Ionizing radiation alters cerebral organoid generation

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INTRODUCTION

Radiation-induced long-term effects, including neurocognitive deficits, pose an immense problem as consequences of radiodiagnostics and multimodal therapies in pediatric brain tumor patients [1,2]. So-called cerebral brain organoids (enCORs) can be used as an adequate *in vitro* model to investigate the mechanisms underlying ionizing radiation-induced cognitive impairments, particularly in combination with other noxa.

METHODS

Cerebral organoids were generated from H9 human embryonic stem cells (hESCs) according to the protocol by Lancaster et al., 2017 [3]. The generated enCORs were characterized on day 66 using quantitative RT-PCR and immunocytochemical analyses (ICC). To study the effects of ionizing radiation on neurogenesis, organoids were exposed to 1, 2 and 8 Gy of X-rays and to 0.5, 1 and 4 Gy of ^{12}C-ions on day 20 of differentiation.

CHARACTERIZATION

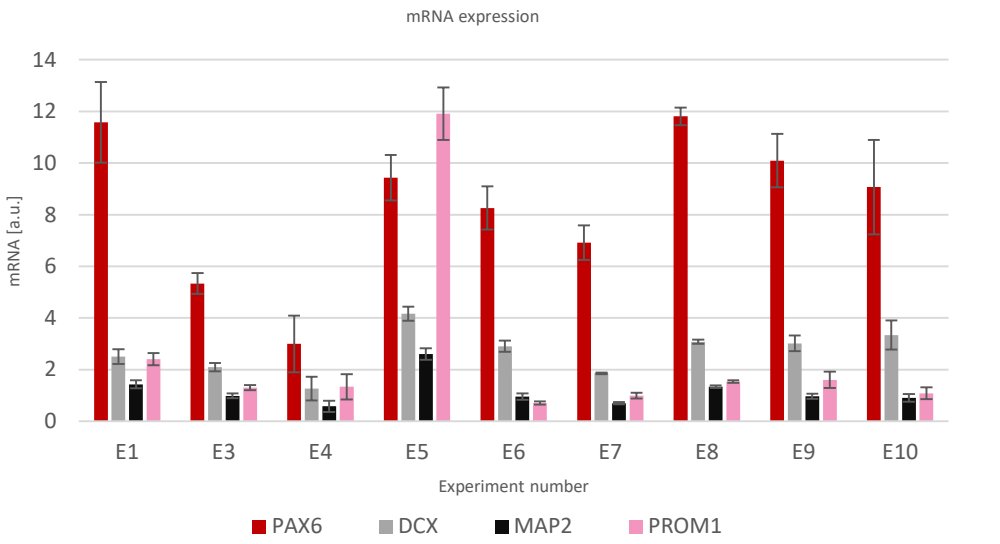

Fig.1. RT-PCR analyses: Relative mRNA expression of neural (PAX6), neuronal (DCX, MAP2) and glial (PROM1) markers on day 66 of differentiation. The error bars represent the determined standard deviations from three technical replicas. Normalization was performed against the housekeeping gene 18sRNA. PAX6**:** paired box protein 6, DCX: doublecortin, MAP2: microtubule-associated protein 2, PROM1: prominin 1.

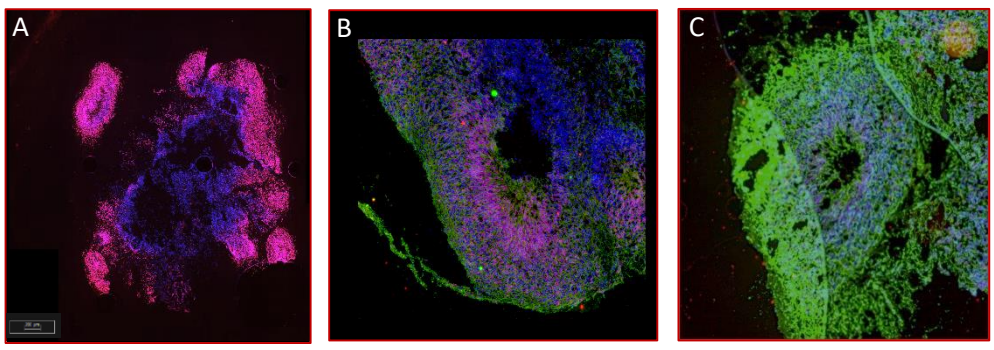

Fig.2. ICC analyses (d66): Immunofluorescence staining against **(A)** the forebrain marker **FOXG1**, **(B)** the neural stem cell markers **PAX6** and **NESTIN** and **(C)** the neuronal marker **ßIIITubulin**. Nuclei were stained with **Hoechst 33342**.

