***Sample preparation***

Human glioblastoma cells (a primary cell line L of glial tumor origin was developed in the lab of Cell Biology, Petersburg Nuclear Physics Institute, NRC "Kurchatov Institute") were cultured in 25-cm² tissue culture flasks in DMEM medium containing 5% fetal calf serum in 5% CO2 at 37°C without antibiotics. Adherent cells were grown to approximately 80% confluence and dispersed by trypsin-EDTA treatment. Cells were pelleted and rinsed twice in Versene, then in 10 mM Tris pH 7.4, 250 mM sucrose. (all centrifugations at 500 x g, 5 min). A final pellet of cells (~107) containing ~2 mg of protein was pipetted up and down to break up the cell clumps in 100 µl of lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 1% dithiothreitol (DTT), 2% ampholytes, pH 3-10, protease inhibitor mixture). After incubation at 4°C for 10 min, the lysate was sonicated on ice using a microtip probe sonicator (2 sec/2 sec, 1 min) and precleared by centrifugation at 15000 x g 15 min. [1-4]. The protein concentration in the sample was determined by the method of Bradford [5].

***Two-dimensional electrophoresis (2DE)*** [6]***.***

Proteins were separated by isoelectric focusing (IEF) using ReadyStrip strips pH 3-10, 7 cm (Bio-Rad), ImmobilineDryStrip pH 4-7 and 3-11, 18 cm (GE Healthcare) following the manufacturer's protocol. The samples in the lysis buffer were mixed with rehydrating buffer (7 M urea, 2 M thiourea, 2 % CHAPS, 0,3% DTT, 0,5% IPG buffer, pH 4-7 or 3-11 NL, 0,001% bromphenol blue) in final volume of 130 µl (150 µg of protein) for 7 cm strip or 300 µl (800 µg of protein) for 18 cm strips. Strips were passively rehydrated for 4 h at 4°C. IEF was performed on a 3100 OFF GEL Fractionator (Agilent technologies) which was programmed as follows: for the strips of 7 cm – 6 kV and 20 kVh, 14 h; for strips of 18 cm – 10 kV and 60 kVh, 14 h; temperature 20°C and maintained at the voltage 500 V. After IEF, strips were soaked 10 min in the equilibration solution (50 mM Tris, pH 6,8, 6 M urea, 2% sodium dodecyl sulfate (SDS) and 30 % glycerol) with 1% DTT. This process was followed by 10-min incubation in the equilibration solution containing 5% (w/v) iodacetamide. The strips were placed on the top of the 11% polyacrylamide gel of the second direction and sealed with a hot solution of 1 ml of 0.5% agarose in electrode buffer (25mM Tris, pH 8.3, 200 mM glycine, and 0.1% SDS) and electrophoresed to second direction under denaturing conditions using the system Hoefer miniVE (gel size 80x90x1mm, GE Healthcare) or Ettan™ DALTsix (180x200x1mm, GE Healthcare). Electrophoresis was carried out at room temperature at constant power 3 W for one gel.

***In gel digestion*** [7,8]

 Proteins were subjected to in-gel trypsinization according to following procedure. After three washes in water, the gel pieces were incubated in 50% (vol/vol) acetonitrile and 100 mM ammonium bicarbonate (pH 8.9) for 20 min, then in 100% acetonitrile for 20 min. The pieces were dried for 1 h. Depending on the original size of the gel-slice, 5–8 μl of trypsin solution (25 ng/μl sequencing grade modified trypsin (Promega) in 50 mM ammonium bicarbonate solution) were added and the protein hydrolysis was carried out at 37 °C overnight. Tryptic peptides were extracted by addition of 15 μl extraction solution (5% acetonitrile, 0.5% formic acid) for 30 min and analyzed by ESI LC-MS/MS or MALDI-TOF.

