Reproductive Toxicology 58 (2015) 45–53

Contents lists available at ScienceDirect

Reproductive Toxicology

journal homepage: www.elsevier.com/locate/reprotox

Comparative proteomic profiling and possible toxicological mechanism of acute injury induced by carbon ion radiation in pubertal mice testes

Hongyan Li[a,b,c], Hong Zhang[a,b,c,*], Cuixia Di[a,b,c], Yi Xie[a,b,c], Xin Zhou[a,b,c], Jiawei Yan[a,b,c], Qiuyue Zhao[a,b,c]

[a] *Department of Heavy Ion Radiation Medicine, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China*
[b] *Key Laboratory of Heavy Ion Radiation Biology and Medicine of Chinese Academy of Sciences, Lanzhou 730000, China*
[c] *Key Laboratory of Heavy Ion Radiation Medicine of Gansu Province, Lanzhou 730000, China*

ARTICLE INFO

Article history:
Received 30 January 2015
Received in revised form 9 July 2015
Accepted 21 July 2015
Available online 6 August 2015

Keywords:
Proteomic
Pubertal mice
Testis
Carbon ion radiation

ABSTRACT

We investigated potential mechanisms of acute injury in pubertal mice testes after exposure to carbon ion radiation (CIR). Serum testosterone was measured following whole-body irradiation with a 2 Gy carbon ion beam. Comparative proteomic profiling and Western blotting were applied to identify potential biomarkers and measure protein expression, and terminal dUTP nick end-labeling (TUNEL) was performed to detect apoptotic cells. Immunohistochemistry and immunofluorescence were used to investigate protein localization. Serum testosterone was lowest at 24 h after CIR, and 10 differentially expressed proteins were identified at this time point that included eIF4E, an important regulator of initiation that combines with mTOR and 4EBP1 to control protein synthesis via the mTOR signaling pathway during proliferation and apoptosis. Protein expression and localization studies confirmed their association with acute injury following exposure to CIR. These three proteins may be useful molecular markers for detecting abnormal spermatogenesis following exposure to environmental and therapeutic radiation.

© 2015 Elsevier Inc. All rights reserved.

1. Introduction

Spermatogenesis requires the precise regulation of sequential developmental and differentiation processes [1–3], and proliferation, differentiation and apoptosis in spermatogenic cells must be balanced accordingly. Changes in the regulation of these processes may lead to testicular diseases [4], and radiation or chemical exposure can promote excessive or ectopic apoptosis in spermatogenic cells [5,6]. The development of nuclear technologies has brought great benefits to medicine, industry, and academic research, and ionizing radiation (IR) is now widely used [7,8]. However, with these benefits comes a significant health risk, IR impairs spermatogenesis and can cause mutations resulting in damage and apoptosis in germ cells, leading to asthenospermia, hypospermia, teratospermia, and even infertility [9]. Radiation-induced apoptosis of germ cells is p53-dependent and involves the Fas/FasL system, and the mitochondrial pathway appears to regulate IR-induced apoptosis in germ cells [10]. However, due to the complexity involved, the molecular mechanisms underlying this are poorly understood.

Puberty is a critical period for the development of the mammalian reproductive system [11], during which time the male reproductive system is more susceptible to the action of external factors that can alter reproductive function [12]. Men of reproductive age undergoing radiotherapy are often concerned about the possible effects on fertility and future children [13]. Thus, it is important to investigate acute injuries to testes that can result from exposure to high linear energy transfer (LET) radiation, and to elucidate the mechanisms underlying apoptosis in spermatogenic cells.

