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Early response of lymphocyte proteins after gamma-radiation

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In radiation biology there is a recent trend of looking for suitable biological parameters to detect early radiation-induced cellular damage. The present paper mentions 29 early response proteins (ERPRO) in human lymphocytes separated by 2 D-SDS-PA gelelectrophoresis 15 minutes after γ-radiation (2 Gy). A third of these proteins including phosphoproteins were identified using Nano-HPLC-MS. Most of the proteins were structural proteins (β-actin, mutant β-actin, talin and zyxin) except phosphoglycerate kinase-1, an enzyme of the glycolysis. For a more complete and detailed picture of ERPRO, the amount of proteins per gel has to be increased in the future by using a preparative gel instead of an analytical one.

Introduction

Recent papers in radiation biology show an increased trend towards looking for suitable biological parameters to detect early radiation-induced cell damage. It is well known that ionizing radiation causes different types of damage to biological cells, especially to the cellular chromatin. The extent of damage is evaluated by measurements of biological endpoints which play an important role in the understanding of cell death, mutation, chromosomal aberrations, etc.[1,2] These analyses have a general drawback in that they show results after a delay ranging from several hours to several days or weeks after the initial exposure. To avoid these disadvantages, a method was applied to estimate the radiation-induced alterations of early response proteins (ERPRO) in human lymphocytes. Recent progress made in the field of proteomics opens up new possibilities of studying cellular response to various stimuli. Proteomic methods are frequently used in studies of cell reaction related to mutagenic and carcinogenic effects of different agents. ZHANG et al.[3] recently demonstrated the application of proteomics in revealing the mechanism of extreme radioresistance and repair of DNA in *Deinococcus radiodurans*, a non-motile bacterium known for its extreme resistance to lethal effects of ionizing radiation. TAPIO et al.[4] recently also showed combined effects of γ-radiation and arsenic, which has been associated with an increased rate of skin and internal cancers on the proteome of human TK6 lymphoblastoid cells. SZKANDEROVA et al.[5] observed 14 up- or down-regulated proteins after exposing human T-lymphocyte leukaemia cells to a single dose of 7.5 Gy. Although these studies were carried out with cell lines and at a higher γ-dose they showed a clear trend towards the radiosensitivity of proteins.

The experiments in the present work describe the alterations of lymphocyte proteins after γ-exposure of human blood in vitro at a dose of 2 Gy. The time interval between the end of irradiation and the beginning of lymphocyte isolation was limited to 15 minutes. Attention was focused on finding those proteins which show either up- or down-regulation or a post-translational modification (in this case phosphorylation) after radiation exposure.

Experimental

Unless otherwise specified all chemicals were purchased from Sigma – Aldrich. The quality was always p.a. except for the purposes of mass spectrometry where the quality was MS grade. The blood was kept at 37 °C except during radiation exposure for 4 minutes when it was kept at room temperature (RT).

Peripheral blood from a healthy human donor was heparinized and irradiated with γ-rays of ^{137}Cs for 4 minutes at a dose rate of 0.5 Gy/min. Fifteen minutes after irradiation the lymphocytes were isolated by density-gradient centrifugation using Ficoll-Paque (GE Health Care) and $3 \cdot 10^6$ of the lymphocytes were suspended in 1 ml of ice-cold lysis buffer containing 30 mM tris-HCl, 2M thiourea, 7M urea, 4% 3-[(3-cholamidopropyl)-dimethylammonio]-1-propane-sulfonate (CHAPS), protease inhibitor cocktail (Roche) and adjusted with 2M HCl (Merck) at pH 8.0. The proteins were purified with the 2D Clean-Up Kit (GE Health Care) and quantified with the EZQ Quantitation Kit (Invitrogen). For isoelectric focusing (IEF), 130 μg of each protein sample were mixed with the IEF buffer containing 12 g urea, 0.5 g CHAPS, 0.5% ampholytes of pH 3–10 (Invitrogen), 20 mM dithiothreitol (DTT), a trace of bromophenol blue and transferred to a 17 cm IPG-strip (pH 3–10 NL) (Biorad).

