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Short Communication

Proteomic identification of sperm from mice exposed to sodium fluoride

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HIGHLIGHTS

- Fluoride altered the sperm proteomics of mice.
- 16 differently expressed spots were picked up to identify using MS.
- Many of the identification proteins are associated to the sperm function.
- Further researches on detoxification, inflammation, membrane structure stability.

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ABSTRACT

Fluoride is a widespread environmental pollutant which can induce low sperm quality and fertilizing ability. However, effect of fluoride on proteomic changes of sperm is unknown. In this study, two-dimensional electrophoresis (2DE) and mass spectrometry (MS) were used to investigate the differently expressed proteins of sperm from mice exposed to fluoride. 180 male mice were randomly divided into three groups, and were administrated with the distilled water containing 0, 25, and 100 mg L^{-1} NaF, respectively. After 45, 90 and 180 day's exposure, mice were sacrificed and sperm from the cauda epididymis and vas deferens were collected for 2DE. 16 differently expressed spots were picked up to identify using MS, 15 of which were successfully identified. Many of them are associated with the sperm function such as sperm motility, maturation, capacitation and acrosome reaction, lipid peroxidation, detoxification, inflammation, and stability of membrane structure. These results could contribute to the explanation and further research of mechanisms underlying sperm damage induced by fluoride.

1. Introduction

Fluoride is a widespread environmental pollutant, which results in dental and skeletal fluorosis, as well as the detrimental effects on nonskeletal systems such as liver, kidney, immune system, central nervous system, and reproductive system (Doull et al., 2006; Jagtap et al., 2012; Perumal et al., 2013). Although mild doses of fluoride are used to prevent dental caries, due to its narrow margin of safety, elevated consumption of fluoride in daily life will increase the body burden and risk of fluorosis through intake of water, toothpastes and other diets containing high fluoride (Doull et al., 2006).

Sperm is highly differentiated cell that is responsible for delivering the paternal genome to the oocyte. It is the end product of spermatogenesis, a differentiation process involving extremely marked genetic, cellular, functional and chromatin changes (Oliva et al., 2009). Previous studies demonstrated that fluoride induced the alterations of sperm morphology (Chinoy and Narayana, 1994), capacitation and acrosome reaction (Dvoráková-Hortová et al., 2008; Izquierdo-Vega et al., 2008; Kim et al., 2015), hyperactivation (Sun et al., 2010), chemotaxis (Lu et al., 2014), and fertilizing ability (Elbetieha et al., 2000; Izquierdo-Vega et al., 2008; Kim et al., 2015).

Omics technologies, such as genomics, proteomics, epigenomics, metabolomics, are useful and powerful tools to explore the toxicological mechanisms. In our previous study, high-throughput sequencing technology was used to analyze miRNA profiling in sperm of mice administrated with sodium fluoride

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(NaF), and 31 differentially expressed known miRNAs and several target genes were identified and predicted in fluoride groups, which provided the new information for further researches (Sun et al., 2016a). High-throughput technologies have revealed that sperm provide both a specific epigenetically marked DNA and a complex population of proteins and RNAs that is crucial for embryogenesis (Jodar et al., 2017). Mature sperm with a minimal amount of cytosol and organelles lost the potential for gene expression, are completely silent transcriptionally and translationally. Compared with many other cell types, this absence of protein synthesis and the reduction of proteins number and dynamic range of protein abundances make sperm particularly well suited for proteomic analysis (Oliva et al., 2009; Brewis and Gadella, 2010; Park et al., 2013). Therefore proteomic study on sperm from mice exposed to fluoride could help to clarify some of the molecular mechanisms involved in fluoride-induced reproductive toxicity.

2. Methods and materials

2.1. Animal treatment

180 male eight-week-old Kunming mice were provided by Laboratory Animal Center of Shanxi Medical University (Taiyuan, China). After one-week acclimatization, the mice were randomly divided into three groups, including the control group (distilled water) and two NaF treated groups (distilled water containing 25 and 100 mg L^{-1} NaF), with 60 mice each group. All animals were raised with standard diet and controlled conditions of temperature (22–25 °C) and 12/12-h light/dark cycle. Day 45, 90, and 180 of exposure were chosen as time points to collect the sperm from twenty mice in every group. The experimental design and implementation were approved by the Ethics Committee of Shanxi Agricultural University (Taigu, China).

2.2. Sperm collection

Mice were sacrificed by cervical dislocation. Subsequently, sperm from the cauda epididymis and vas deferens were collected and dispersed into 1 ml phosphate buffer solution (PBS). Then the sperm were stored at −80 °C until further experiments.