Advances in proteomics and mass spectrometry provide appropriate methods for identifying proteins involved in cellular injury

Abbreviations: CIR, carbon ion radiation; 2-DE, two-dimensional gel electrophoresis; MALDI-TOF-TOF, matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry; HIRFL, Heavy Ion Research Facility in Lanzhou; CCB, Colloidal Coomassie Blue G 250; TBS, tris buffer saline; DAB, diaminobenzidine; HRP, horseradish peroxidase; TCPZ, T-complex protein 1 subunit zeta; MAOX, NADP-dependent malic enzyme; PGK2, phosphoglycerate kinase 2; CAPZB, F-actin-capping protein subunit beta; RANG, Ran-specific GTPase-activating protein; GLO2, hydroxyacylglutathione hydrolase (mitochondrial); GSTM2, glutathione S-transferase Mu 2; eIF4E, eukaryotic translation initiation factor 4E; PRDX2, eukaryotic translation initiation factor 3 subunit K (eIF3K) and peroxiredoxin-2.

* Corresponding author at: Department of Heavy Ion Radiation Medicine, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China.
E-mail address: zhangh@impcas.ac.cn (H. Zhang).

http://dx.doi.org/10.1016/j.reprotox.2015.07.079
0890-6238/© 2015 Elsevier Inc. All rights reserved.

and apoptosis. In this study, we used a comparative proteomics approach based on a two-dimensional electrophoresis (2-DE) reference map to determine alterations in protein expression in the testes of pubertal Swiss-Webster mice exposed to CIR. We found that eIF4E may play an important role in proliferation and apoptosis in spermatogenic cells, and the mTOR signaling pathway appears to mediate CIR-induced injury. Integration of proteomics and signaling pathway indicated aspects of the underlying molecular mechanisms of acute injury following CIR.

2. Materials and methods

2.1. Animals

Healthy male Swiss-Webster mice aged 3 weeks and weighing 12–18 g were obtained from Lanzhou Medical College (Lanzhou, China) and randomly assigned to a control (0 Gy) and irradiated (2 Gy) groups ($n = 24$ in each group). Animals were housed in polycarbonate cages with cellulose fiber chips and maintained in a conventional animal facility at 22 ± 2 °C, 60 ± 10% humidity and a 12 h light–12 h dark photoperiod, with 3 mice per cage. All animals were given free access to deionized water in glass bottles with rubber stoppers and rodent feed (Keaoxieli laboratory animal feed, Co., Beijing, China). Animals were acclimatized to the same laboratory conditions for 1 week before use, and all experiments were approved by the Institutional Animal Care Committee.

2.2. Irradiation procedure and animals treatment

4-week-old mice were whole-body irradiated with a 2 Gy carbon ion beam at 300 MeV/U and 31.3 keV/μm (at the beam entrance), to give a dose of approximately 0.5 Gy/min, at the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China). The carbon ion was equipped with a passive beam delivery system. Six mice from each group were killed by cervical dislocation at 6, 12, 18, and 24 h after irradiation. Control animals were housed in the same laboratory conditions and killed in the same way. Blood was collected and centrifuged at 2000 × g, 4 °C for 15 min, and serum was stored at −80 °C until analysis. Testes were removed, and fat and connective tissues were separated. Left testes were fixed in 4% paraformaldehyde solution (4 g paraformaldehyde in 100 mL PBS) and embedded in paraffin blocks, and right testes were frozen immediately in liquid nitrogen and stored at −80 °C until analysis.

2.3. Serum testosterone levels

Serum testosterone levels were measured using an ELISA kit (Elabscience Co., Wuhan, China).

2.4. Sample preparation and measured protein concentration

Right testes were treated with lysis buffer containing 7 M urea, 2 M thiourea, 4% (w/v) 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane sulfonate (CHAPS), 2% (w/v) dithiothreitol (DTT) and 1% (w/v) protease inhibitor cocktail (Sigma Chemical, St. Louis, MO, USA). Protein concentration was measured by the Bio-Rad Bradford protein assay with bovine serum albumin (Sigma) as a standard.