***Mass spectrometry analysis***

All procedures were performed according to the protocol described previously [9,10]. After separation by 2DE and staining with Coomassie R350, the gel pieces with a diameter of 1.5 mm, corresponding to a protein spots were excised using a micropipette tips, partially destained for 15-minute incubation in 500 µl of 50% aqueous acetonitrile (ACN) with 25 mM ammonium bicarbonate. Further, the gel pieces were dehydrated by 10-minute incubation in 200 µl of 100% ACN. ACN was removed, and the gel was dried for 20 minutes in a centrifugal evaporator Speed Vac. The dried gel pieces were rehydrated for 25 min on ice in a 12 µl solution of 25 mM ammonium bicarbonate (ABC) containing trypsin (“Trypsin Gold”, 10 µg/ml) and proteolysis of the protein was performed by incubation at 37°C for at least 4 h. Tryptic peptides were eluted from the gel with extraction solution (5% (v/v) ACN, 5% (v/v) formic acid) and dried in a vacuum centrifuge. For MS analysis peptides were dissolved in 5% (v/v) formic acid.

 ***MALDI-MS analysis*** [3]

 The digest (typically a 1 μL aliquot) was mixed with the same volume of a matrix solution, 10 mg/mL (α-Cyano-4-hydroxycinnamic acid, Fluka, Switzerland) in 0.1% trifluoroacetic acid in 50% acetonitrile, spotted onto the MALDI plate and allowed to crystallize. Mass spectra were acquired in positive reflector mode (voltage of 20 kV in the source 1 and laser intensity ranged from 5800 to 6200). Typically, 500 shots per spectrum were accumulated.

***ESI LC−MS/MS analysis***

Using an Agilent HPLC system 1100 Series (Agilent Technologies), 4 µg of peptides in 5% formic acid were injected onto a trap column Zorbax 300SB–C18, 5 ×0.3 mm (Agilent Technologies). After washing with 5% acetonitrile containing 0.1% formic acid, peptides were resolved on a 150 mm x 75 µm Zorbax 300SB-C18 reverse phase analytical column (Agilent Technologies) over a 30 min organic gradient of 5-60% ACN, 0.1% formic acid with a flow rate of 300 nL/min. Peptides were ionized by nano-electrospray ionization at 2.0 kV using a fused silica emitter with an internal diameter of 8 µm (New Objective). MS/MS analysis was carried out in duplicate on an Orbitrap Q-Exactive (Thermo Scientific). Mass spectra were acquired in positive ion mode. High resolution data was acquired in the Orbitrap analyzer with a resolution of 30 000 (m/z 400) for MS and 7500 (m/z 400) for MS/MS scans. Survey MS scan was followed by MS/MS spectra of the five most abundant precursors. For peptide fragmentation, higher energy collisional dissociation (HCD) was set to 35 eV, the signal threshold was set to 5000 for an isolation window of 2 m/z, and the first mass of HCD spectra was set to 100 m/z. Fragmented precursors were dynamically excluded from targeting for 90 s. Singly charged ions and ion with not defined charge state were excluded from triggering MS/MS scans. The automatic gain control target value was regulated at 1×106 with a maximum injection time of 100 ms and at 1 × 105 with a maximum injection time of 250 ms for MS and MS/MS scans, respectively.

***Protein identification.***

Identification of proteins was performed using Mascot “2.4.1” (Matrix Science, London, UK) by searching UniProt/Swiss-Protein sequence database (October 2014, 20,196 total sequences). The following search parameters were used: trypsin - as the cutting enzyme, mass tolerance for the monoisotopic peptide window was set to ±20 ppm, missed cleavages - 1. Cysteine carbamidomethyl was chosen as a fixed and oxidized methionine as a variable modification. NeXtProt database was used as a protein sequence database. For FDR assessment, a separate decoy database was generated from the protein sequence database. False positive rate of 1% was allowed for protein identification. A minimum Mascot ion score of 30 was used for accepting peptide MS/MS spectra. Data were also searched, using the SearchGUI, an open-source graphical user interface [11]. Two unique peptides per protein were required for all protein identifications. Exponentially modified PAI (emPAI), the exponential form of protein abundance index (PAI) defined as the number of identified peptides divided by the number of theoretically observable tryptic peptides for each protein, was used to estimate protein abundance [12]

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