Original Articles

Proteomic Analysis of Radiation-Induced Alterations in L929 Cells

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Abstract. In this study we examined the protein expression profiles in X-irradiated L929 cells to get insight into how mammalian cells respond to radiation-induced cell damage. L929 cells were irradiated with the dose of 6 Gy and cell lysates were collected at different time intervals (20 min, 12, 24, 36, 48 and 72 h). The extracted proteins were separated by 2-DE and quantified using computerized image analysis. Proteins exhibiting significant abundance alterations when comparing irradiated to unirradiated cells were identified by mass spectrometry. Using the proteomics approach we detected 47 proteins that exhibited a significant radiation-induced increase or decrease in the course of 72 h. From this group of spots 28 proteins were identified by mass spectrometry and of these 24 proteins exhibited minimally 2-fold differences in mean abundance values in comparison to controls. The identified proteins represent diverse sets of proteins participating either in protective and reparative cell responses or in induction of apoptosis and oncogenesis. The results document that proteomics is a useful method for unravelling the molecular mechanisms involved in cell reaction to ionizing radiation.

One of the crucial aims in radiobiological research is the search for biological parameters applicable for early detection of radiation-induced cell damage (biological indicators) and estimation of the absorbed radiation dose (biological dosimetry). Recently, it has been shown that the sum of different modes of cell death, namely micronucleation, apoptosis and abnormal cells (MAA) (e.g. necrotic cells; MAA-assay) correlates with the radiation-induced cell damage, but independently of the cell model in use (Abend et al., 2000). This suggested that MAA might be suitable as a biological indicator in tissues of different origin. However, the assay has its limitations (delayed onset of measurements, multiple measurements necessary and time consuming). From the clinical point of view, morphological changes after irradiation appear too late. In the case of radiation accidents it would also be desirable receiving dose estimates within a short period of time using a simple assay. Furthermore, there are indications that not only apoptosis, but also other modes of cell death like micronucleation (Abend et al., 1999) and even necrosis represent actively controlled processes of cell destruction induced by the damaged cells. It is very likely that those processes are controlled at the gene expression level.

Nowadays, monitoring of radiation-induced alterations in gene expression represents the most rapidly developing area of research aimed at identification of molecular markers for radiation exposure. Several methods have been developed for the comprehensive analyses of gene expression in complex biological systems. Until now the major effort was focused on application of procedures based on genomic and transcriptomic profiling. Ongoing experiments based

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Abbreviations: ALDR – aldose reductase, DTT – dithiothreitol, eIF 5A – initiation factor 5A, HINT – histidin triad nucleotide-binding protein, IMPDH-II – inosin-5'-monophosphate dehydrogenase 2, IPG – immobilized pH gradient, MAA – micronucleation, apoptosis, abnormal cells, MN – micronuclei, MTF2 – metal response element-binding transcription factor, NDK B – nucleoside diphosphate kinase B, PDI – protein disulphide isomerase, RhoGDI 1 – rho GDP-dissociation inhibitor 1, RhoGDI 2 – rho GDP-dissociation inhibitor 2, SDS-PAGE – sodium dodecylsulphate polyacrylamide gels, TCP-1 – T-complex protein 1, TCTP – translationally controlled tumour protein, VEGF-D – vascular endothelial growth factor D, 2-DE – two-dimensional gel electrophoresis.

on macro- or microarray hybridization protocols have been identifying large numbers of potential biomarkers (Admundson et al., 2001). However, these approaches exhibit various limitations (Celis et al., 1998); therefore, the ultimate profiling of gene expression necessitates incorporation of a third procedure, named proteomics, which encompasses qualitative and quantitative analyses of real biological effectors – proteins (Hanash et al., 2002). In the presented study we applied proteomics for the measurement of protein expression during the first three days after cell irradiation. Using a comparative proteomics procedure, the proteins exhibiting significant radiation-induced down- or upregulation were detected and their possible roles in radiation-induced cell response were suggested.

Material and Methods

Cells and cell culture

Mouse fibrosarcoma cells (L929) (Flow Laboratories, Meckenheim, Germany) were grown as a monolayer culture and subcultured twice per week. Cells were maintained in the MEM medium (Sigma, Deisenhofen, Germany) supplemented with 20% heat-inactivated foetal calf serum (Boehringer Mannheim, Mannheim, Germany). Culture conditions were 37˚C in a humidified atmosphere buffered by 5% CO_2 in air and hydrogen carbonate (Merck, Darmstadt, Germany), at a pH 7.4.

Radiation conditions

Twenty-four hours after passaging, exponentially growing cell cultures were irradiated at room temperature with single doses of 240 kV X-rays (Isovolt 320/10; Seifert, Ahrensberg, Germany) filtered with 3 mm Be. The absorbed dose was measured with a Duplex dosimeter (PTW; Freiburg, Germany). The dose rate was ~ 1 Gy/min at 13 mA: L929 cells were irradiated with 6 Gy.

