Effects of heavy ionizing radiation on neuronal development, as analyzed in the retina of chick embryos

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Introduction

The developing central nervous system (CNS) is well known to be particularly sensitive against ionizing radiation. However, only little is known about the impact of high LET radiation on CNS development. Therefore we have developed an experimental approach to analyze the consequences of high LET radiation on the retina of chick embryos, as a comparably simple structured part of the CNS. Hereby, fertilized chicken eggs of different developmental stages become bombarded with heavy ion radiation, allowing us to investigate high LET effects on survival, proliferation and differentiation in an *in vivo* situation. To compare these effects with low LET radiation, control experiments with X-radiation have been carried out.

Materials and Methods

Fertilized chicken eggs were incubated for five or seven days, allowing chick embryos to grow to an appropriate developmental stage that is ideal for the analysis of the effects of radiation on retinal development. After five to seven days, irradiation was carried out with nickel or titanium ions (1GeV/u, see Fig. 1) or X-ray (135kV, 19mA), respectively. Doses that were used for the irradiation accounted between 1-2Gy. For the investigations of the effects, embryos were sacrified at defined time points after the treatment and eyes were harvested for retinal analysis by immunohistochemistry in paraffin sections. Determination of apoptotic events was done by counting of pyknotic nuclei, visualized by DAPI staining.

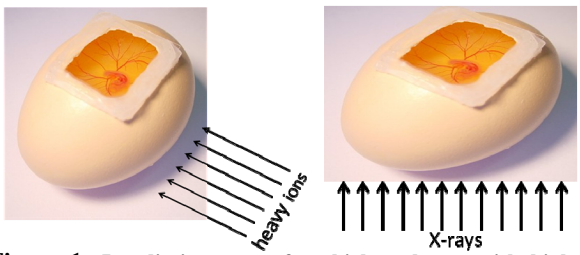

Figure 1: Irradiation setup for chick embryos with high and low LET-radiation. *For high LET treatment, fertilized chicken eggs were laterally scanned with the nickel- or titanium-beam, whereas for X-irradiation the X-ray-source was placed below the egg. Note that the embryo is localized at the top of the yolk. Therefore, a dosimeter was placed directly above the embryo to determine the applied dose during X-irradiation (not shown).*

Results and Conclusion

Preliminary results of our experiments showed that chick embryos are an adequate model system for heavy ion irradiation experiments. Both, five (E5) and seven (E7) day old embryos survived the high LET treatment and could be further incubated until the seventeenth day after fertilization.

No deviations from the phenotype of control embryos could be detected by first glance. However, the results from retinal analysis are still pending.

Control experiments with X-rays instead have already been analyzed for the impact of low LET radiation on cellular survival within the different embryonic stages of the retina, showing not only a time- but also an age-dependency in the occurrence of radiation-induced apoptotic events (see Fig. 1).

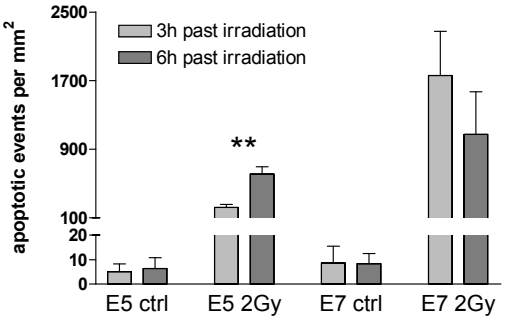

Figure 2: Time- and age-dependent occurrence of apoptotic events within the embryonic chick retina after low LET radiation. *Numbers of apoptotic events within were determined 3 and 6 hours after exposure to 2 Gy of X-rays by counting of pyknotic nuclei in DAPI-stained paraffin sections of the 5 and 7 day-old chick retinae.*

In fact, after 2 Gy of X-rays, the number of apoptotic events per mm^2 was doubled from 3 to 6 hours in 5 day old embryos. In contrast, apoptosis dropped from 1760 to 1072 events per mm^2 within the same time period in seven day old embryos.

Conclusion and Outlook

Our preliminary results show that the chick embryo is an appropriate model system to investigate the impact of high and low LET-radiation on the developing CNS in an *in vivo* situation. In further analyses, differences between comparable doses of high and low LET exposures will be determined, regarding not only survival, but also cell cycle regulation and/or radiation-induced cell cycle arrests as well as differentiation of retina-specific cell types.

Aknowledgements

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