Dose-dependent changes after proton and photon irradiation in zebrafish model

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Introduction

The laser-driven ionizing (LDI) beams have unique property of ultra-high dose rate, ultra-short pulses and carry the potential toward special clinical application. In our previous studies we have established an *in vivo* vertebrate model for radiation biology research. Zebrafish embryos are highly tolerant to extreme enviromental conditions and prove to be reliable in assessement of special radiaton qualities.

Objective

In the present investigation our aim was to optimize both biological model and irradiation parameters for research on future laser-driven proton beams, testing radiation sensitivity at different embryonal ages, assessing dose-dependent changes at different timepoints and developing further quantitative endpoints apart from survival and simple malformation analysis at conventional proton and photon sources.

Methods

24 and 72 hours post-fertilization (hpf) zebrafish embryos were irradiated at the University Proton Therapy Dresden with escalated doses (5, 10, 15, 20 and 30 Gy) at two positions along the proton depth-dose curve, at the plateau and at the middle of Spread Out Bragg Peak (mSOBP), and with reference 6 MV photon beams from a clinical linac (n=96 in each group). The experiment was 3 times repeated under the same conditions. On the 3th (96 hpf) and 4th (120 hpf) days after irradiation morphological malformations were documented (photo) and determined quantitatively. Two independent observers measured the length of the embryos, the degree of the yolk sac edema and the diameter of the eyes. Additionally, we have detected the DNA double-strand breaks (DSB) immunohistochemically (γ-H2AX foci) after 30 min of the irradiation at the proton (mSOBP and plateau position) and photon beams, at 5 Gy dose level.

Results

Dose-dependent organ developmental deteriorations could be detected morphologically at >10 Gy dose levels.

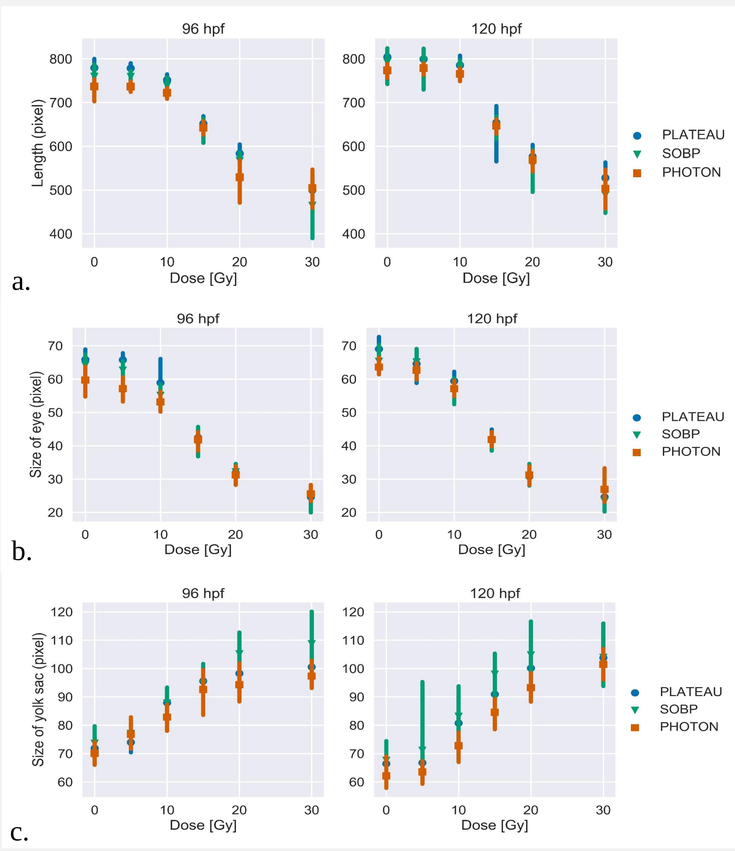

Fig. 1. The length of the embryo (a.) and the size of the eyes (b.) is reduced, while the yolk sac edema (c.) is increased significantly in dose-dependent degree after 10 Gy, 15 Gy, 20 Gy and 30 Gy irradiation, at both developmental stages. Statistics: Kruskal-Wallis one-way ANOVA p<0.05 was considered statistically significant.