Sample preparation for two-dimensional gel electrophoresis

The L929 cells were lysed in 500 µl of lysis buffer (137 mM NaCl, 10% glycerol, 1% n-octylglucopyranosid, 50 mM NaF, 20 mM TRIS, pH 8, Complete ^{TM}Mini, 1 mM Na_3VO_4) at 20 min, 12, 24, 36, 48 and 72 h after irradiation. Extracted proteins were precipitated overnight in 20% trichloroacetic acid (TCA) in acetone (-18˚C) containing 0.2% dithiothreitol (DTT) (Görg et al., 1997) and then solubilized in a buffer for isoelectric focusing (IEF) (9 M urea, 4% CHAPS, 70 mM DTT and 2% carrier ampholytes pH 9–11). The lysates were centrifuged (5 min; 15 000 x *g*; 4˚C) and protein concentration in the IEF buffer was determined by the modified bicinchoninic acid (BCA) assay (Brown et al., 1989).

Two-dimensional gel electrophoresis and analysis of gel images

Immobilized pH 3–10 gradient (IPG) gels (Immobiline DryStrip 3–10 NL, Amersham Pharmacia Biotech, Uppsala, Sweden) were used for first dimension-isoelectric focusing. Commercial strips were swollen in rehydration buffer containing 2 M thiourea, 5 M urea, 2% CHAPS, 2% SB 3–10, 2 mM tributyl phosphine (TBP), 40 mM Tris base and 0.5% Ampholine, pH 3–10, overnight. For analytical two-dimensional gel electrophoresis (2-DE), 100 µg of proteins were loaded in the first dimension. The sample was applied at the cathodic side of the gel and the IPG separation was carried out using a Multiphor II apparatus (Amersham Pharmacia Biotech) overnight (100 kVh, 20˚C). Immediately after being focused, IPG gels were equilibrated for 15 min in 6 M urea, 2% sodium dodecylsulphate (SDS), 50 mM Tris-HCl, pH 6.8, 30% v/v glycerol and 1% DTT, then for 15 min in the same solution except that DTT was replaced by 5% w/v iodoacetamide. In the second dimension the vertical slab gradient 9–16% SDS polyacrylamide gels (SDS-PAGE) were used. These gels were cast in a Protean^{TM} multi-gel casting chamber and SDS-PAGE electrophoresis was performed using a Protean^{TM} 2-D multi-cell apparatus (Bio-Rad, Hercules, CA) at 40 mA per gel for 5 h. Immediately after electrophoresis, proteins were visualized by silver staining (Hochstrasser et al., 1988) and 12-bit monochromatic images at 50 µm resolution were obtained by scanning gels with a laser densitometer (Molecular Dynamics, Palo Alto, CA). Two gels were run for each sample. The computerized image analysis was carried out using a Melanie 3 software package (Bio-Rad). Proteins separated by 2-DE were quantitated in terms of their relative spot volumes (% vol), i.e., digitized staining intensity integrated over the area of an individual spot divided by the sum of integrated staining intensities of all spots and multiplied by 100. The quantitative data from two independently prepared samples were submitted to Student's *t*-test implemented in Melanie 3 software for determination of significant differences at the levels of $P < 0.05$. From this group of spots only proteins having a positive or a negative fold change with magnitude of ≥ 2 were further taken into account.

In-gel digestion and mass spectrometry

For the micropreparative 2-DE gels, 1 mg of protein was loaded on IPG strips. Selected spots stained with Coomassie blue R-250 were then excised and covered with 500 µl of 100 mM Tris-HCl, pH 8.5, in 50% acetonitrile for 20 min at 30˚C. Five hundred µl of equilibration buffer (50 mM ammonium bicarbonate, pH 7.8 in 5% acetonitrile) were added to the gel pieces. After that the gel pieces were vacuum-dried, covered with 0.1 µg of sequencing grade trypsin (Promega,

Madison, WI) in 30 µl of 50 mM ammonium bicarbonate, pH 7.8, and 5% acetonitrile and mildly shaken overnight at 37°C. The mass spectra were recorded in reflector mode in a MALDI mass spectrometer Voyager-DE STR (Perseptive Biosystems, Framingham, MA) equipped with delayed extraction. The peptide mixture samples were interfused with the same volume of the matrix solution α-cyano-4-hydroxycinnamic acid (in 50% acetonitrile with 0.5% trifluoroacetic acid (TFA)), 2 µl of the solution were applied to a plated sample holder and introduced into the mass spectrometer after drying. The instrument was calibrated externally with a two-point calibration using peptide standards. Proteins were identified by peptide mass fingerprinting (PMF) using ProFound and PeptIdent programs. Liquid chromatography-tandem mass spectrometry data were recorded in a LC-MS/MS mass spectrometer Q-TOF Ultima™ API (Micromass, Manchester, UK) fitted with a nanospray ionization source (Z-spray). The mass spectrometer combines the selectivity of a quadrupole (MS 1) with the ultra high efficiency of an orthogonal acceleration time of flight (TOF) mass analyzer (MS 2). The in-gel protein digest samples were separated by means of a Micromass modular CapLC system (Micromass) connected directly to the Z-spray source. The MS/MS spectra were acquired on the four most intense ions. All data were processed automatically by means of ProteinLynx software. Proteins were identified by searching the Non Redundant Data Base (NRDB) using the ProteinLynx Global Server engine.

