The role of p53 and primary cilia in the radiation-induced DNA damage response of neural progenitor cells

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Introduction

The exposure of the embryonic mouse brain to ionizing radiation is known to induce the DNA damage response leading to apoptosis and premature differentiation in a p53-dependent way (Mfossa et al., 2020, bioRxiv), culminating in a reduction of the neural progenitor cells (NPCs) and as a consequence microcephaly, a disease marked by a reduction of the head circumference. It was observed that when p53 is knocked out (KO) the apoptosis ratio and premature differentiation are reduced, partially rescuing the microcephaly phenotype revealing a critical role for p53 as a regulator of NPCs fate. Besides that, we believe that the primary cilium, a cellular structure whose biogenesis influences cell cycle progression (Han et al., 2018), could have an important role in the microcephaly phenotype induced by ionizing radiation (Chen et al., 2021).

Objectives

- ✓ Investigate the effects of ionizing radiation exposure on fibroblasts and mouse NPCs evaluating the
  - (1) Cellular proliferation;
  - (2) DNA damage response activation;
  - (3) Primary cilium status;
  - (4) Cellular differentiation

Methodology

Irradiation. Human fibroblasts and mouse NPCs (WT and p53 KO) were irradiated with a single X-ray dose of 2 Gy.

Immunohistochemistry. Fibroblasts, WT and p53 KO mNPCs were fixed after 2 h, 6 h and 24 h following irradiation, and were stained to assess cell proliferation (BrdU, Ki67), the primary cilium status (acetylated tubulin, Arl13b), the DNA damage response (γ-H2AX, p-p53), and cellular differentiation (Nestin, TUJ1 and GFAP). The signal was captured under fluorescence microscopy.