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After 16 hours of incubation at RT, the strips were focused in the Protean IEF Cell (Biorad) according to the standard protocol recommended by the manufacturer. The focused IPG strips were reduced with 50 mM DTT and alkylated with 125 mM iodoacetamide.

The second dimension (SDS-PAGE) was carried out with a PROTEAN II system (Biorad) using 12% SDS gels and tris/glycine/SDS buffer. The IPG strips containing the protein sample were transferred to the gels and sealed with 1% LMP (low melting point) agarose solution. The gels were used at 19 °C and 24 mA/gel for about 4 hours. They were stained with the phospho-protein-sensitive fluorescence dye Pro-Q Diamond (Invitrogen) and with Sypro Ruby (Invitrogen) for the analysis of total proteins. The gels were imaged on a FLA 5000 Laser Scanner (Fuji) with 100 μm resolution. The Pro-Q Diamond stain was imaged using the 535 nm laser and 570 nm LP filter while the 475 nm laser and 530 nm BP (20 nm) filter was used for the Sypro Ruby stain. The images were analyzed with Delta 2D software (version 3.3, Decodon). Only spots showing significant changes (Student's t-test, $p \leq 0.05$) and a greater than twofold increase or decrease in magnitude were evaluated and picked for MS analysis with the Proteineer SP II spot-picking robot (Bruker). The gel pieces were washed in 50 mM bicarbonate buffer and digested in 15 μl trypsin (Promega) solution (10 μg/ml 25 mM bicarbonate buffer). The mixture was left at 37 °C overnight (16 h) to digest. The peptides in the gel spot were extracted three times with 20 μl of 0.5% formic acid and 20% acetonitrile in ultra-pure water. The peptides (ca. 5 fmol/μl) were analyzed using a 1D Nano-HPLC (Eksigent) coupled with the ESI-QTrap 2000 MS (Applied Biosystems). In detail, the peptides were desalted on a 300 μm×5 mm C-18 trap column (Dyonex) using a mixture of ultra-pure water with 2% acetonitrile and 0.1% formic acid and separated on a 75 μm×150 mm C-18 Nano-HPLC column (Dyonex) by acetonitrile/water gradient elution (5 to 40% acetonitrile in 45 min). Eluted peptides were subjected to MS/MS analysis and the proteins were identified by the Mascot 1.7 search engine (Matrixscience).

Results and discussion

The ERPRO analysis of lymphocytes was performed 15 minutes after the termination of γ-irradiation in vitro at 2 Gy ($n=3$) and compared with the results of the non-irradiated samples ($n=3$). The total 2-DE protein patterns of the irradiated and non-irradiated lymphocytes are shown in Figs 1 and 2 where 15 spots in the pH range of 4–10 show significant radiation-induced alterations (spots Nos 1–9: decrease in protein abundance by factors of 0.1 to 0.5; spots Nos 10–15: increase in protein abundance by factors of 2.8 to 9.6), while Figs 3 and 4 represent the phosphoprotein patterns with and without irradiation also ranging from pH 4–10. Twelve of the 14 spots increase in protein abundance by factors of 2 to 62 and only 2 of them (spot Nos 1 and 13) decrease significantly by a factor of 0.3. The relatively high frequency of phosphoproteins after γ-irradiation may indicate activation of DNA repair-relevant enzymes and their poly-ADP-ribosylasted reaction products. SZKANDEROVA et al.[6] found proteins (KCIP-1 and Rab 1) in murine fibroblasts (L 929) with an increase in protein abundance 20 minutes after 6 Gy X-ray irradiation. In human leukemia cells,[5] the same group detected 14 up- or down-regulated proteins after exposure to 7.5 Gy γ-irradiation and incubation times of 2, 5 and 12 hours.

Table 1 shows the identified protein spots:

The remaining proteins could not be identified with a significant level of certainty. Therefore, further experiments are required to be undertaken. These include preparation of preparative gels for a higher concentration of proteins per gel spot, resulting in better identification by mass spectroscopy.

