Cerebral organoids to mimic the human developing brain and radiation-induced microcephaly

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Methodology

Brain organoid culture. Human embryonic stem cell (hESC)-derived brain organoids were obtained using the STEMdiff™ Cerebral Organoid kit based on Lancaster *et al.*, 2014 (Fig. 1).

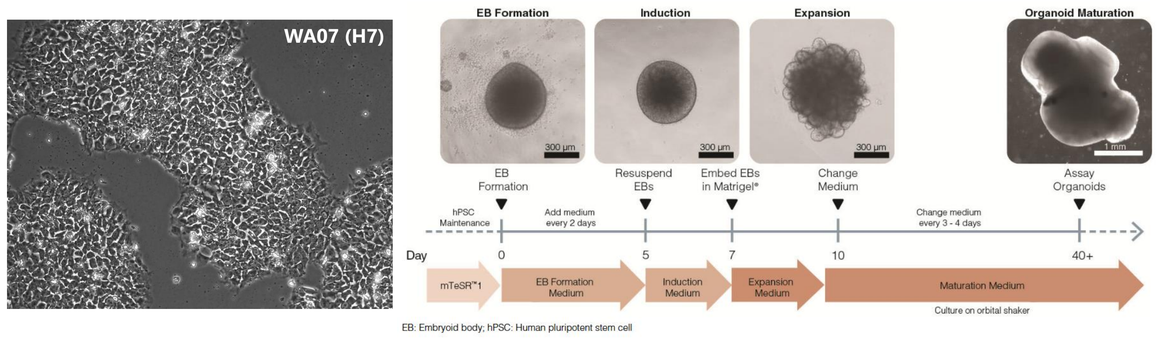

Figure 1. Obtaining brain organoids from hESCs. The hESCs (WA07) were maintained with mTeSR medium. To obtain EBs the cells were detached, transferred to a low-adherent plate and cultured with EB formation medium for 5 days. To achieve neuroephitelia induction and expansion the EBs were cultured for 2 days with induction medium, then embedded in matrigel and cultured for 3 days with expansion medium. To induce organoid maturation they were transferred to a 6-well plate and cultured with maturation medium at least until day 40 on an orbital shaker.

H&E staining. Cryosections were incubated with hematoxylin (3 min), washed, and then incubated with eosin (10 min). Next, dehydration with ethanol/xylene was performed.

Immunohistochemistry. Cryosections were incubated with primary antibodies against DCX (1:300), PAX6 (1:200), Ki67 (1:500), yH2AX (1:200) and pP53 (1:500) overnight, and then incubated with secondary antibodies conjugated to Alexa Fluor 488 or 568 (1:300), or conjugated with HRP (1:200). Ki67 signal was amplified using a TSA Plus Fluor kit.

Irradiation. After 14 days in culture brain organoids were irradiated with a single X-ray dose of 2 Gy.

Introduction

Exposure of fetuses to ionizing radiation during the neurogenesis period may affect brain development, leading to microcephaly and mental disability. However, much of the mechanisms behind neurodevelopmental disorders after radiation exposure remain unclear. Thus, the radiation protection policies for pregnant women are very strict. Although mice are excellent models and widely used to study the effects of radiation exposure, they are not perfect to mimic human brain development. Important differences exist between mouse and human neurogenesis, like the progenitor zone organization and the timing of neurogenesis which is reflected by the massive expansion of the human brain. To fill this gap and better mimic human brain development physiology, we will use a 3D-brain organoid model generated from hESCs. This model recapitulates many features of cellular differentiation and human cortical development (Fig. 2).

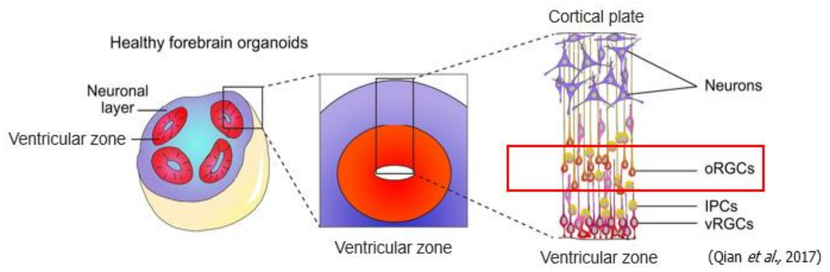

Figure 2. hESC-derived brain organoids recapitulate features of the developing human brain. The ventricular zone organization rich in human-enriched outer radial glial cells (oRGCs) makes the brain organoids a very good model of the human fetal brain.

