DNA damage, protein expression and migration of melanoma cells irradiated with proton beam

M. Elas[1], S. Kędracka-Krok[1], U. Jankowska[1], Ł. Skalniak[1], J. Jura[1], Ewa Zuba-Surma[1], K. Jasińska[1], A. Pawlak[1], U. Sowa[2], P. Olko[2], K. Urbańska[1], B. Romanowska-Dixon[3]

[1]*Faculty of Biochemistry, Biophysics and Biotechnology, JU, Gronostajowa 7, Kraków, Poland* email: martyna.elas@uj.edu.pl

[2]*Institute of Nuclear Physics, PAS, Radzikowskiego 152, Kraków, Poland*

[3]*Department of Ophthalmology and Ophthalmic Oncology, Jagiellonian University Medical College, Kopernika 38, Kraków, Poland*

Aim

The goal of our study was to determine the cellular response to low, sublethal doses of proton beam irradiation, in particular DNA damage, cell cycle arrest, changes in expression of proteins, and effect on metastases in vivo.

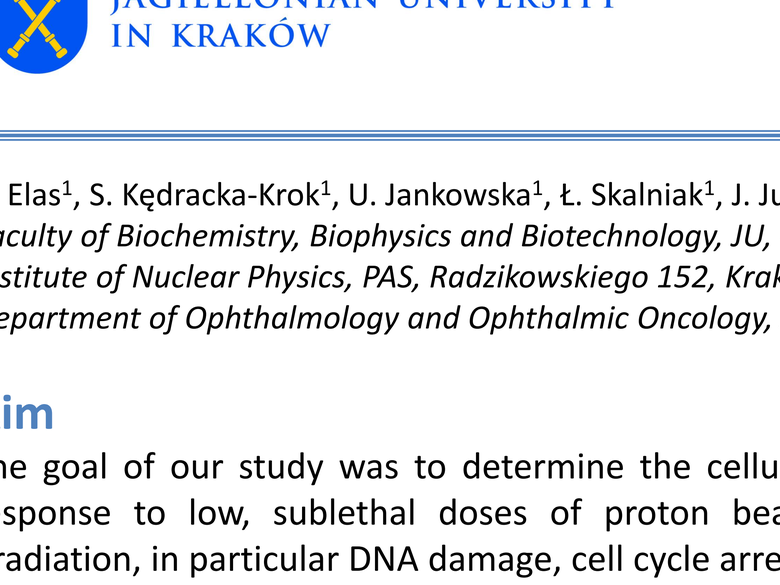


Fig. 2 Delayed apoptosis on day 9-10 after irradiation in S-91 murine melanoma cells.

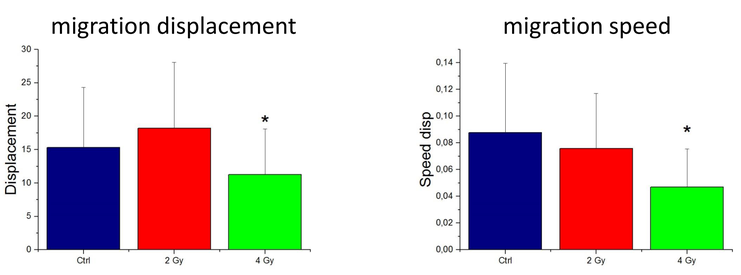


Fig. 3 Migration properties of irradiated Blm cells was inhibited, provided that they were seeded at high density.

Methods

BLM cells were irradiated with 1-7 Gy of proton beam irradiation. The source of the 58 MeV proton beam was the AIC-144 cyclotron at Institute of Nuclear Physics, Polish Academy of Sciences, Kraków. The dose rate was 0.15 Gy/s). DNA content was evaluated by flow cytometry (Becton Dickinson). The level of DNA damage was tested by electrophoresis of single cells in agarose gel (comet assay). Protein expression were determined by 2D protein electrophoresis and mass spectroscopy. Tumors of Bomirski Hamster Melanoma (BHM) implanted into the anterior chamber of the hamster eye grew aggressively and completely filled the anterior chamber within 8-10 days. Metastases, mainly in the lung, were found in 100% of untreated animals 30 days after enucleation. The protons were accelerated using AIC-144 isochronous cyclotron, operating at 60 MeV and BHM tumors located in the anterior chamber of the eye were irradiated with 10 Gy, for the depth of 3.88 mm.