Results

Comparative proteomics analysis of unirradiated and X-irradiated L929 cells

Proteome analysis was performed with cells 20 min, 12, 24, 36, 48 and 72 h after irradiation with the dose of 6 Gy. As demonstrated previously (Abend et al., 2000), this dose induces the formation of micronuclei (MN), whose level starts to accrue at 18 h and peaks from 30 to 42 h after irradiation. Approximately 2000 spots were resolved with Melanie 3 software in gels of both control and irradiated cells. Globally, computer-assisted comparative analysis of 2-DE protein patterns of unirradiated and irradiated L929 cells revealed 47 proteins exhibiting significant radiation-induced alterations. All these differentially expressed proteins were submitted to peptide mass fingerprinting or LC-MS/MS analysis resulting in successful identification of 28 proteins (60%). The positions of all identified protein spots are indicated in the reference silver-stained gels of unirradiated and X-irradiated L929 cell

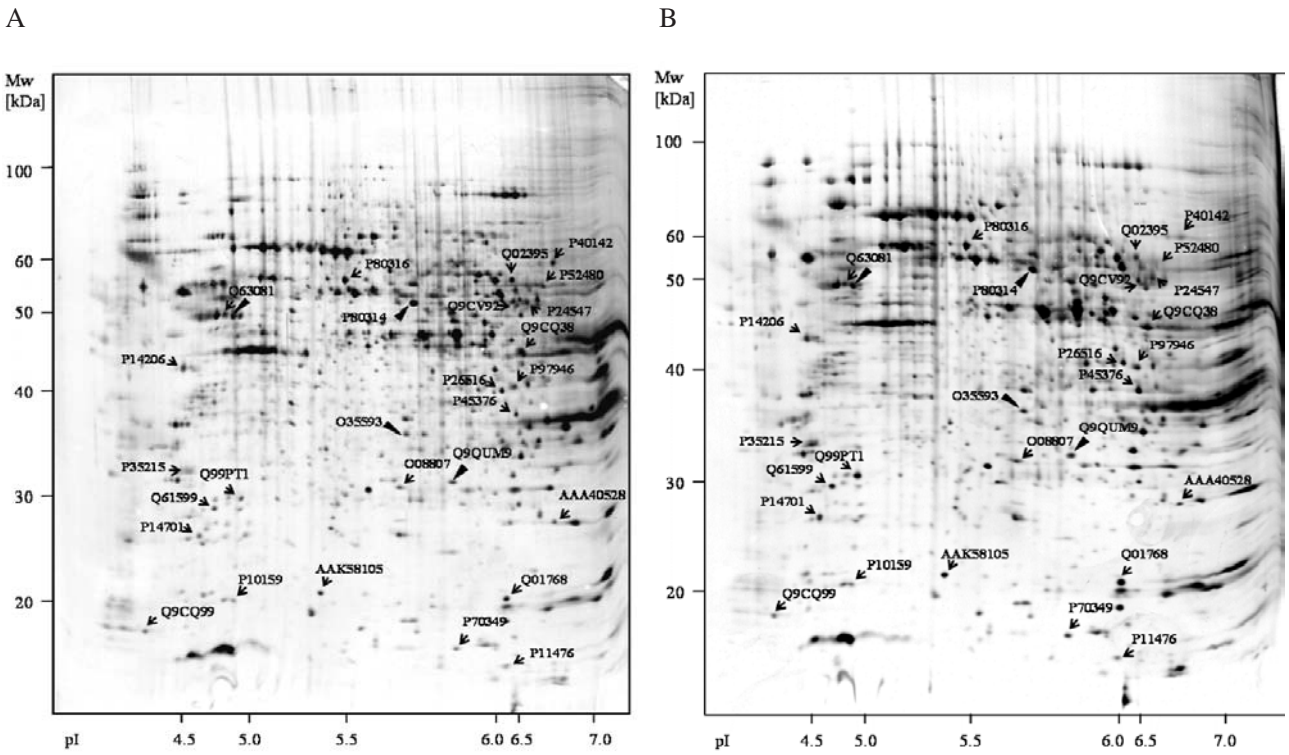


Fig. 1. Silver-stained 2-DE protein pattern of unirradiated (A) and X-irradiated (B) murine fibroblast L929 cell line. All identified spots are labelled with their accession numbers. Arrows mark the proteins whose levels exhibited a minimally 2-fold change comparing to controls; arrowheads then denote the proteins whose radiation-induced abundance alterations were also found to be statistically significant, but the magnitude of the mean abundance change was less than 2.

line (Fig. 1A, 1B). The annotations for 28 differentially expressed features are listed in Table 1. Of the 28 differential features identified, we further selected proteins whose mean abundance values differed by ≥ 2 fold. In total, 24 proteins met this criterion, and their expression profiles in the course of 72 h after X-irradiation are depicted in Fig. 2.