Objectives

- ✓ To mimic developing human brain using hESCs-derived brain organoids
- ✓ To identify defective cellular mechanisms that causes microcephaly due to ionizing radiation exposure

Results

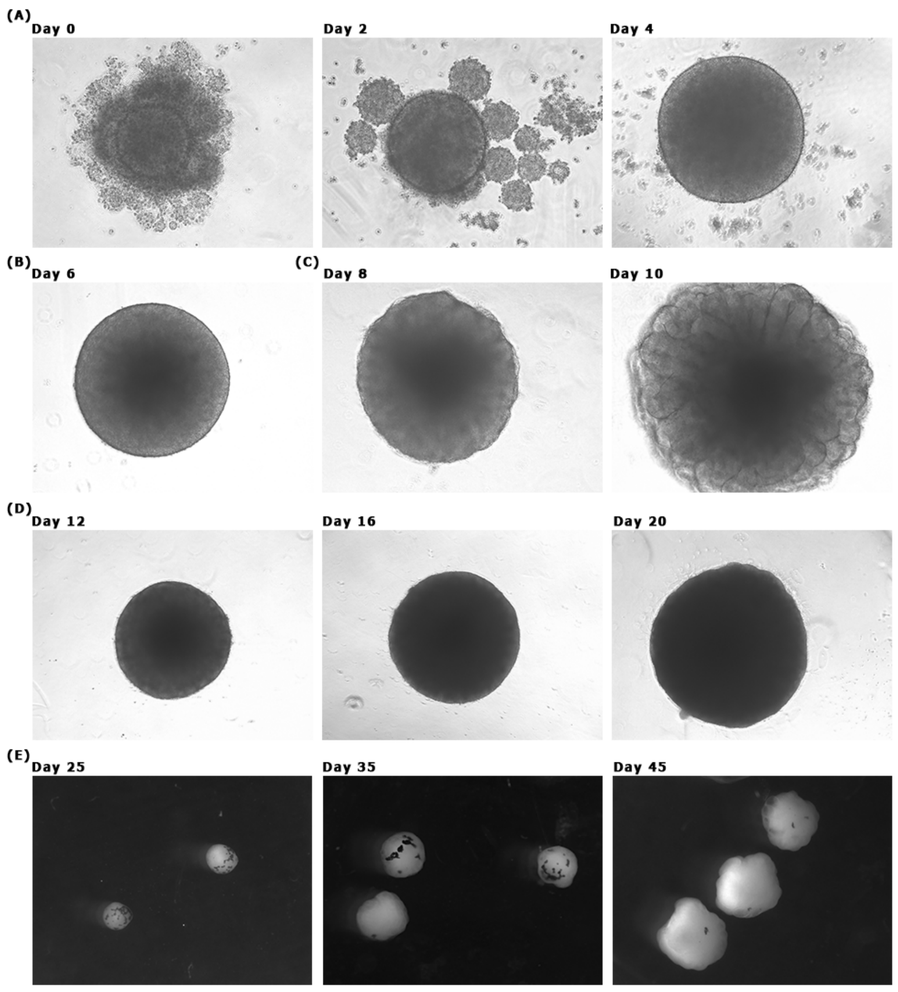

Figure 3. Brain organoid culture. (A) Embryoid body (EB) formation; **(B)** Neuroepithelia induction; **(C)** Neuroepithelia Expansion; **(D)** Organoid maturation; **(E)** Mature organoids. Pictures were taken under bright-field microscopy.