Although only about 1/3 of all proteins that showed radiation-induced changes were identified and any definite conclusion will have to be drawn after identifying the rest of the proteins, a trend is apparent. Of the five proteins identified, four are structural proteins. Talin is known as a high-molecular-weight cytoskeletal protein playing a key role in the assembly of actin filaments. BAČÁKOVÁ et al.[7] reported a 6% decrease in talin concentration in rat aortic smooth muscle cells after exposing collagen III to ultraviolet light radiation. In this work, we report talin decrease by a factor of 0.3, which is much more than the decrease observed by BAČÁKOVÁ et al. This could be explained by the fact that BAČÁKOVÁ et al. irradiated only the collagen III on which the rat muscle cells were grown. This means that the irradiation was applied indirectly. The new findings published by BÉCAM et al.[8] show that talin is also involved in the gene regulation of other structural proteins.

Table 1. Identified early response proteins after exposing human lymphocytes to a single γ-radiation dose (2 Gy)

Spot No.	Protein	Accession no.	MW / pI	Ratio (increase/decrease)	t-Test
4	Talin	gi|6739602	271,653/5.77	0.30	95.72
7	Mutant beta-actin	gi|28336	42,128/5.22	0.33	95.35
7P	Zyxin (zyxin-2)	gi|2497677	62,436/6.22	2.70	98.97
10P	Actin (beta-actin)	gi|4501885	42,052/5.29	4.71	99.01
13P	Phosphoglycerate kinase 1	gi|4505763	44,854/8.30	0.32	96.94