Results

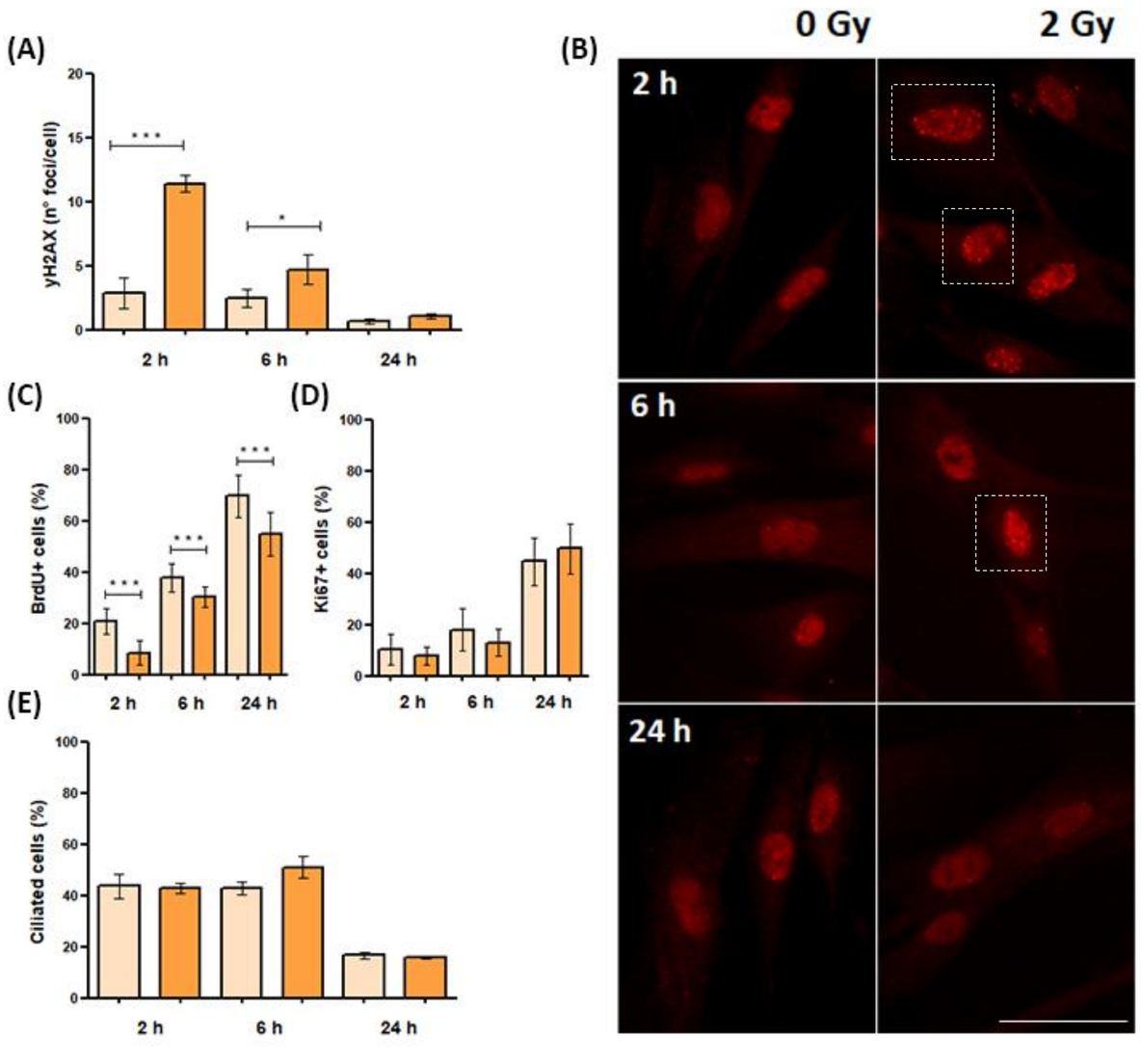

Figure 1. Exposure to ionizing radiation induces DNA damage in human fibroblasts and reduces BrdU incorporation. Following radiation exposure human fibroblasts presented **(A)** marked DNA damage induction specially after 2 h, measured by the number of γ-H2AX positive foci; And presented **(B)** p53 activation that can be observed by p53 foci in the nucleus indicating DNA damage response activation; And a reduction in BrdU incorporation could be observed **(C).** No significant changes could be observed in the percentage of **(D)** Ki67+ cells and **(E)** ciliated cells. Each graph is representative of two experiments with two replicates, and the data is displayed as the means ± standard deviation. ANOVA one-way test followed by Tukey's test for multiple comparisons was performed, *p<0 .01; **p<0 .001. Scale bar = 50 µm

