detected during spermatogenesis using proteomic approaches [18, 19]. Methylation of histone H3 variants, primarily at N-termini, has also been reported to be related to transcription and chromatin remodeling. In particular, H3K9 methylations are a well-described factor that associate with condensed chromatin during spermatogenesis [18–21].

Researchers have focused on characterizing sites of K9...
PTMs on H3 variants to understand their biological functions during spermatogenesis [5, 20–25]. Studies have identified several H3K9 PTMs using antibodies, including acetylation and methylation modifications; however, it is difficult to identify each H3 variant in detail [20–23]. Other approaches using MS have identified H3K9 PTMs during spermatogenesis in H3 variants that were previously poorly characterized [5, 24]. Garcia et al. separated and characterized mono-, di-, tri-methylation on K9 of H3.2 and H3.3 in rat testes using a middle-down analysis [5]. Recent research using LC-MS/MS after prior trypsin digestion has reported comprehensive identification of several K9 methylations on histone H3 variants (H3.1 and H3.3) during spermatogenesis and in mouse sperm [24]. These studies required specific prior results to identify methylations on histone H3 variants, such as H3t, during spermatogenesis.

Our approach has recently identified K9 methylations on H3 variants via a top-down analysis using matrix assisted laser desorption/ionization (MALDI)-in source decay (ISD) (Figure 1). We were able to successfully characterize H3K9 mono-, di-, and tri-methylations from each histone H3 variant (H3.1, H3.2, H3.3, and H3t). These variants were separated by HPLC using reversed-phase C4 column with an ion-pairing reagent (Figure 1b). In the initial experiment using our strategy, novel PTM patterns from histone K9H3t were detected in mouse testes (Figure 1d). We were also able to specifically examine sequences of N- and C-termini of intact histone H3 variants using MALDI-ISD [25]. MALDI-ISD is both an accurate and sensitive top-down approach, providing data on sequence and the PTMs of protein tails, without a prior digestion step [26–30]. Although there are already some approaches to examine specific and novel proteins, and their modifications [27–30], the characterization of PTMs on histone variants using MALDI-ISD has not been fully explored. To date, several top-down, MS-based analytical strategies for characterizing histone H3 variants and their PTMs have been conducted by electrospray ionization. This approach generates large and complicated data-sets using multi-charged ions [7, 13–16]. Our approach using MALDI-ISD, which utilizes single-charged ions, is a simple, specific, and rapid top-down analysis for N- and C-terminus analysis of histones. We have used our technique to successfully characterize H3 variants and their K9 modifications in mouse testes [25].

The characterization of PTMs on histones that are associated with epigenetic control has become an important factor in understanding biological functions. The combination of various analytical strategies will be needed to comprehensively study the structure and PTMs of histone variants. Our approach using MALDI-ISD is a novel paradigm for a top-down MS-based analysis and will be applicable for further research examining histone tails.

Conflicting interests

The authors have declared that no conflict of interests exist.

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Abbreviation

MS: Mass Spectrometry; PTM: Post-Translational Modification; MALDI: Matrix Assisted Laser Desorption/Ionization; ISD: In-Source Decay; HPLC: High Performance Liquid Chromatography.

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