RBE of 20 MeV Protons for Induction of Micronuclei and γ-H2AX Foci in HeLa Cells at Continuous and Pulsed Irradiation Modes at SNAKE

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Introduction: In the context of radiotherapy hadrons exhibit some physical and potentially biological advantages over photons like a favorable dose distribution and, in case of high-LET irradiation like carbon ions, less dependency of cell cycle distribution or tissue oxygenation. Due to building and cost requirements so far the availability of facilities for ion beam radiotherapy is limited. Laser generated ion beams might be a more space- and cost-effective alternative. As technology providing high intensity ultra short pulsed lasers develops rapidly, application in the field of radiotherapy seems possible. The excellence cluster Munich-Centre for Advanced Photonics (MAP) of the "Deutsche Forschungsgemeinschaft" addresses these issues, aiming for the development of a biomedical laser beam line, which will be used to explore the physical, radiobiological and clinical aspects of laser-generated particle irradiation. So far, the radiobiological impact of such a laser-generated particle beam on cells or tissues is unclear, as the pulse structure and peak intensity of dose delivery differs from current radiotherapy modalities by several orders of magnitude. First investigations on cell cultures have been initiated to compare the relative biological effectiveness (RBE) of X-rays and protons in continuous irradiation mode with nanosecond proton pulses. The current subject of debate is the question of whether or not pulsed irradiation with high-energy particles or protons may induce a different amount of damage in cells or even changes in repair or apoptosis pathways relative to continuous irradiation. The short-pulse effects are subject to our current investigations utilizing pulsed ion beams at the SNAKE (*S*uperconducting *N*anoprobe for *A*pplied nuclear (*K*ern) physics *E*xperiments) microprobe of the Munich tandem accelerator, where 10^5 high energy protons can be bunched into a single nanosecond pulse at a beam diameter of about 100 μm. These beam parameters are sufficient to irradiate cell cultures or tissue up to a dose of 5 Gy by a single pulse of protons. The aim of the present experiments is to compare the RBE of irradiation with high-energy protons at 20 MeV in continuous versus pulsed mode, relative to a reference irradiation with 70 kV X-rays, using micronuclei and γ-H2AX assays in HeLa cells.

Methods: Experiments were performed with the ion microprobe SNAKE at a Van-de-Graaff-tandem accelerator designed to generate 20 MeV proton beams in continuous mode or single pulses of nanosecond duration. For reference, dose-response curves were derived from treatments with 70 kV X-rays. A) Micronucleus assay: HeLa cells attached to a mylar foil were exposed to X-rays (1000 cells per dose level) or 3 Gy of protons in continuous mode or by single pulses (each 3 replicates of 500 – 1000 cells). 24 h after cytochalasin B was added, cells were fixed and stained with acridine orange and micronuclei were counted. Tests were repeated in an independent experiment with slightly different pulse duration (1.7 ns and 0.9 ns). B) γ-H2AX assay: HeLa cells attached to a mylar foil were exposed to X-rays (1000 cells per dose level) or 1 Gy of protons in continuous or pulsed mode (each 3 replicates of > 100 cells). Cells were stained for γ-H2AX and the frequencies of γ-H2AX foci were determined.

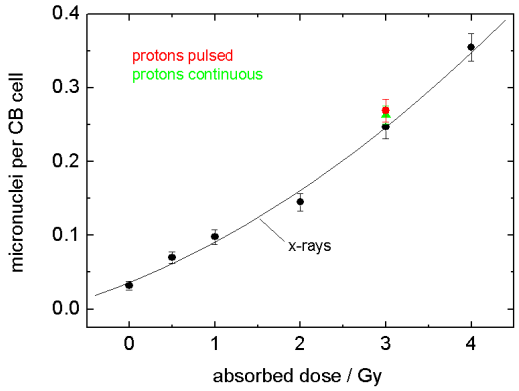

Fig. 1: Reference dose-response X-ray curve and proton data for micronuclei induction.