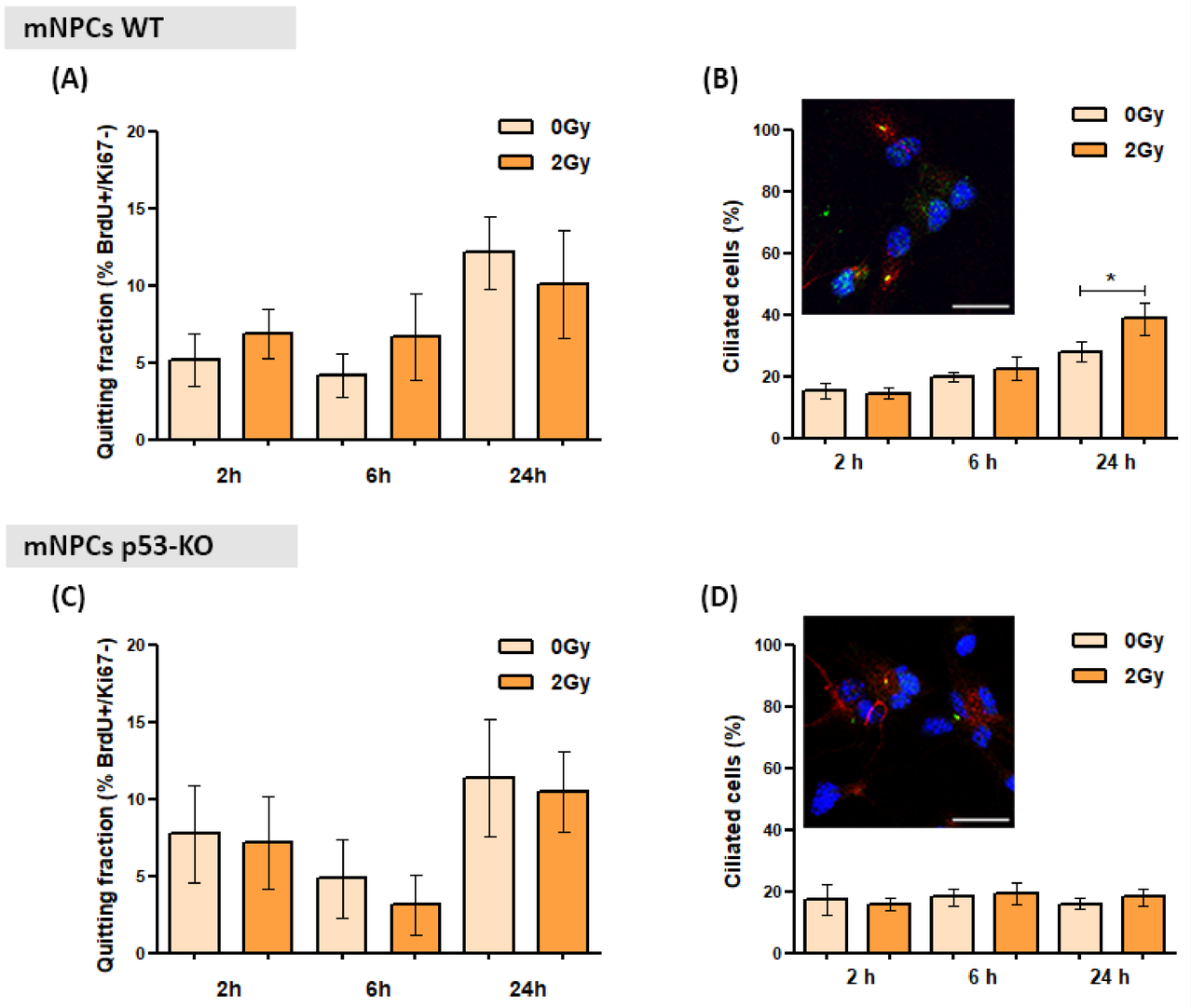

Figure 2. Radiation exposure increases ciliogenesis after 24 h in WT mNPCs, which was not observed in absence of p53. Following radiation exposure the number of cells exiting the cell cycle measure by **(A, C)** the quitting fraction (Brdu+ / Ki67- cells) seems to reduce after 24 h for the WT mNPCs. **(B)** An increase in the percentage of ciliated cells was noticed after 24 h in the WT mNPCs, which was not observed in the p53 KO mNPCs **(F)**. Each graph is representative of two experiments with three replicates, and the data is displayed as the means ± standard deviation. ANOVA one-way test followed by Tukey's test for multiple comparisons was performed. *p<0 .01; **p<0 .001. Scale bar = 20 µm

