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Insights into the lysine acetylproteome of human sperm

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ABSTRACT

Protein lysine acetylation is a dynamic and reversible post-modification that is known to play diverse functions in eukaryotes. Nevertheless, the composition and function of non-histone lysine acetylation in gametes remain unknown. In humans, only capacitated sperm have the capacity to fertilize an egg. In the present study, we found complex composition of lysine acetylated proteins in capacitated human sperm. In vitro fertilization inhibition assay by anti-acetyllysine antibody showed essential roles of lysine acetylation in fertilization. And inhibition of lysine deacetylases, the histone deacetylases, by trichostatin A and nicotinamide, could significantly suppress sperm motility. After immunopurification enrichment of acetylpeptides with anti-acetyllysine antibody and high-throughput liquid chromatography–tandem mass spectrometry identification, we characterized 1206 lysine acetylated sites, corresponding to 576 lysine acetylated proteins in human capacitated sperm. Bioinformatics analysis showed that these proteins are associated with sperm functions, including motility, capacitation, acrosome reaction and sperm–egg interaction. Thus, lysine acetylation is expected to be an important regulatory mechanism for sperm functions. And our characterization of lysine acetylproteome could be a rich resource for the study of male fertility.

Biological significance

Mature sperm are almost transcriptionally and translationally silent, thus post-translational modifications play important roles in sperm functions. Till now, only two types of PTMs, phosphorylation and glycosylation, are well studied in normal human sperm based on large scale proteomics. In the present study, we established the acetylproteome of capacitated human sperm. Over 1000 lysine acetylated sites were identified. Bioinformatics analysis shows that lysine acetylated proteins participate in many biological events of sperm functions. We further provided functional data that the lysine acetylation is essential for sperm motility and fertilization using histone acetylase inhibitors and anti-acetyllysine antibody. These data can be strong evidences for the important function of lysine acetylation in human sperm.

Abbreviations: Ac-BSA, acetylated bovine serum albumin; COCs, cumulus-oocyte complexes; FDR, false discovery rate; HDAC, histone deacetylase; IVF, in-vitro fertilization; SCX, strong cation exchange

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1. Introduction

The main function of sperm is to fertilize the egg. In physiological condition, sperm should first undergo capacitation to acquire the ability to fertilize an egg. Only capacitated sperm can complete fertilization [1]. Besides, the capacitated sperm also needs to have normal motility, and undergo successful acrosome reaction, and sperm–egg interaction to finish the fusion between sperm and egg. These complex processes are highly regulated and the mechanisms are still not well known. As a terminal cell, sperm is transcriptional silent due to condensation of the nucleus during spermiogenesis. Most of the spermatid cytoplasm is discarded, and the translational activity is very low in sperm [2]. Thus, sperm functions are regulated at protein level, mainly by post-translational modifications of proteins. Till now, phosphorylation and glycosylation of proteins are known to be important in sperm [3,4]. But the roles of other modifications are less known.

Traditionally, reversible lysine acetylation is mainly studied on histones and is known to involve in the regulation of transcription [5]. But recent studies showed that lysine acetylation of non-histone proteins has diverse functions in neuroprotection, angiogenesis, reversible cholesterol transport, glucose production and so on [6]. It has become the research focus due to development of proteomic technologies [7,8]. However, the compositions and functions of lysine acetylation in gametes are still not well studied.

In our previous proteomic study of human sperm, we identified many deacetylases including HDAC1, HDAC2, HDAC6, HDAC11, SIRT2, SIRT3, and SIRT5 [9]. Several of them are known to regulate non-histone acetylation. For example, HDAC6 and SIRT2 are microtubule associated deacetylases [10,11], while SIRT3 is a mitochondrial deacetylase [12]. Normal mitochondrial functionality in sperm is essential for male fertility [13], and sperm motility is associated with cytoskeletal proteins located in the sperm flagella, a structure similar to cilia. In order to study the roles of acetylated proteins in human sperm with the capacity to fertilize the ovum, we performed proteome-wide analysis of lysine acetylation sites in normal capacitated human sperm. A total of 1206 lysine acetylation sites were identified unambiguously, corresponding to 576 lysine acetylated proteins. Additionally, the inhibition by histone deacetylase (HDAC) inhibitors and anti-acetyllysine antibody showed that the acetylation was indeed important for sperm motility and fertilization.