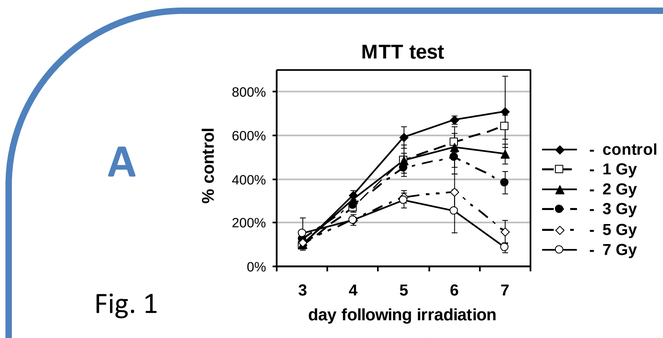
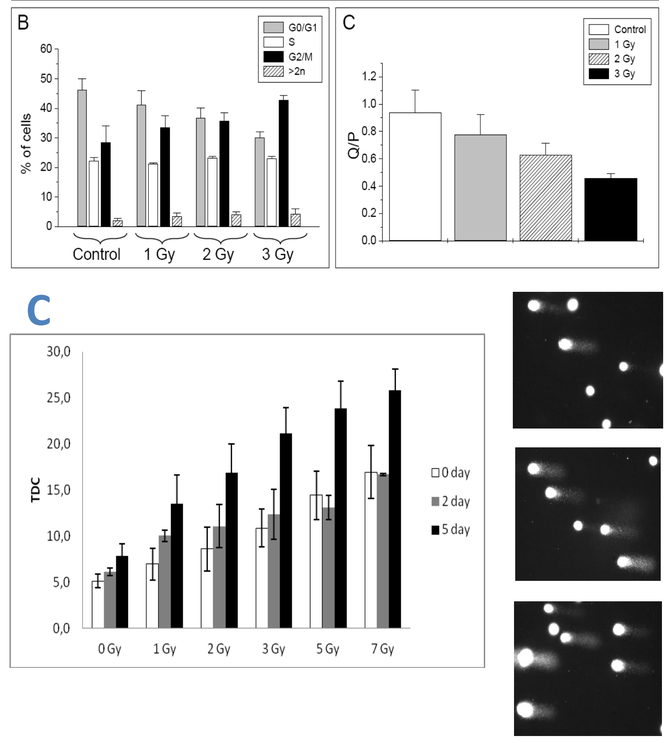
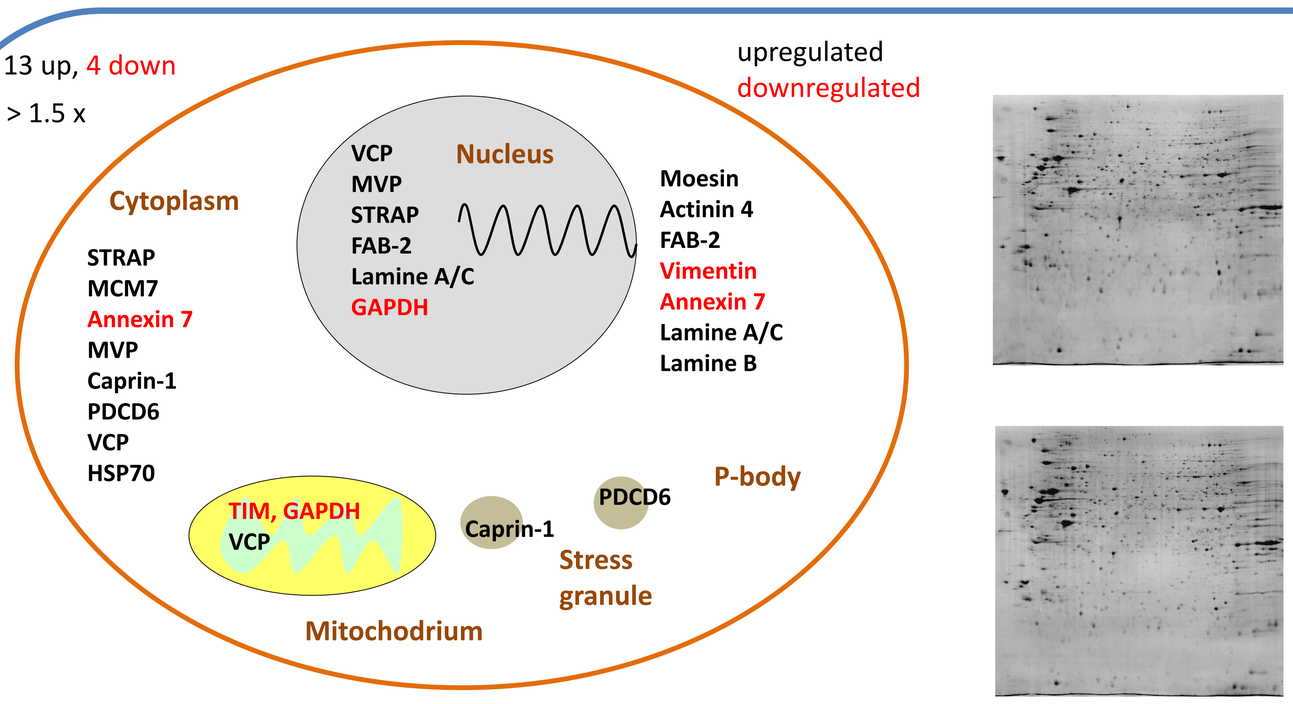