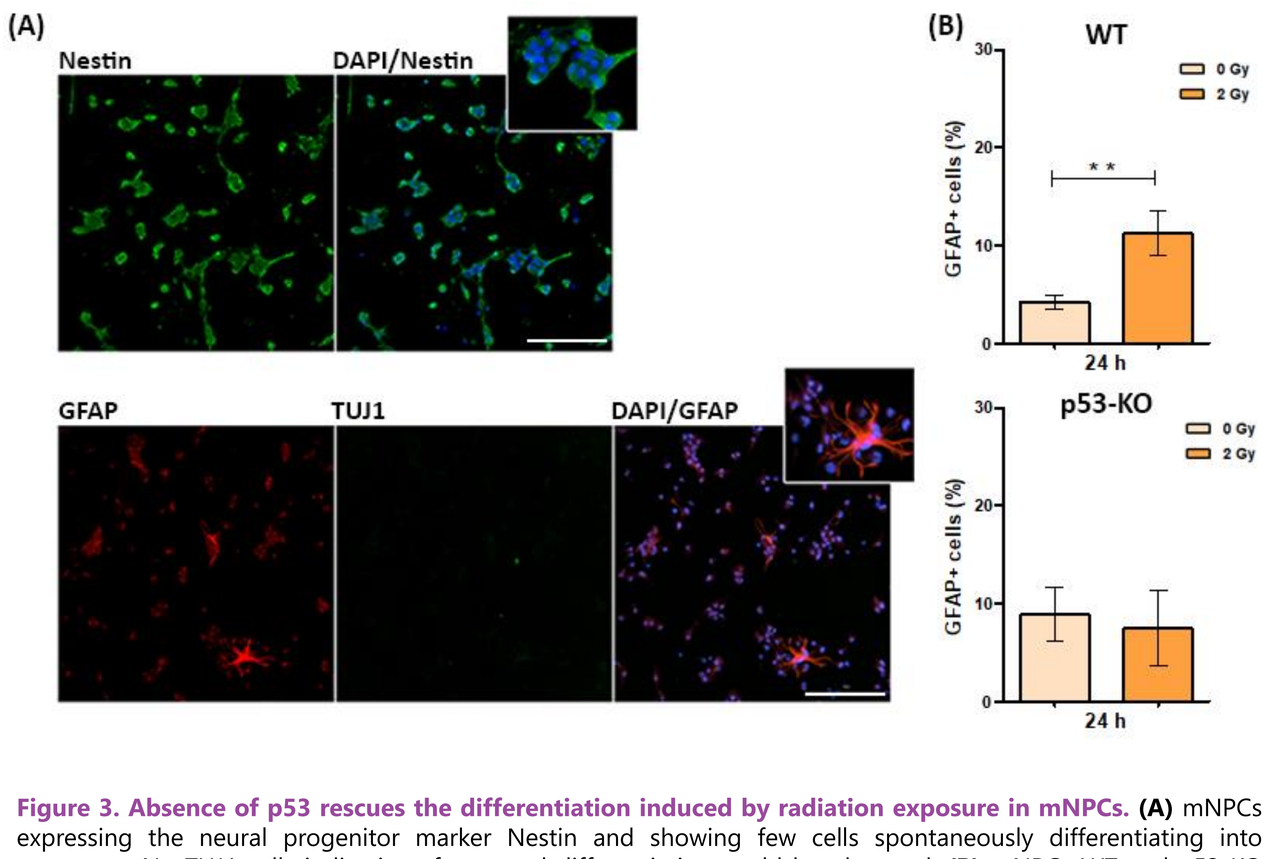

Figure 3. Absence of p53 rescues the differentiation induced by radiation exposure in mNPCs. (A) mNPCs expressing the neural progenitor marker Nestin and showing few cells spontaneously differentiating into astrocytes; No TUJ1 cells indicative of neuronal differentiation could be observed; **(B)** mNPCs WT and p53 KO were exposed to X-ray (2 Gy) and an increase in the differentiation ratio could be observed after 24 h in the WT cells, which was not observed in absence of p53. Each graph is representative of two experiments with two replicates, and the data is displayed as the means ± standard deviation. T-test was performed, **p<0 .002. Scale bar = 100 µm.

Conclusion and future perspectives

These findings demonstrated that radiation exposure of WT mNPCs induces ciliogenesis which may constitute an additional checkpoint to halt the cell cycle allowing cells to repair the DNA damage, and in p53 KO mNPCs there was no increase in ciliogenesis, indicating that cells maintain their proliferation status in the absence of p53 even when its DNA is damaged. However, it is also possible that the increased number of ciliated cells is a consequence of the observed cellular differentiation after radiation exposure. Furthermore, the absence of p53 rescued the cellular differentiation indicating a role for p53 in mNPCs fate. The increased differentiation into astrocytes (rather than neurons) is probably because these cells were isolated from a E15 mouse embryo which corresponds to the gliogenesis stage. These preliminary findings are a starting point to understand the role of the primary cilium and p53 in the DNA damage response, and how cilia could be modulated in an attempt to avoid a reduction of the NPC pool during brain development and ultimately microcephaly.

Based on these results, our future perspectives are to:

(1) Evaluate through FUCCI labelling whether radiation exposure induces cell cycle arrest in mNPCs and if so, whether this is p53-dependent; (2) Evaluate the DNA damage response through NHEJ and HR pathway proteins (XRCC4, RAD51, KU70/80); (4) Evaluate in the absence of cilia (IFT88 silenced mNPCs) the DNA damage response, the differentiation potential and cellular proliferation; (5) Translate these findings to human brain organoids.