Eleven proteins exhibited significant abundance alterations in late time intervals from 24 to 48 h, in which the formation of MN is started and reaches its maximum. This group of spots involved rho GDP-dissociation inhibitor 2 (RhoGDI 2), two variants of RIK protein, epsilon subunit of T-complex protein 1 (TCP-1), regulatory subunit of proteasome complex, enzyme transketolase, metal response element-binding transcription factor (MTF2), translationally controlled tumour protein (TCTP), histidine triad nucleotide-binding protein (HINT), aldose reductase (ALDR) and nucleoside diphosphate kinase B (NDK B). The expression of seven proteins (protein disulphide isomerase (PDI) A6 precursor, Rab 1, 14-3-3 protein zeta/delta, pyruvate kinase, initiation factor 5A (eIF 5A), rho GDP-dissociation inhibitor 1 (RhoGDI 1) and L04851 putative protein) was altered in both early and late examined periods. Epididymal 17 kDa precursor was then an example of early radiation-induced abundance alteration and, conversely, the level of five proteins (2610511I09RIK protein, peroxiredoxin 4, vascular endothelial growth factor D (VEGF-D), inosin-5'-monophosphate dehydrogenase 2 (IMPDH-II) and 40S ribosomal protein) was altered in the last examined interval 72 h. Partial 2-DE images showing differences in the protein expression level of spots exhibiting significant 2-fold abundance alterations after irradiation are denoted in Fig. 3A and 3B.

Discussion

Identification of new molecular biomarkers for radiation exposure assessment can have profound beneficial applicability in radiation epidemiology, therapy and biodosimetry. Our strategy is to identify radiation-responsive gene expression targets using a comparative proteomics procedure. The main goal of this approach is the determination of qualitative and quantitative differences in the protein composition of two or more samples followed by identification of selected proteins (Smolka et al., 2002). Using an *in vitro* model system of murine L929 fibroblasts we searched for differentially expressed proteins in the course of three days after X-irradiation. We identified 24 proteins whose abundances were significantly altered by radiation. The identified proteins can be divided into seven functional groups referring to which cellular systems were targeted by ionizing radiation.

The first group involves five proteins engaged in cell signalling pathways. RhoGDI 1 and 2 are cytosolic proteins that participate in the regulation of both the GDP/GTP cycle and the membrane association/dissociation cycle of Rho/Rac proteins (Olofsson et al., 1999). Radiation regulates, especially in late intervals, protein levels of both RhoGDIs differentially. So, while the RhoGDI 2 level exhibits severe suppression from 24 to 48 h after irradiation, the amount of RhoGDI 1 exerts strong induction two days after treatment. The opposite regulation of RhoGDI 1 and RhoGDI 2 abundances was also described for drug-induced apoptosis (Essman et al., 2000). Rab 1 is a member of the Ras family of GTPases and it controls transport events through early Golgi compartments (Martinez and Goud, 1998). Rab 1 is exceptional among identified proteins because its level is besides 24 h upregulated by radiation in all examined time intervals. There is no information about the particular role of Rab 1 in irradiated cells. Nevertheless, there is substantial evidence to demonstrate that increased radiation resistance is accompanied with increased expression of other members of the Ras protein family (Jones et al., 2001). A further identified protein included in this group, the 14-3-3 zeta/delta, is probably a multifunctional regulator of the cell signalling processes mediated by both protein kinase II and protein kinase C. The 14-3-3 family members are dimeric phosphoserine-binding proteins, and primary function of mammalian 14-3-3 proteins is to inhibit apoptosis (Xing et al., 2000). Other authors (Hermeking et al., 1997) discovered that 14-3-3 sigma protein levels are strongly induced by gamma irradiation and other DNA-damaging agents in a colorectal cancer cell line. Our data correspond with the published finding and the increased level of 14-3-3 zeta/delta protein can substantiate the reluctance of L929 cells to activate the apoptotic process. HINT is an intracellular receptor for purine mononucleotides and it is an inhibitor of protein kinase C. Recently, 2-fold downregulation of HINT was observed in a human bronchial epithelial cell line irradiated by low doses of gamma-rays (Gamble et al., 2000). In our study, a significant decrease of HINT concentration was found 24 h after irradiation of L929 cells.

The second group of the identified proteins comprises two enzymes of carbohydrate metabolism, transketolase and pyruvate kinase, M2 isozyme. While transketolase was significantly upregulated in X-irradiated cells, the level of pyruvate kinase, M2 isozyme shows a more complicated pattern of expression, with two peaks at 12 and 36 h followed by the decline in the last two examined intervals 48 and 72 h. The effect of ionizing radiation on the increase of glycolytic metabolism was recently described in cultured tumour cells (Fujibayashi et al., 1997).

A further group is formed with two enzymes that are engaged in the detoxification process. ALDR exhibits a broad substrate specificity for many endogenous and xenobiotic carbonyl compounds. The upregulation of ALDR can reflect the accumulation of both saturated and

Table 1. The list of 28 identified proteins exhibiting significant radiation-induced abundance alterations. Proteins were annotated by MALDI-TOF MS; LC-MS/MS was applied only in the case of PDI, A6 precursor.