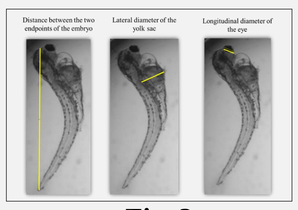

Fig. 2.
Manual measurements by Image J

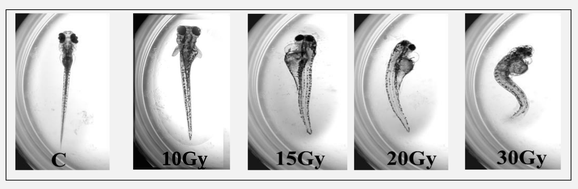

Fig. 3.
Morphological dose-dependent organ developmental deteriorations

We have found significant elevation at 5 Gy dose irradiation in the number of DNA-DSB, as compared to the unirradiated control groups. Furthermore, significant difference between photon and proton groups for positions along the proton depth-dose curve at the earlier time points (30 and 60 min.).

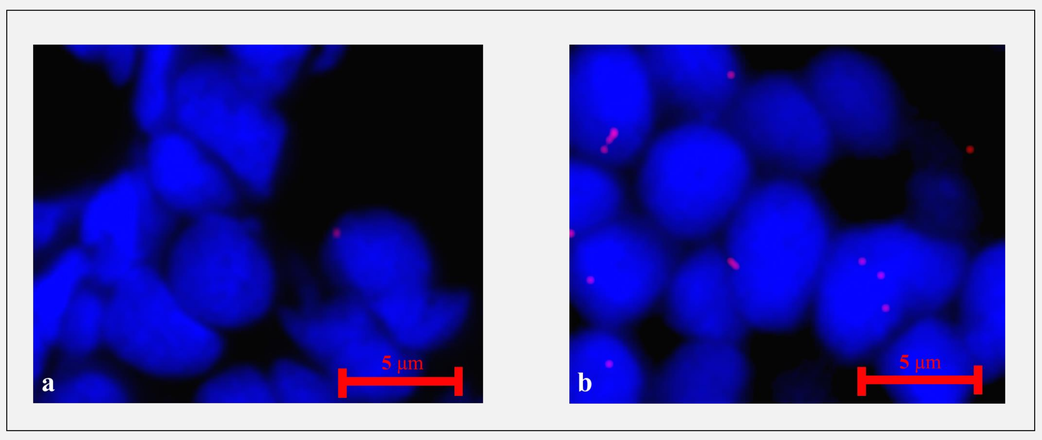

Fig.4. Immunhistochemical staining of a Control (a) and after 5 Gy irradiation (b). DAPI (blue color) stained the nucleus, while ALEXA 647 (pink color) the γ-H2AX foci which indicated the number of DNA-DSB in zebrafish embryo cells.

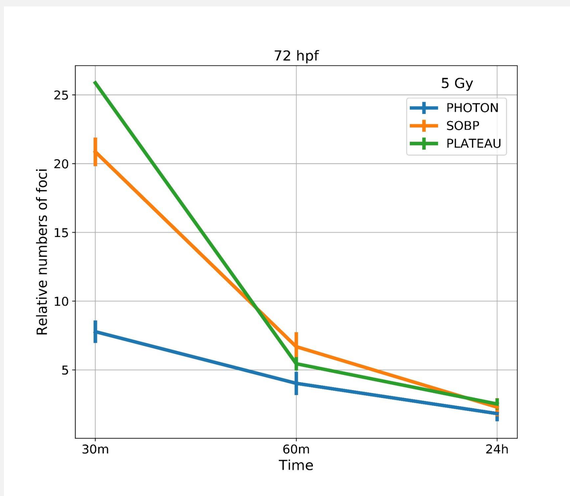

Fig. 5.
The data show that after proton irradiation the degree of the γ-H2AX foci indicating DNA-DSB was higher, as compared to the photon irradiation. Statistics: one-way ANOVA p<0.05 was considered statistically significant.

Conclusions

We could establish the zebrafish model for future LDI experiments optimizing the age of the embryos, developing reliable quantitative morphological analysis methods of dose-dependent organ malformations and a LET sensitive molecular endpoint, and defining the appropriate sampling time for different biological endpoints assessment. The zebrafish embryo model proved to be appropriate for complex evaluation of the irradiation-caused damages, molecular changes and for comparison of the biological effects of different radiation qualities.

References

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