2.5. Two-dimensional gel electrophoresis (2-DE) and image analysis

Testis protein samples (400 μg) were dissolved in 350 μL rehydration buffer and loaded onto 17 cm Immobiline IPG (pH 3–10) strips (Bio-Rad Laboratories, Hercules, USA) and separated on an IPGphor isoelectric focusing system (IEF) using the following settings: 14 h at 50 V; 1 h at 250 V; 1000 V for 1 h; 9000 V for 6 h; 9000 V for 8 h. Thereafter, strips were equilibrated and subjected to 12% SDS-PAGE on a Protean II xi Cell (BioRad) [14]. After electrophoresis, gels were stained with Coomassie Brilliant Blue (CBB)-G250 overnight and destained with ultrapure water three times for 10 min. Three 2-DE gels were prepared for each sample and were scanned with an Epson scanner and stored as TIF files. PDQuest 8.0 software (Bio-Rad) was used to detect and match spots. Spots were selected if the difference in protein quantity was ≥1.5-fold (normalized spot volume) between control and irradiated groups.

2.6. In-gel trypsin digestion of proteins and MALDI-TOF/TOF MS Analysis

Spots were manually cut from 2-DE gels, destained for 20 min in 30 mM potassium ferricyanide/100 mM sodium thiosulfate (1:1 v/v) and washed with Milli-Q water until fully destained. Samples were incubated in 0.2 M NH_4HCO_3 for 20 min, lyophilized, and digested overnight in 25 mM NH_4HCO_3 containing 12.5 ng/mL trypsin. Peptides were extracted three times with 60% acetonitrile (ACN)/0.1% trifluoroacetic acid (TFA). Extracts were pooled and dried completely by a vacuum centrifuge.

MS and MS/MS data for protein identification were obtained using a MALDI-TOF-TOF instrument (4800 proteomics analyzer; Applied Biosystems). Instrument parameters were set using the 4000 Series Explorer software (Applied Biosystems). MS spectra were recorded in reflector mode across a mass range of 800–4000 with a focus mass of 2000. CalMix5 standards were used to calibrate the instrument (ABI 4700 Calibration Mixture). For each spectrum, 25 sub-spectra comprising 125 shots per sub-spectrum were accumulated using a random search pattern. Trypsin autolysis peaks ([M+H]+ 842.5100 and 2,211.1046) were used as internal calibrators, and up to 10 of the most intense ion signals were selected as precursors for MS/MS acquisition, excluding trypsin autolysis peaks and matrix ion signals. In MS/MS positive ion mode, each spectrum comprising 50 sub-spectra and 50 shots per sub-spectrum were accumulated using a random search pattern. The collision energy was 2 kV, the collision gas was air, and the default calibration was set using the Glu1-Fibrino-peptide B ([M+H]+ 1,570.6696) spotted onto Cal 7 positions of the MALDI target. Combined peptide mass fingerprinting PMF and MS/MS queries were performed using the MASCOT 2.2 search engine (Matrix Science, Ltd.) embedded into GPS-Explorer Software 3.6 (Applied Biosystems) in concert with the SwissProt database. The parameter settings were as follows; mass accuracy = 100 ppm, one missed cleavage allowed, carbamidomethylation set as a fixed modification, oxidation of methionine was allowed as a variable modification, MS/MS fragment tolerance was set to 0.4 Da. A GPS Explorer protein confidence index ≥95% was used for further manual validation.

2.7. Observation of spermatogenic cells apoptosis

The in situ Cell Death Detection POD Kit (Roche, Mannheim, Germany) was used to carry out the terminal dUTP nick end-labeling (TUNEL) assay. Briefly, paraffin blocks of testis material were cut into 4 μm thick sections and treated with 20 mg/mL proteinase K solution for 20 min at 37 °C. The TUNEL reaction was then performed in TdT buffer in the presence of dUTP-biotin for 60 min at 37 °C, then incubated with the secondary antifluorescein-POD-conjugate for 30 min. The signal was visualized using diaminobenzidine (DAB). Sections were then counterstained with hematoxylin, dehydrated, cleaned, mounted and observed under a light microscope (Axio10, Zeiss, Germany).

Download English Version:

https://daneshyari.com/en/article/5858040

Download Persian Version:

https://daneshyari.com/article/5858040

Daneshyari.com