Protein name	Accesion No.	Mr[kDa] / pI theoretical	Mr[kDa] / pI measured	Sequence coverage
RHO GDP-dissociation inhibitor 1 [a)]	Q99PT1	23.4/5.1	29.3/4.9	35.3 %
RHO GDP-dissociation inhibitor 2 [a)]	Q61599	22.9/4.9	28.2/4.7	63.0 %
Ras-related protein Rab 1A [a)]	P11476	22.7/5.9	17.0/5.9	23.0 %
14-3-3 protein zeta/delta [a)]	P35215	27.8/4.7	31.9/4.5	17.1 %
Histidine triad nucleotide-binding protein [a)]	P70349	13.9/6.4	18.0/5.8	52.0 %
Transketolase [a)]	P40142	61.1/6.5	60.9/6.8	25.0 %
Pyruvate kinase, M2 isozyme [a)]	P52480	57.8/7.4	54.2/6.7	13.6 %
Aldose reductase [a)]	P45376	36.1/6.7	36.6/6.5	44.0 %
T-complex protein 1, epsilon subunit [a)]	P80316	59.6/5.7	57.0/5.5	33.1 %
Protein disulphide isomerase A6 precursor [a)]	Q63081	47.2/5.0	49.7/4.8	17.2 %
Nucleoside diphosphate kinase B [a)]	Q01768	17.4/7.0	20.8/6.0	27.6 %
Vascular endothelial growth factor D [a)]	P97946	40.9/6.3	38.6/6.0	31.3 %
Inosine-5'-monophosphate dehydrogenase 2 [a)]	P24547	56.2/6.9	53.9/6.1	13.0 %
Translationally controlled tumour protein [a)]	P14701	19.5/4.8	25.4/4.6	30.2 %
40S Ribosomal protein SA [a)]	P14206	32.7/4.7	41.6/4.5	19.0 %
Initiation factor 5A [a)]	P10159	15.6/4.3	20.7/4.8	58.2 %
26S Proteasome regulatory subunit S12 [a)]	P26516	36.5/6.3	39.0/6.0	28.7 %
Metal-response element-binding transcription factor 2 [a)]	Q02395	42.0/8.1	54.3/6.0	15.0 %
Epididymal 17 kDa protein precursor [a)]	AAK58105	20.0/5.7	21.3/5.4	13.0 %
(L04851) putative protein [a)]	AAA40528	26.0/7.9	26.7/6.8	19.0 %
2610008O03RIK protein [a)]	Q9CV92	51.3/8.9	52.4/6.0	38.5 %
2700049I22RIK protein [a)]	Q9CQ99	11.7/4.4	19.2/4.3	69.6 %
2610511I09RIK [a)]	Q9CQ38	47.0/6.9	45.5/6.1	25.9 %
Peroxiredoxin 4 [a)]	O08807	31.3/6.7	30.5/5.7	24.0 %
Protein disulphide isomerase A6 precursor [b)]	Q63081	47.2/5.0	49.3/4.9	24.8 %
Proteasome subunit, alpha type 6 [b)]	Q9QUM9	27.8/6.3	30.8/5.8	21.0 %
T-complex protein 1, beta subunit [b)]	P80314	57.4/5.9	53.2/5.7	51.6 %
26S Proteasome, non-ATPase subunit [b)]	O35593	34.6/6.2	34.6/5.7	24.3 %

a) Proteins whose radiation-induced abundance alterations exhibited either a positive or a negative fold change with magnitude of ≥ 2.

b) Proteins whose radiation-induced abundance alterations exhibited a statistically significant either positive or negative fold change with magnitude of < 2.