Results: A) The dose-response curve for reference was established in a dose range from 0 – 4 Gy (0, 0.5, 1, 2, 3 and 4 Gy). The X-ray response curve for micronucleus induction in HeLa cells can be described with the LQ-model (Fig. 1). Results for the independent proton experiments were highly reproducible with induction of 0.263 and 0.262 micronuclei per cell for continuous and 0.269 and 0.272 for pulsed irradiation mode. The RBE of 20 MeV protons was calculated as the ratio between the dose of the reference radiation (70 kV X-rays) and the dose of protons which produced equal response and resulted in 1.06 ± 0.07 and 1.05 ± 0.07 for continuous and 1.07 ± 0.07 and 1.09 ± 0.08 for pulsed irradiation modes, respectively. B) The dose-response curve for reference was established between 0 and 1.25 Gy (0, 0.25, 0.5, 0.75, 1, 1.25 Gy). In this dose range, the number of phosphorylated γ-H2AX foci per cell followed a linear relationship (Fig. 2).

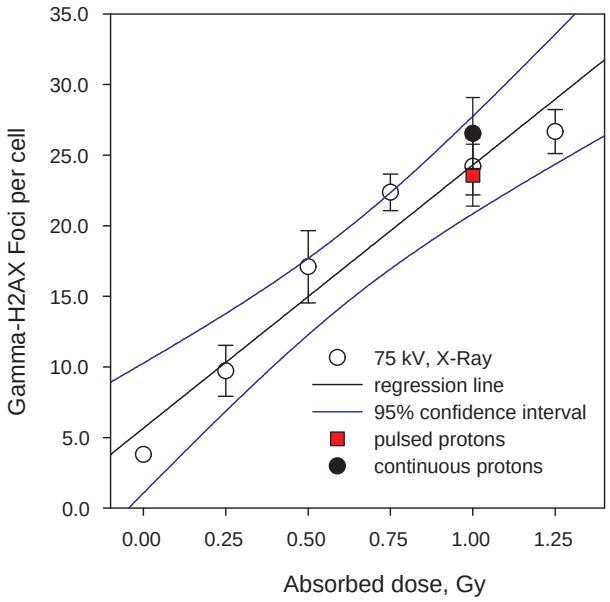

Fig. 2: Reference dose-response X-ray curve and proton data for γ-H2AX induction.

In the sham-treated samples the average number of foci per cell was 3.8 ± 0.16. From the dose-response curve, a yield of 24.8 ± 2.01 foci per Gy was measured. In the proton experiments with a proton dose of 1 Gy at pulsed and continuous irradiation modes, mean numbers of γ-H2AX foci per cell of 23.29 ± 2.04 and 26.54 ± 2.54 were obtained. The corresponding RBE values relative to the reference radiation of 70 kV x-ray were 0.96 ± 0.18 and 1.13 ± 0.21, respectively. The difference was not significant ($p = 0.21$, t-test). Although absolute numbers of γ-H2AX foci formation per cell revealed no significant difference after irradiation with pulsed or continuous proton beams ($p = 0.16$, t-test), the percentage of foci smaller than 5 – 10 pixels was slightly reduced after irradiation with the pulsed proton beam. Instead, foci generated with pulsed irradiation tended to be bigger and more aggregated.

Conclusions: For the chosen endpoints, our first results on direct comparison of the effects of 20 MeV proton irradiation in pulsed mode of nanosecond duration to continuous irradiation do not show a difference in HeLa cells. The RBE measured is well in the range of the values used in conventional proton radiotherapy. The high reproducibility of our results from three independent proton experiments, deriving from different beam access times, underlines the stability of the experimental system and the validity of these findings. Our data support the idea, that the amount of genetic damage inflicted is not markedly affected by the ultrahigh dose-rate of a pulsed delivery mode when compared to irradiation times close to a second. Further studies on different endpoints, including normal and tumor tissues, are necessary before it is safe to handle dose prescriptions for pulsed proton irradiation similarly to continuous irradiation mode.

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