2. Materials and methods

2.1. Collection and preparation of capacitated human and mouse spermatozoa

Seminal specimens were obtained from healthy adult males who had proven normal semen quality according to the 5th World Health Organization standard andrology criteria [14]. Prior to sample collection, approval was granted by the Committee of Nanjing Medical University on Human Rights in Research. The semen were ejaculated into sterile containers and allowed to liquefy for at least 30 min before being processed next. Percoll (GE Healthcare, Waukesha, WI, USA) was diluted to 60% with 1 × BWW medium (114.00 mM/L NaCl, 4.78 mM/L KCl, 1.71 mM/L $CaCl_2$, 1.19 mM/L $MgSO_4$, 1.19 mM/L KH_2PO_4, 21.58 mM/L sodium lactate, 5.56 mM/L glucose, 10.00 mM/L HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and 25.07 mM/L $NaHCO_3$ (pH 7.6)). The semen was gently stratified on top of the 60% Percoll gradient and centrifuged at 350 ×*g* for 10 min to remove round cells and seminal plasma. The sperm were separated in the precipitate and then washed in 1 × BWW medium two times. Sperm were incubated to capacitation in HTF containing 10 mg/mL BSA for 2.5 h in a humidified CO_2 incubator at 37 °C under 5% CO_2/95% air (v/v) according to published condition [15]. Significant increase of tyrosine phosphorylation indicating capacitation was observed after 2.5 h culture (Supplementary Fig. S2). Sperm samples were collected from two groups of volunteers and subsequent proteomic analyses were performed based on these two biological replicates.

The sperm of 8-week male ICR mice were dissociated and washed in 1 × BWW three times. Then, the mouse sperm were cultured to be capacitated in HTF-BSA with the same method mentioned above. All experiments requiring the use of animals received prior approval from Nanjing Medical University and were performed according to USDA-approved protocols.

2.2. Protein extraction

The in-vitro capacitated human sperm were lysed in 8 M urea, 75 mM NaCl, 50 mM Tris, pH 8.2 and 1% (v/v) protease inhibitor cocktail. The lysates were sonicated on ice using Sonics Uibra Cell (SONICS&MATERIALS. INC, CT, USA), and clarified by centrifugation at 16,000 ×*g*, 20 min, 4 °C. The concentration of the supernatant was determined by BCA method and the protein supernatant was stored at −80 °C until it was used.

2.3. Western blots

The extracted proteins were separated by SDS-PAGE (4–20% acrylamide gel) and transferred to PVDF membrane (GE Healthcare). The membranes were incubated for 2 h at room temperature in TBS containing 5% nonfat milk powder. Then, the membranes were incubated with anti-acetyllysine antibody or anti-phosphotyrosine antibody (Millipore Corp., Billerica, MA, USA). For anti-acetyllysine antibody incubation, home-made or commercial anti-acetyllysine antibody (Cell Signaling Technology, Danvers, MA, USA) was diluted in TBS/5% BSA overnight at 4 °C with or without acetylation competition acetylated bovine serum albumin (Ac-BSA). After washing 4 times in TBS, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Beijing ZhongShan Biotechnology Co., Beijing, China) for 2 h at room temperature. The proteins were detected using an ECL reagent (GE Healthcare) and ChemiDoc™ (Bio-Rad Laboratories, Hercules, CA, USA).

2.4. Indirect immunofluorescence

Both human and mouse capacitated sperm were washed in 1 × PBS three times, resuspended in PBS, and air-dried on