Differential expression of serum proteins in rats subchronically exposed to arsenic identified by iTRAQ-based proteomic technology——14-3-3 ζ protein to serve as a potential biomarker

Supplementary Materials

1. Details of serum pretreatment before iTRAQ reagents labeling

Total-protein samples (200μg) diluted in 4% SDS, 100 mM Tris-HCl pH 7.6, and 100 mM dithiotreitol solution were heated at 95℃ for 5 min. After each sample was cooled to room temperature 200 μL UA buffer (8 M Urea and 150 mM Tris-HCl pH8.0) was added into the samples and mixed. Then the mixture was transferred to an ultrafiltration filter (30 kDa cutoff, Sartorius, German) and centrifuged at 14 000×g for15 min. The filtrate was discarded and the filter was washed again with UA buffer. Subsequently, 100 μL of iodoacetamide solution (50 mM iodoacetamide in UA buffer) were added to the filter. The filter unit was mixed at 600 rpm for 1 min, followed by incubation for 30 min at room temperature in the dark and centrifuged at 14 000×g for 10 min. Two wash steps with 100 μL UA buffer were performed with centrifugation at 14 000×g for 10 min after each wash step. Then, 100 μL dissolution buffer (Applied Biosystems, USA) was added to the filter and centrifuged at 14 000×g for 30 min, and this step was repeated twice. Finally, 40 μL of trypsin buffer (2 μg trypsin in 40 μL dissolution buffer) was added and the samples were digested at 37℃ for 16-18 h. Then, the filter unit was transferred to a new collecting tube and centrifuged at 14 000×g for 20 min. Resulting peptides were collected as a filtrate and the peptide concentration was analyzed at OD280.

2. Details of chromatography separation

Buffer A was 0.1% (v/v) formic acid and buffer B was 84% acetonitrile with 0.1% (v/v) formic acid. The column was equilibrated for 20 min with 95% (v/v) buffer A. Samples were auto pumped into EASY column (2cm ×100μm, 5μm-C18, Thermo scientific, USA) and then separated using EASY-nLC column (100 mm×75 μm, 5 μm-C18, Agilent Technologies, USA) at 250 nL/min. Peptides were separated with buffer B using a segmented gradient from 0–55% (v/v) in 220 min, from 55–100% (v/v) in 8 min, and then at 100% (v/v) for 12 min.

3. Instrument parameters of the Q-Exactive mass spectrometer

The mode was set as positive ion, with a selected mass range of 300-1800 mass/charge (m/z). Resolving power for the Q-Exactive was set as 70 000 for the MS scan and 17 500 for the MS/MS scan at m/z 200. MS/MS data were acquired using the top 10 most abundant precursor ions with charge≥2 as determined from the MS scan. These were selected with an isolation window of 2 m/z and fragmented by higher energy collisional dissociation with normalized collision energies of 29eV.The maximum ion injection times for the MS scan and the MS/MS scans were 20 and 60 ms, respectively, and the automatic gain control target values for both scan modes were set to 3e6. Dynamic exclusion for selected precursor ions was set at 25 s. Underfill ratio was defined as 0.1% on the Q-Exactive.

4. The details of parameters for MASCOT software

The following parameters were set: monoisotopic mass, MS/MS tolerance at 0.1 Da and peptide mass tolerance at ± 20 ppm, trypsin as the enzyme and allowing up to two missed cleavages. Fixed modifications were defined as iTRAQ labeling and carbamidomethylation of cysteine; oxidation of methionine was specified as a variable modification. The decoy database

pattern was set as reversed version of the target database. All reported data were based on 99% confidence for proteins and peptides identification as determined by false discovery rate (FDR) of no more than 1%. Protein identification was supported by the identification of at least one unique peptide.