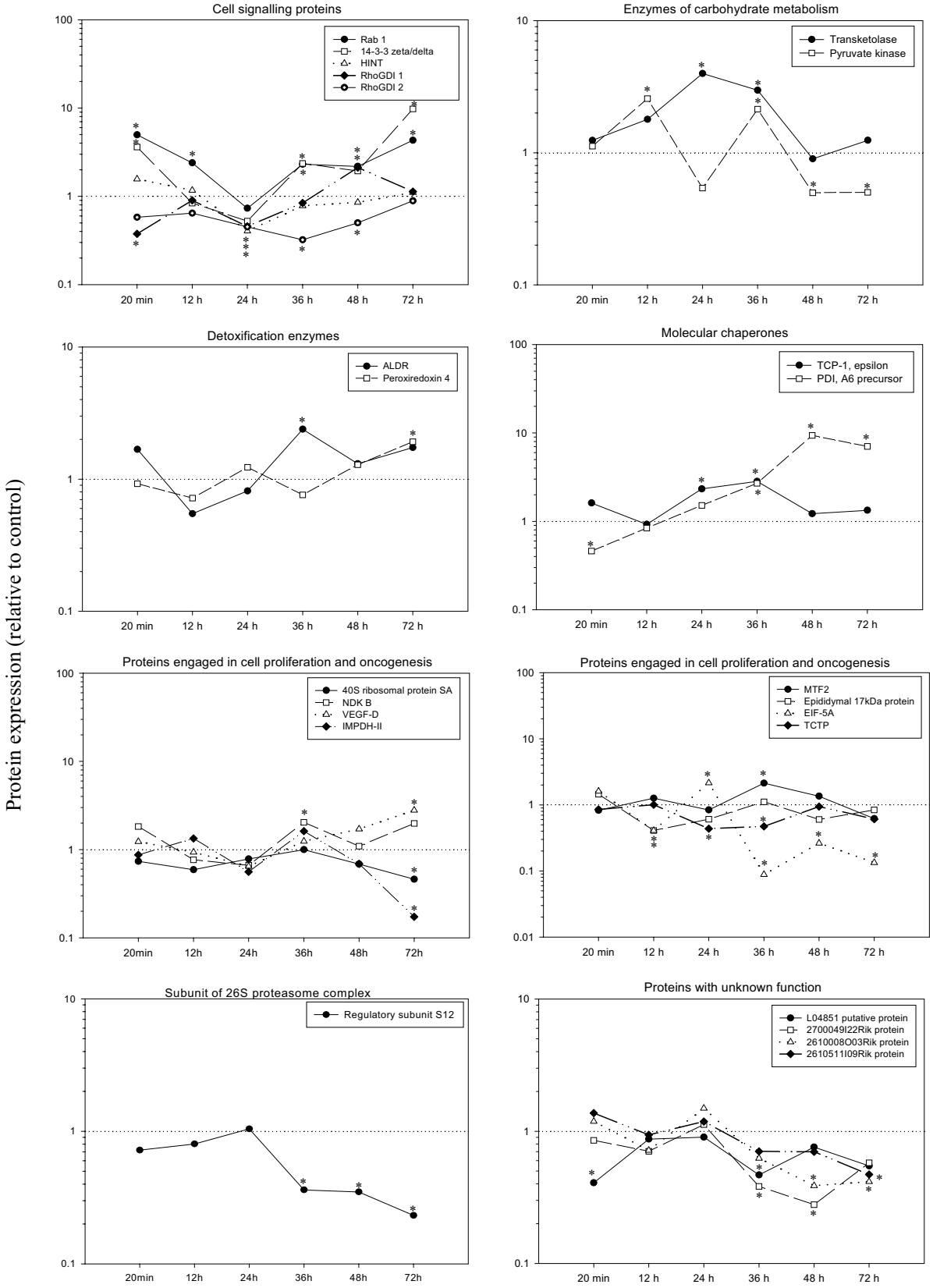


Fig. 2. Radiation-induced abundance alterations of identified 24 proteins in the course of 72 h. Each data point represents the mean values of relative spot intensities from two independent experiments (N = 2). Asterisks indicate the protein levels exhibiting a radiation-induced 2-fold increase or decrease.