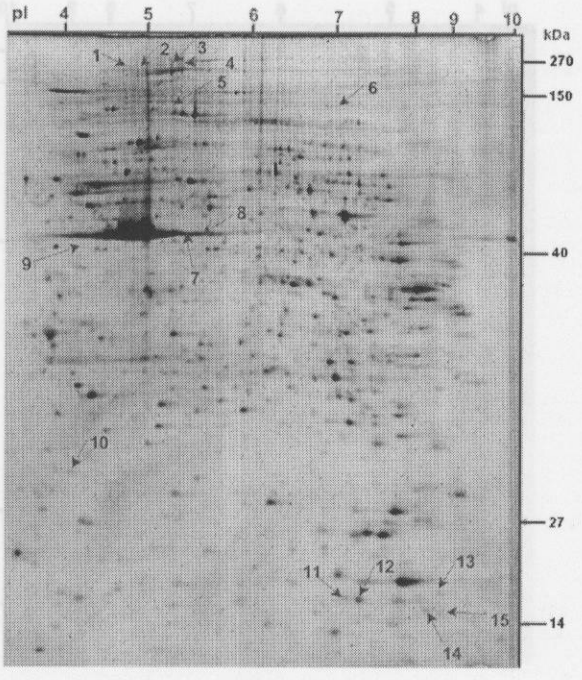

Fig. 1. 2-D Sypro Ruby stained protein pattern: non-irradiated lymphocytes

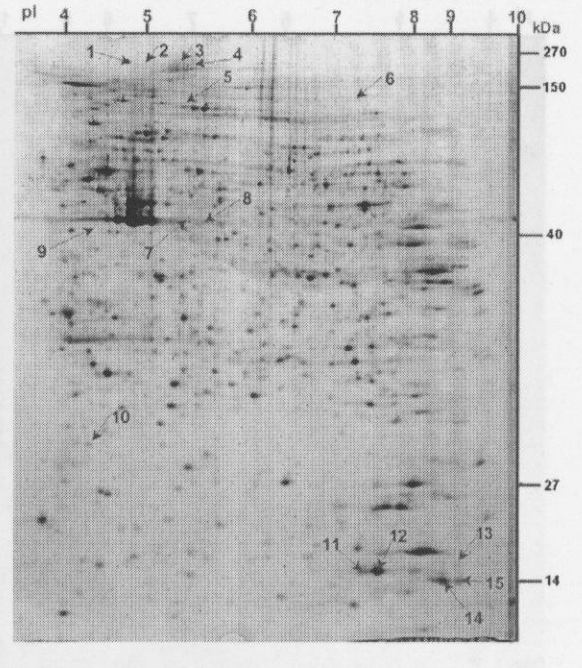

Fig. 2. 2-D Sypro Ruby stained protein pattern: 2 Gy γ-irradiated lymphocytes

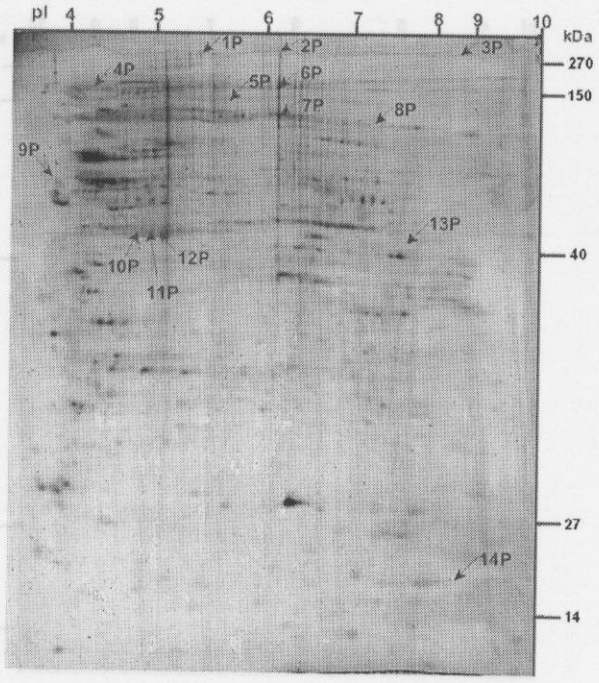

Fig. 3. 2-D Pro-Q-Diamond stained protein pattern: non-irradiated lymphocytes

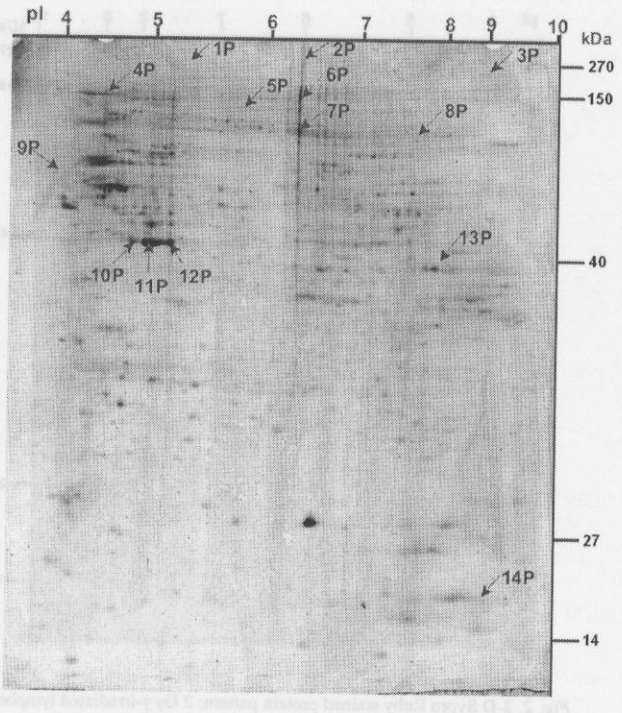

Fig. 4. 2-D Pro-Q-Diamond stained protein pattern: 2 Gy γ-irradiated lymphocytes

Beta-actin and its mutant form are further structural proteins found to be altered in the irradiated cells with respect to the controls. An increasing number of authors reported the involvement of the cytoskeleton in cellular apoptosis and necrosis. Cleavage of β-actin into 15 kDa and 31 kDa fragments is a way of inducing apoptosis. Mutant β-actin impairs apoptotic pathways originating from the tumour necrosis factor (TNF)-α (LI et al.).[9] Hence, β-actin and its mutated form play an important role in cell death regulation. Here, a decrease was observed in the concentration of the mutated β-actin and an increase of the phosphorylated form of the normal β-actin. According to KULMS et al.[10] cytoskeletal alterations are increasingly regarded as the cause and not only as a consequence of apoptosis. Radiation-induced cellular alterations of cytoskeletal proteins could, therefore, cause an activation of the suicide mechanism in the irradiated lymphocytes.