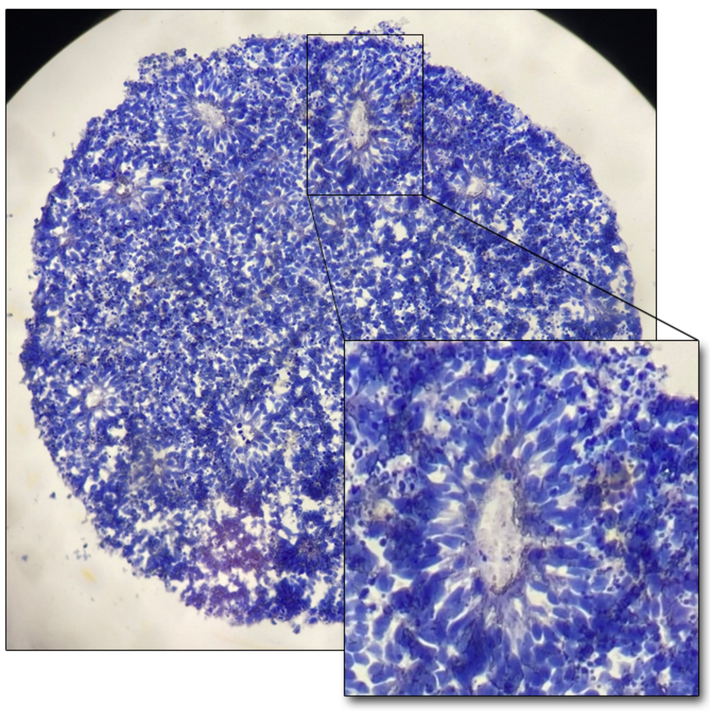

Figure 4. Cerebral organoids modeling the ventricular architecture found in developing human brain. After 14 days in culture the organoids were cryosectioned and stained with hematoxylin/eosin (H&E). Pictures were taken under bright-field microscopy (20x magnification).

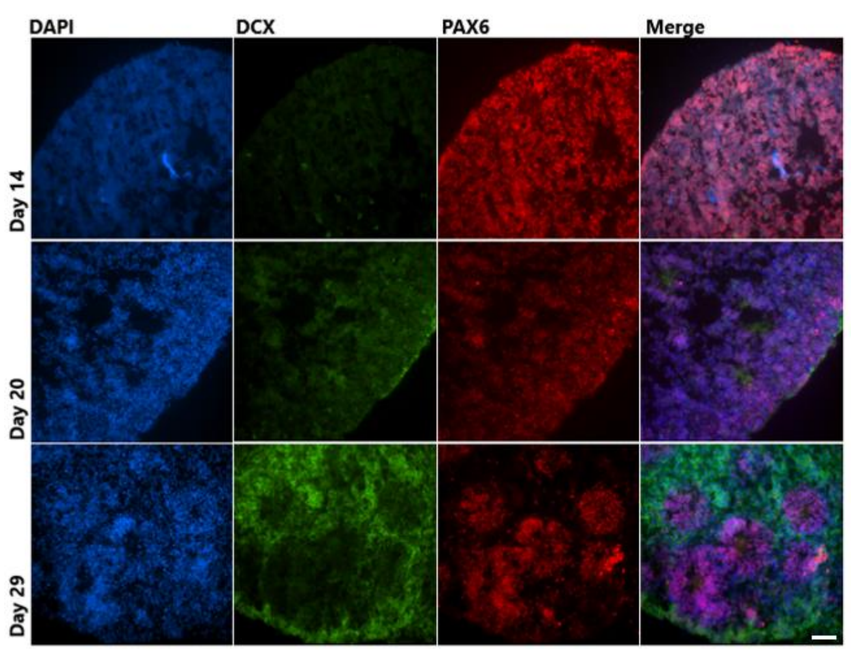

Figure 5. Brain organoids showing a time-dependent increase of the neurogenic marker DCX and a decrease of the radial glia marker PAX6, mimicking developmental changes in cellular composition. After 14, 20 and 29 days in culture brain organoids were cryosectioned and stained for DCX and PAX6. Scale bar = 50 µm

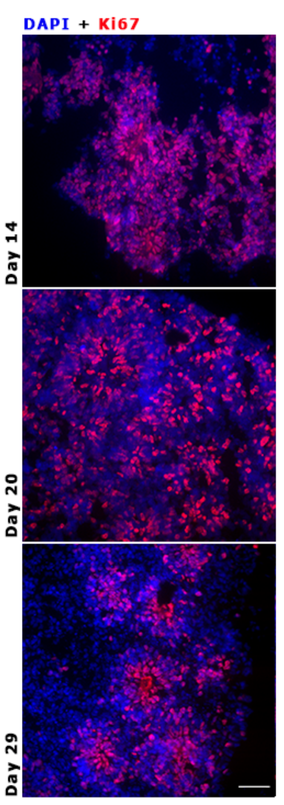

Figure 6. Brain organoids showing a time-dependent reduction in cell proliferation. Cells positive for the proliferation marker Ki67 are restricted to the proliferative ventricular zones in Day 29 organoids. After 14, 20 and 29 days in culture brain organoids were cryosectioned and stained for Ki67 to assess cell proliferation. Scale bar = 50 µm.