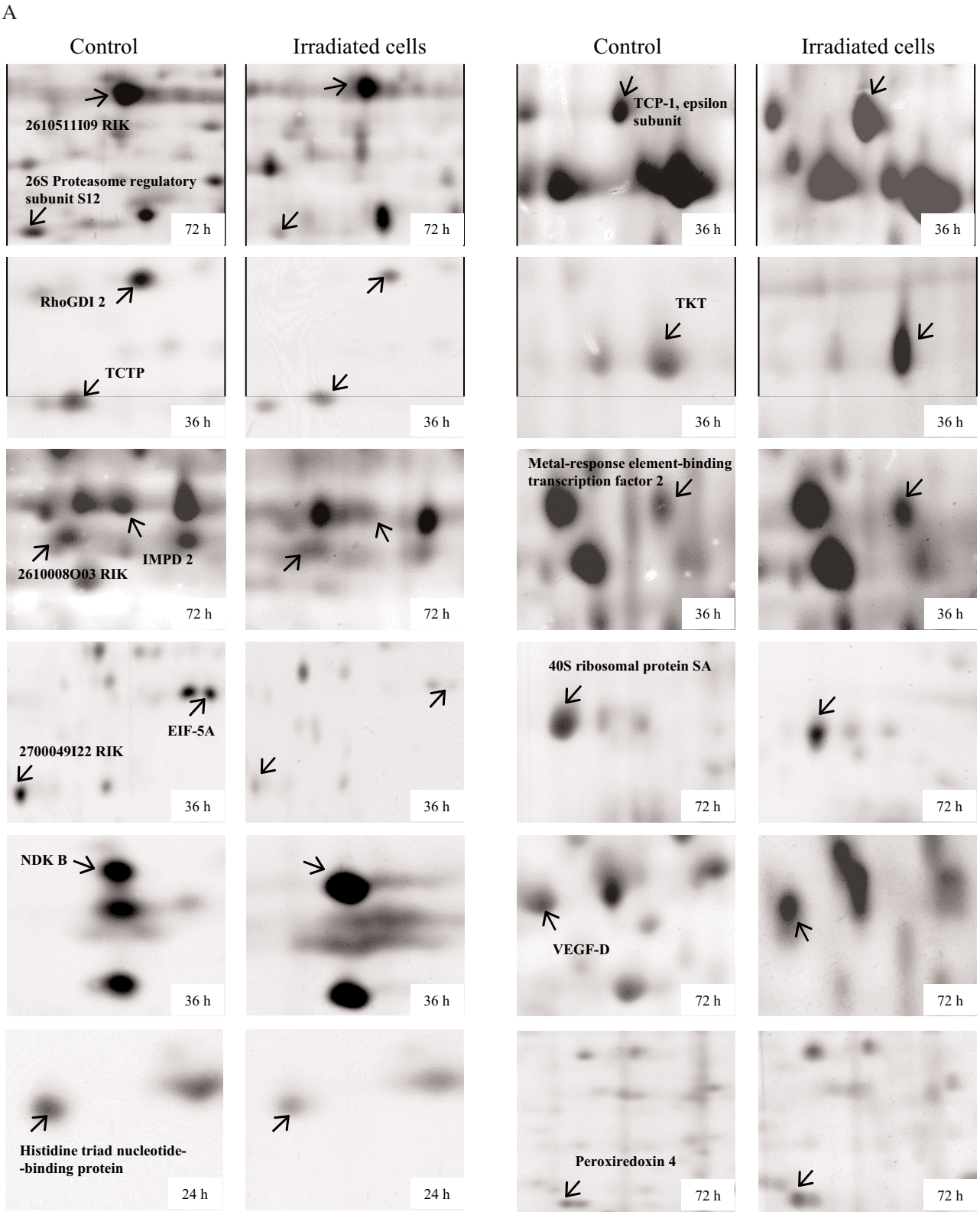
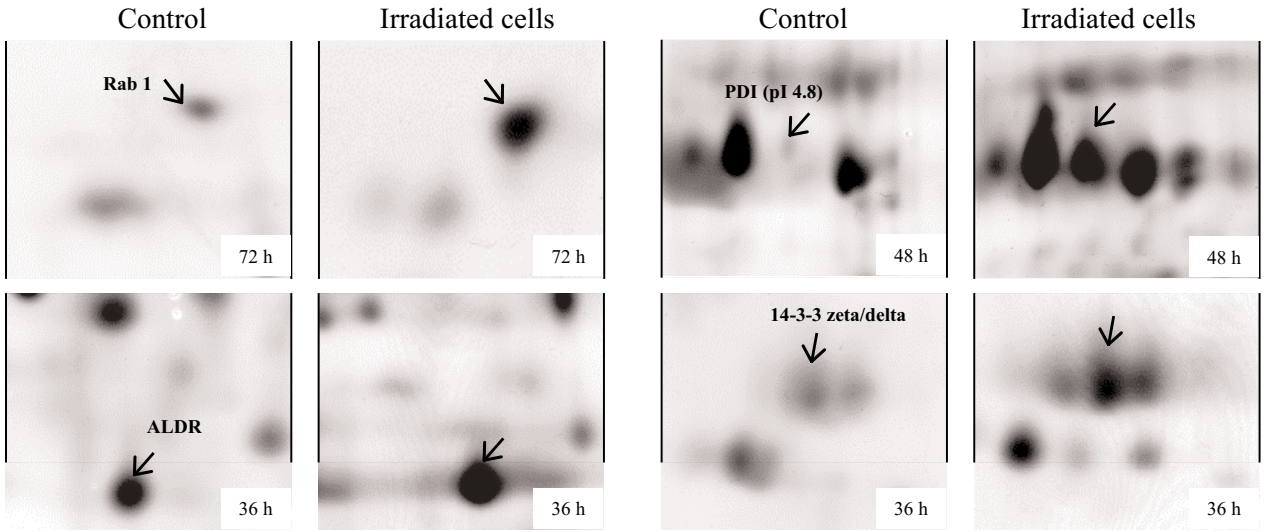
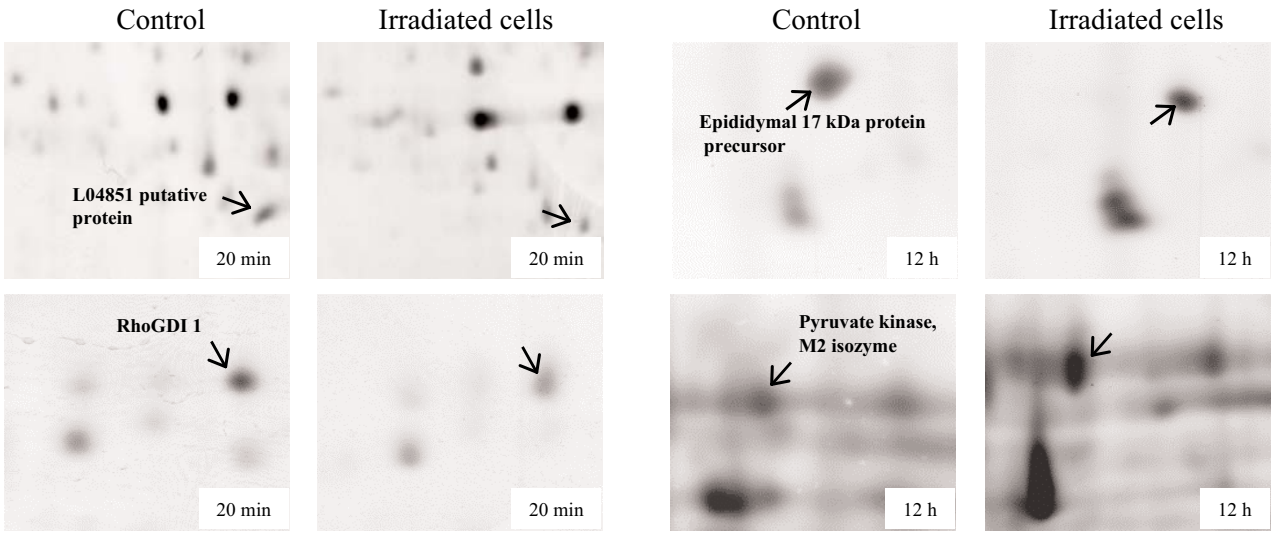


Fig. 3. The sections of two-dimensional images showing differences in protein expressions between control (left panel) and irradiated L929 cells (right panel) in the late (A) or early (B) time intervals. The position of protein spots exhibiting significant 2-fold abundance alterations is denoted with arrows in selected sections.