As a complement to these findings, zyxin was found in an increased phosphorylated condition in the irradiated lymphocytes. Zyxin is a well-known regulator of the actin filament assembly primarily responsible for cell-to-cell adhesion and interaction. VOZENIN-BROTONS et al.[11] reported an increased concentration of zyxin in human late radiation enteritis. It seems that increased amounts of this protein correlate most probably with its function as a component of the signal transduction pathway. Zyxin and talin (mentioned above) could both play a role in controlling the gene expression of other structural proteins.[8]

Finally, one protein which is not a structural protein showed radiation induced alteration. Phosphoglycerate kinase 1 (PGK1) was found in the irradiated sample in a decreased phosphorylated condition with respect to the control. This protein plays an important role in the second phase of glycolysis by phosphorylating 3-phospho-D-glycerate. The significance of this under-phosphorylated condition of PGK1 in irradiated lymphocytes remains unknown. Nevertheless, most important cellular processes (glycolysis and ATP synthesis) seem to be affected at an early stage of the cell-radiation interaction process.

Conclusions

As more ERPRO are identified and further experiments show results regarding the γ-dose-dependent behavior of these proteins, a better picture of early cellular response to irradiation can be obtained. These preliminary findings showed that structural proteins play an important and active role during cellular defence processes. The family of proteins around the major structural protein, actin, seem to be affected. The fact that structural proteins are a significant part of the entire proteome may make them appropriate for such a purpose. Not only do they contribute to the physical structure but they join molecules together, form aggregates and provide a matrix for important reactions. On the other hand, as expected, it was found that an important glycolysis enzyme (PGK1) was affected by the γ-radiation damage. Irrespective of whether the cell repairs DNA breaks, stops the cell cycle or induces apoptosis, adenosine-tri-phosphate (ATP) as an energy source is needed. Therefore, changes in the production process of ATP and energy household as a result of γ-radiation-induced cellular lesions are more than probable.

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References

1. J. O. HUMSTOE, F. H. A. SCHNEEWEISS, A. SRIVASTAVA, A. SARMA, R. N. SHARAN, Radiat. Eff. Defect. S., 158 (2003) 603.
2. R. N. SHARAN, in: Advances in Radiation Biology and Peace, S. C. GOEL (Ed.), U.P. Zool. Soc., India, 1999, p. 19.
3. C. ZANG, W. JIANFENG, Z. ZHIGUO, Y. NANJIAO, S. DUOHONG, H. YUEJIN, Proteomics, 5 (2005) 138.
4. S. TAPIO, J. DANESCU-MAYER, M. ASMUSS, A. POSCH, M. GOMOLKA, S. HORNHARDT, Mutat Res – Rev Mutat., 581 (2005) 141.
5. S. SZKANDEROVA, J. VAVROVA, L. HERNYCHOVA, V. NEUBAUEROVA, J. LENCO, J. STULIK, Radiat. Res., 163 (2005) 307.
6. S. SZKANDEROVA, L. HERNYCHOVA, I. KASALOVA, J. VAVROVA, J. STULIK, M. ABEND, D. VAN BEUNINGEN, Folia Biol. Prague, 49 (2003) 15.
7. L. BAČÁKOVÁ, V. LISA, L. KUBINOVA, J. WILHELM, J. NOVOTNA, A. ECKHART, J. HERGET, Virchows Arch., 440 (2002) 50.
8. I. E. BÉCAM, G. TANENTZAPF, J. A. LEPESANT, H. B. NICHOLAS, J. R. HUYNH, Nat. Cell. Biol., 7 (2005) 510.
9. J. LI, Q. LI, C. XIE, H. ZHOU, Y. WANG, N. ZHANG, H. SHAO, S. C. CHAN, X. PENG, S. C. LIN, J. HAN, J. Cell. Sci., 117 (2004) 4673.
10. D. KULMS, H. DÜßMANN, B. PÖPPELMANN, S. STÄNDER, A. SCHWARZ, T. SCHWARZ, Cell Death Differ., 9 (2002) 598.
11. M. C. VOZENIN-BROTONS, F. MILLIAT, C. LINARD, C. STRUP, A. FRANCOIS, J. C. SABOURIN, P. LASSER, A. LUSINCHI, E. DEUTSCH, T. GIRINSKY, J. AIGUEPERSE, J. BOURHIS, D. MATHE, Radiat. Res., 161 (2004) 299.