Fig. 1

A) Blm cell growth inhibition after proton beam irradiation of cells in suspension. Graph represents mean ± SD from three independent experiments normalized to untreated control at day 3 following proton treatment.
B) Cell cycle redistribution: incerase in G2/M phase with dose.
C) DNA content of melanoma cells following proton irradiation. Delayed dose-dependent DNA damage shown by comet assay.
D) Caspases 3 and 7 were activated strongly on 6 days post irradiation, indicating delayed entering into apoptosis .





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3 Gy

Fig. 4 2D electrophoresis (right panel) and mass spectroscopy revealed 17 differentially regulated proteins in proton irradiated Blm cells. Abbreviations: ACTN 4 - α Actinin 4, Caprin-1 - Cytoplasmic activation/proliferation-associated protein-1, FAB-2 - Far upstream element binding protein 2, G3BP1 - RasGAP SH3-domain-binding protein 1, GADPH - Glyceraldehyde 3-phosphate dehydrogenase, MCM-7 – Minichromosome Maintenance Protein 7, Moesin - Actin-regulatory protein, MVP - Major Vault Protein, PDCD6 - Programmed cell death 6, or apoptosis-linked gene-2, STRAP - Serine-threonine kinase receptor-associated protein, TIM - Triosephosphate isomerase, VCP – Transitional endoplasmic reticulum ATPase [1].

Results

Slow accumulation of damage was observed, reflected in slowing of the proliferation rate (Fig. 1A), and increase in caspases activity with time (Fig. 1D). The number of cells in G2/M and >2n increased with proton beam dose (Fig. 1B). Proton beam irradiation caused upregulation of proteins involved in: DNA repair, RNA functioning (i.e. stress granule and P-bodies components), apoptosis and survival processes and downregulation of enzymes engaged in glycolysis (Fig. 4). Of particular interest was heavy downregulation of vimentin (2.4 times), involved in structural integrity of cells and tissues, adhesion and migration, and other processes. Irradiation led to changes in cell migratory properties (Fig. 3). Proton beam irradiation caused inhibition of tumor growth by about 10 days and inhibition of metastastic spread in a hamster melanoma tumor growing in the eye[2].

Conclusions

Low doses of proton beam irradiation cause significant DNA damage in human melanoma metastatic cells. Arrest in G2/M phase in response to DNA damage may lead to apoptosis (5 and 7 Gy), increase in polyploidy (>2n) or to DNA repair and cell survival (1-3Gy). Four groups of proteins were differentially regulated after proton beam irradiation: i) DNA repair and stress, ii) pro-survival response, iii) metabolic and iv) connected to motility and cytoskeleton. 10 Gy of proton beam irradiation given to melanoma growing in the hamster eye inhibited metastases growth in the lung 4.3 times.

Bibliography

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Acknowledgements

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