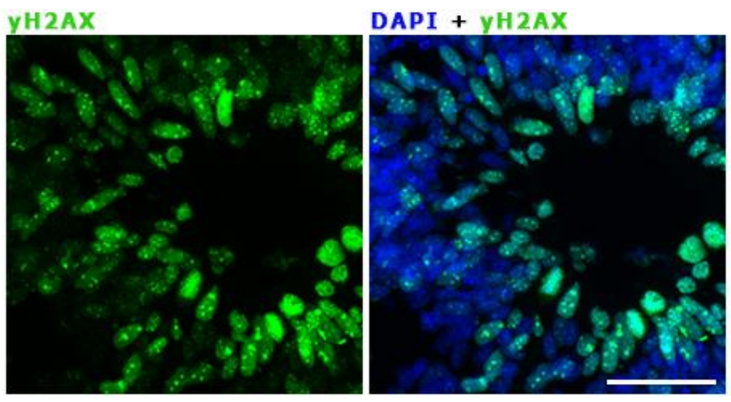

Figure 7. Brain organoids show DNA damage foci especially in the ventricular zone after radiation exposure. Brain organoids were irradiated (2 Gy), fixed after 1 h, cryosectioned and stained for yH2AX to assess DNA damage. Scale bar = 50 µm.

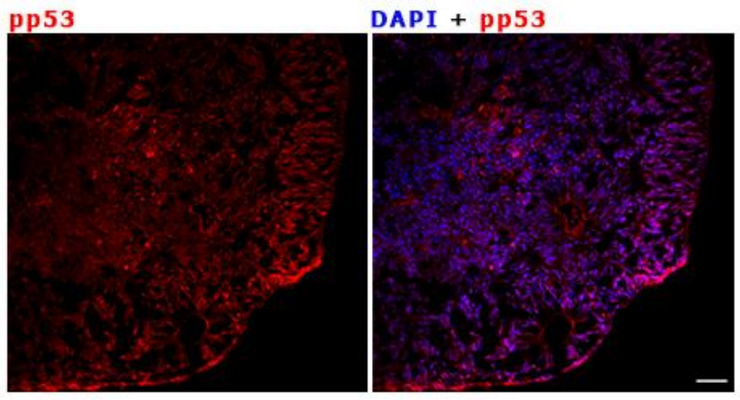

Figure 8. Brain organoids showing a DNA damage response through p53 activation after radiation exposure. Brain organoids were irradiated (2 Gy), fixed after 6 h, cryosectioned and stained for phosphorylated P53 (pP53). Scale bar = 50 µm.

Discussion and future perspectives

As presented here, hESC-derived brain organoids are able to mimic important aspects of the developing human brain organization and also respond to radiation exposure. Having established the experimental model, it will be used to study cellular and molecular mechanisms underlying radiation-induced microcephaly. For this, brain organoids will be irradiated with different X-ray doses (0, 0.1, 1 and 2 Gy) and evaluate possible effects at different time points (Fig. 9).

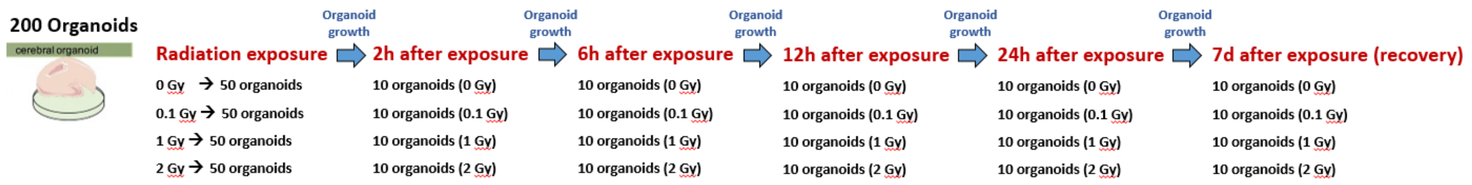

Figure 9. Irradiation plan.

Aiming to investigate the mechanisms behind microcephaly, IHC techniques will be used to assess organoid growth, morphology, cellular diversity, premature differentiation and cell death. Flow cytometry will be used to assess cell proliferation, cell cycle length and DNA fragmentation. To study the molecular mechanisms, single cell analysis methods (sc-RNAseq and sc-ATACseq) will be applied. These mechanisms will be further validated through reverse genetics techniques like CRISPR-Cas9-mediated genome editing to rescue radiation-induced defects.