A – continued



B



unsaturated aldehydes in irradiated cells (Przybyszewski et al., 2002). Similarly, the level of peroxiredoxin 4 that acts as a scavenger of intracellular H_2O_2 and inhibitor of apoptosis (Zhang et al., 1997; Kang et al., 1998) was augmented 72 h after X-irradiation.

TCP-1, epsilon subunit and PDI A6 belong to the group of proteins with chaperone activity. TCP-1 is a hetero-oligomeric molecular chaperone that mediates protein folding in the cytosol of eukaryocytes (Kubota et al., 1999). Induction of apoptosis in Jurkat cells by treatment with anti-CD95 antibody diminished the TCP-1 level in cytosol and prompted its translocation to the nuclear fraction (Gerner et al., 2000). In our case we measured the radiation-induced increase in the amount of TCP-1 in time intervals 24 and 36 h after treatment. PDI A6 exhibits both chaperone and isomerase/foldase activities. The radiation-induced 2-fold increase of PDI abundance was first detected in the time interval 36 h after irradiation; at this time point it overlapped with accumulation of the TCP-1 protein, the maximal level was observed at 48 h and the increased level was still maintained 72 h after treatment. Recently, radiation-induced PDI A6 level was measured in irradiated human prostate epithelial cells (Prasad et al., 1999).

A further group includes eight proteins that take part in the regulation of cell proliferation and oncogenesis. NDK B was identified as a transcription factor stimulating the transcription of the c-*myc* oncogene (Agou et al., 1999). Ionizing radiation has a positive effect on NDK B activity as it was documented by Savitskii and Nagier (1983). We also proved a significant up-regulation of the NDK B level in L929 cells 36 h after X-irradiation. VEGF plays an important role in angiogenesis and cell proliferation of cancer cells (Mori et al., 2000). Radiation-induced alterations usually lead to accumulation of the VEGF protein in many carcinoma cell lines

(Gorski et al., 1999; Park et al., 2001). Our results show a 2-fold increase in the VEGF D protein level at 72 h after cell irradiation. MTF2 is believed to play a generalized role in regulating genes involved in protection against oxidative stress (Solis et al., 2002). This finding may be supported by our result describing overexpression of the MTF2 protein level at 36 h after X-irradiation. Inosine 5'-monophosphate dehydrogenase is a rate-limiting enzyme for guanine nucleotide biosynthesis. Two isoforms of this enzyme have been identified. IMPDH-1 is present in normal cells, whereas type 2 is predominant in malignant cells (Jayaram et al., 1999). Modulation of these isoforms by ionizing radiation was not described yet. Our results show a significant radiation-induced decrease in the amount of IMPDH-II at 72 h. TCTP is a calcium-binding protein occurring in several healthy and malignant cells (Sanchez et al., 1997). The cDNA of the TCTP gene is up-regulated during vitamin D-induced rat glioma cell death, indicating the more general role of TCTP in induction of programmed cell death (Baudet et al., 1998). So, TCTP radiation-induced decrease is also in accordance with the apoptosis-resistance character of L929 cells. The 40S ribosomal protein and initiation factor 5A (eIF 5A) are components of the protein synthetic apparatus. Deletion of the gene encoding the 40S ribosomal protein abrogated 40S ribosome biogenesis and induced a checkpoint control that prevented cell cycle progression (Volarevic et al., 2000). In our study we detected a significant decline in the 40S ribosomal protein level three days after evaluation. The kinetics of eIF 5A production, after an initial decline, exerts an accretion 24 h after irradiation, which is replaced by a steep fall in following time intervals. Takeuchi et al. (2002) published the results showing that the loss of eIF 5A is associated with a decreased cell growth rate and finally with diminished cell viability without chromosomal DNA fragmentation. Regarding the last member in this group, epididymal 17 kDa protein precursor, currently there is no information about the relationship between gamma-irradiation and its abundance alterations.

Regulatory subunit S12 of 26S proteasome forms the sixth group of identified proteins. Regulatory subunit S12 exhibits an abundance suppression from the time interval 36 h after irradiation. This finding coincides with the described inhibitory effect of both low- and high-dose irradiation on proteasome activity (Pajonk and McBride, 2001). Furthermore, the 26S proteasome complex acts as a negative regulator of genomic nucleotide excision repair (Lommel et al., 2000). Hence, radiation-induced inhibition of proteasome function can be beneficial for the DNA repair mechanism.

The last group consists of the L04851 putative protein and three variants of the RIK protein, whose function is unknown. The levels of all these proteins exhibit radiation-induced downregulation.

In conclusion, in this study we utilized the proteomics approach for a comprehensive analysis of X-irradiated mouse fibrosarcoma L929 cells. We have identified 28 proteins that exhibited significant radiation-induced alterations. From the functional point of view, most of these proteins seem to affect various events induced by ionizing radiation such as antioxidant reaction, changes in cell cycle proliferation, DNA repair, apoptosis and oncogenesis. These results document the applicability of the proteomics approach for such type of study. The steps are underway to enrich current 2-DE protein profiles about low-abundance proteins, proteins with extreme pI values and hydrophobic membrane proteins.

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