IRRADIATION

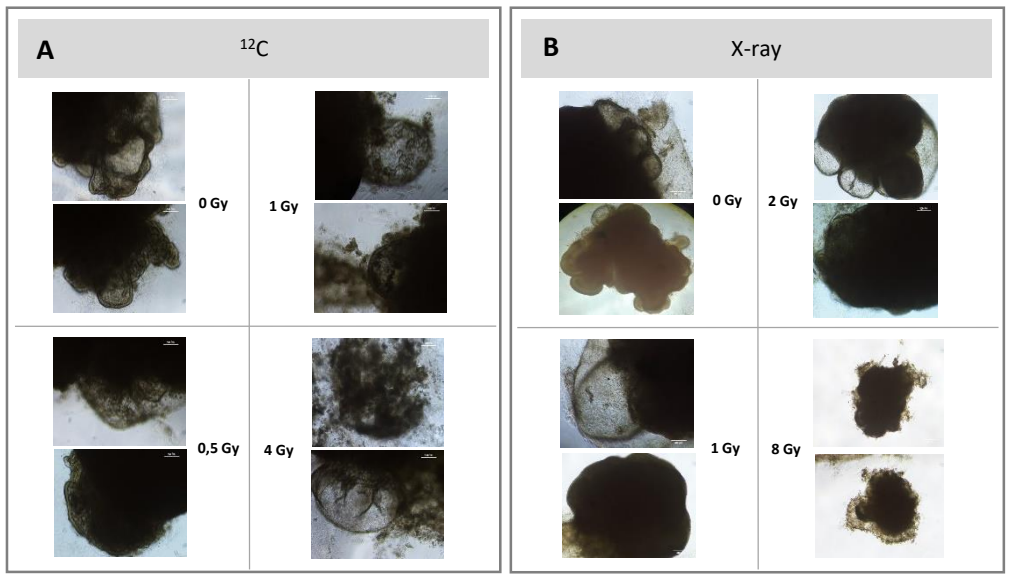

Fig.3: (A) Pictures of irradiated enCORs (d68) in comparison with non-irradiated enCORs (d68). Organoids were irradiated with 0.5, 1 and 4 Gy ^{12}C-ions. **(B)** Pictures of irradiated enCORs (d40) in comparison with non-irradiated enCORs (d40). Organoids were irradiated with 1, 2 and 8 Gy X-rays. Controls were sham-irradiated.

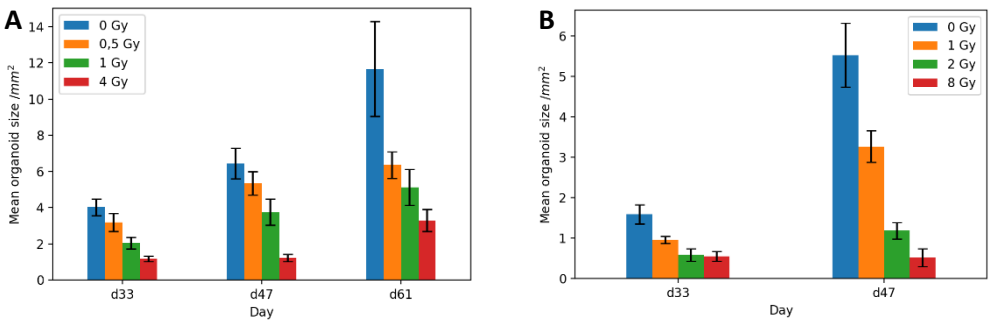

Fig.4: Size measurement of irradiated enCORs on day 33, 47 and 61. **(A)** Organoids were irradiated with 0.5, 1 and 4 Gy ^{12}C-ions. **(B)** Organoids were irradiated with 1, 2 and 8 Gy X-rays. Controls were sham-irradiated. Error bars represent the standard error.

CONCLUSION & OUTLOOK

In summary, different neural and neuronal markers were expressed at the RNA level and thus different CNS-cell types were detected within the generated enCORs. Furthermore, these various neural cell types, that were grouped organ-like in different cell layers, could be stained by ICC. First data show a dose-dependent growth inhibition of the irradiated enCORs as well as morphological changes. PCR and ICC analyses of irradiated samples taken on day 66 and day 88 are currently performed.

Functional analyses by measuring the electrophysiological potential of the enCORs using microelectrode array (MEA)-chips are planned in cooperation with the University of Applied Sciences Aschaffenburg.

Our results demonstrate, that enCORs are an ideal *in vitro* tool to investigate the effects of ionizing radiation on the CNS-cell types and neurogenesis of the human brain.

References

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[3] Lancaster, M. A., Corsini, N. S., Wolfinger, S., Gustafson, E. H., Phillips, A. W., Burkard, T. R…& Knoblich, J. A. (2017). Guided self-organization and cortical plate formation in human brain organoids. *Nature biotechnology*, *35*(7), 659.

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