2.3. Extraction of protein

Extraction of sperm protein was carried out as previous studies with minor modification (Kirkland et al., 2006; Young and Truman, 2012). Briefly, sperm concentration was adjusted to 1.5×10^7/ml. 1 ml Trizol and 20 μl β-mercaptoethanol were added into the EP tube containing sperm, and then incubated for 1 h at 65 °C. After adding 0.2 ml chloroform, sperm were centrifuged at 12,000 g for 15 min at 4 °C. Protein was collected carefully from the bottom. Absolute ethyl alcohol was added to remove DNA, and the supernatant was collected in a new tube. 1.5 ml isopropanol was added, followed by the incubation for 10 min at 28 °C. After that, the mixture was centrifuged at 12,000 g for 10 min. The deposit was washed with 95% ethanol solution containing 0.3 M guanidine hydrochloride and absolute ethyl alcohol for three times respectively. Protein concentration was measured by Bradford method.

2.4. Protein separation by two-dimensional electrophoresis (2DE)

Sperm protein samples from five mice of each group were separated in the first dimension by isoelectric focusing (IEF). Protein samples of each 120 μl were covered by a pH 5–8, 7 cm immobilized pH gradient (IPG) strip, for 15 h rehydration at room temperature. IEF was running as following conditions: (1) 250 V for 2 h linear gradient, (2) 500 V for 2 h rapid gradient, (3) 4000 V for 3 h linear gradient, (4) 4000 V rapid gradient until reaching total 20,000 Vh, and (5) 500 V rapid gradient for 24 h. Then the strip was equilibrated in solution A (50 mM Tris-HCl, pH 8.8 6 M urea, 2% SDS, 30% glycerol, and 1% DTT) for 15 min, and in solution B (50 mM Tris-HCl pH 8.8, 6 M urea, 2% SDS, 30% glycerol, and 4.5% iodoacetamide) for another 15 min. 10% SDS-PAGE gel was used for second dimension electrophoresis at 15–20 mA for 30 min and then at 20–30 mA for 100 min. Gels were stained with coomassie blue G-250 for 5 h, and washed with ultrapure water for 3 times. Spot detection, background subtraction and spot matching were carried out by PDQuest 8.0 image analysis software (Bio-Rad, Hercules, CA). Spots with the same trend in two fluoride groups were chosen for the mass spectrometry analysis.

2.5. Mass spectrometry (MS) analysis

Samples were analyzed using the matrix-assisted laser desorption-time of flight (MALDI-TOF-MS) provided by Applied Biosystems (Shanghai, China). The proteins were identified through MASCOT (http://www.matrixscience.com) with the help of National Center for Biotechnology Information (NCBI) mouse database. Score >60 and CI% > 95 are considered as the threshold value for successful identification.

3. Results

With the help of 2DE, sperm proteomic changes from mouse administrated with fluoride were investigated in this study. Fig. 1 presents the representative 2DE images of control group and fluoride treatment groups. 16 differently expressed spots were picked up to identify with MALDI-TOF-MS, 15 of which were successfully identified in NCBI mus musculus database (seen in Supplementary file in detail). Table 1 shows the information of mass spectrometry identification result, and the fore three proteins were chosen for every spot under the condition of score >60 and CI% > 95. As shown in Table 2, many of them are associated with the sperm function such as sperm motility, maturation, capacitation and acrosome reaction, lipid peroxidation, detoxification, inflammation, and stability of membrane structure.

4. Discussion

Recently through proteomic techniques such as 2D polyacrylamide gel electrophoresis (2D-PAGE), MS, and differential in gel electrophoresis (DIGE), numerous sperm-specific proteins have been identified from different species (du Plessis et al., 2011; Li et al., 2016). Because proteins are the main functional effectors of cell, more proteomic approaches are applied to compare the differential expression proteins between immature and mature sperm, or before- and after-capacitated sperm, or normal and defective sperm, or sperm from infertility and fertility (du Plessis et al., 2011; Park et al., 2013; Li et al., 2016; Jodar et al., 2017). In the current study, we found 16 differential expression protein spots in fluoride-treatment groups at different exposure times, and 15 of which were successfully identified using MS.

Mammalian sperm fertility-related proteins are divided into six groups, including (1) sperm motility and differentiation related proteins; (2) sperm-zona pellucida interaction and sperm-oolemma penetration proteins; (3) acrosome biogenesis and acrosome reaction proteins; (4) nuclear proteins; (5) peripheral proteins; (6) post-translational modification proteins (Ashrafzadeh et al., 2013). Our identified proteins of 15 differential expression spots were involved in the above category. Due to the complexity of protein functionality, a protein can be classified into different