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

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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.

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, Krakow. 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 B6D2F1 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.

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 metastatic spread in a hamster melanoma tumor growing in the eye².

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 (1-3 Gy). 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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