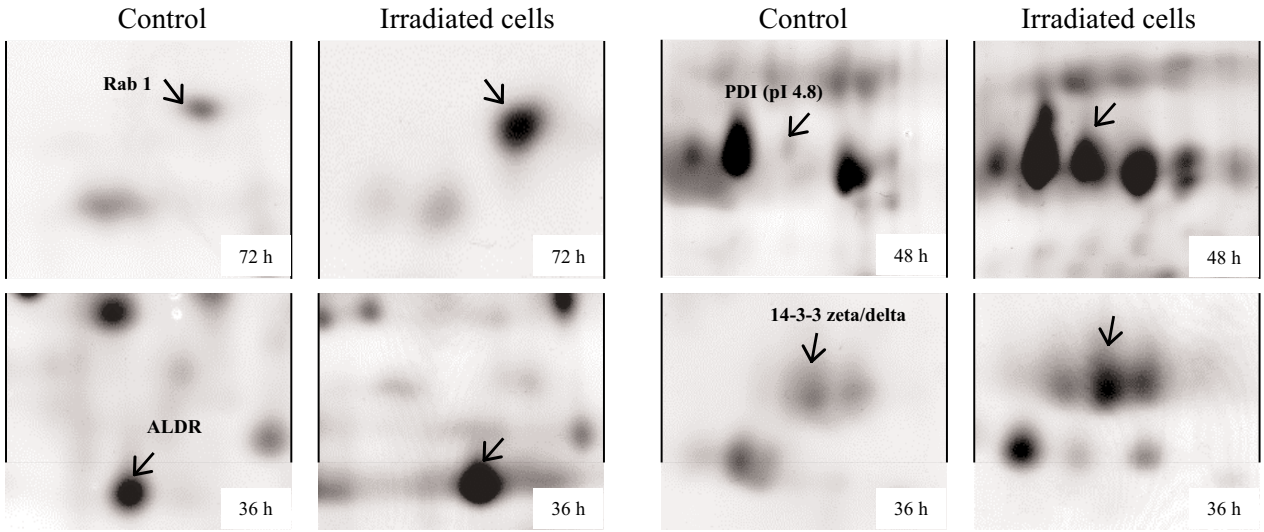
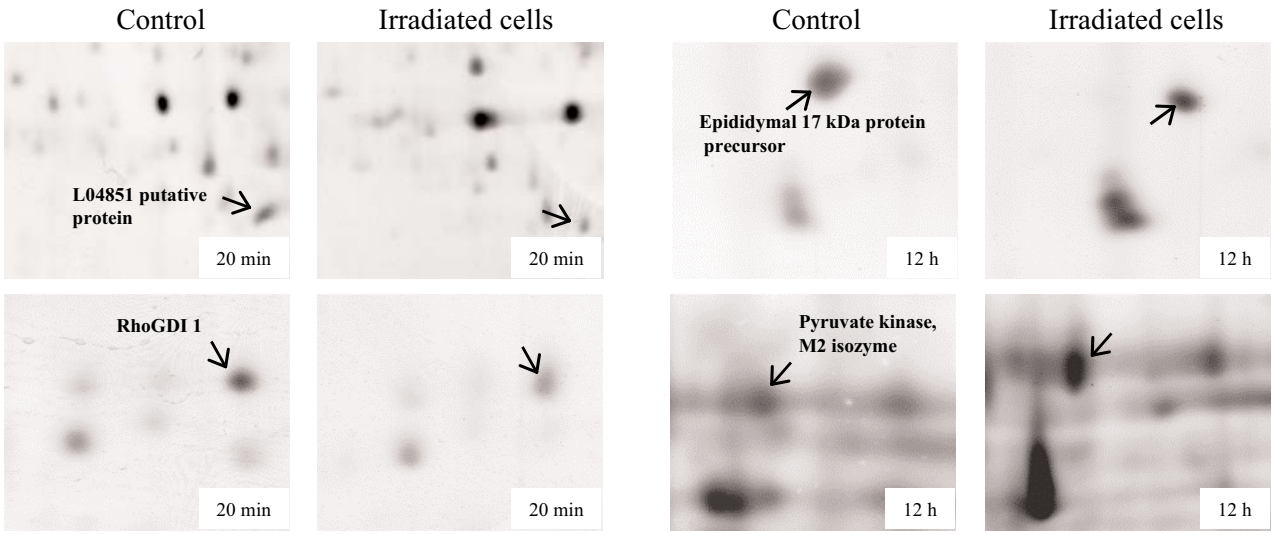
A – continued



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.

Acknowlegemens

The authors thank Jana Michaličková, Alena Fitychová and Inge Nuyken for excellent technical assistence.

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