DNA double-strand breaks repair kinetics in mammalian and human cells after proton and nitrogen irradiation

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Introduction

•DNA repair is a foundational and highly conservative process in mammalian cells that, when damaged, can cause severe functional impairment or cell death

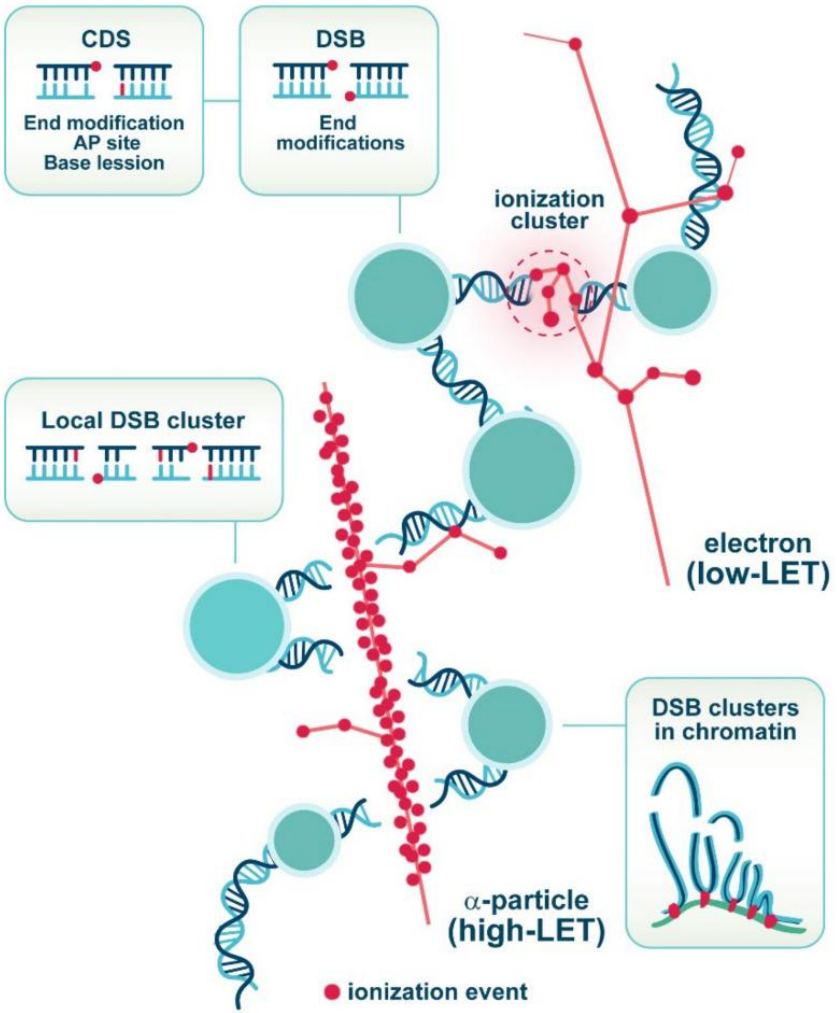
•Disruption of the DNA repair is often associated with malignant transformation of normal cells

•Affected DNA repair can cause severe neurological disorders, like ataxia telangiectasia, manifested in altered brain development

•Revealing of DNA repair features in different cell types can bring better understanding of interactions among repair proteins that can be used as a target in cancer therapy

DNA repair process and repair protein foci generation

Radiation-induced foci is an accumulation of DNA repair proteins at the site of DNA DSBs or other DNA lesions caused by ionizing radiation



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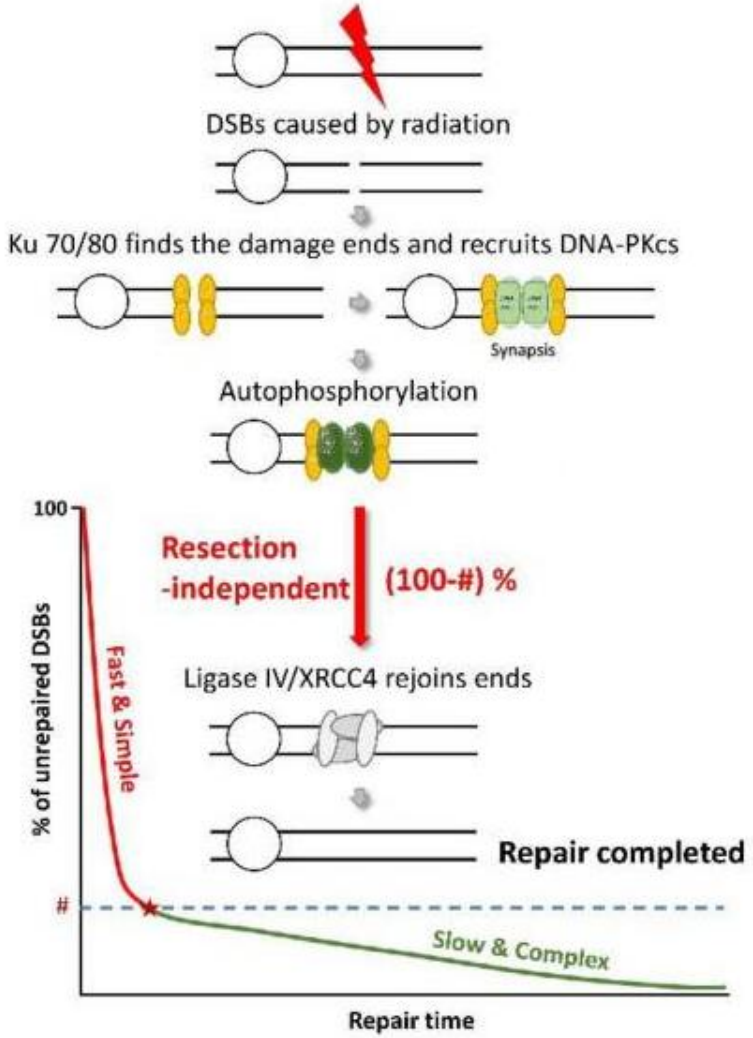

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Materials and methods

Experimental procedure

Objects: 4 cell cultures in vitro

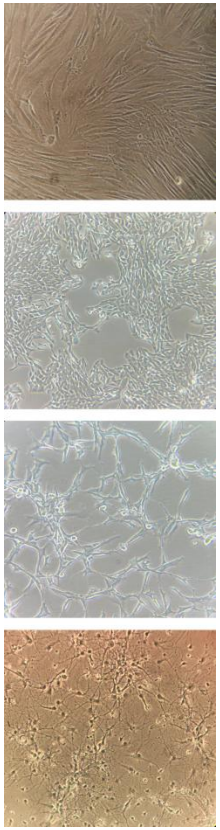
1. Preliminary cell culture preparation: thawing/extraction, subculturing and cultivation
2. Irradiation: protons (1.25 Gy, 150 MeV, ~10 keV/μm) or nitrogen ions (1.25 Gy, 14 MeV, 180 keV/μm)
3. Formalin fixation: 15, 30 min, 1, 4, 24 h post-irradiation, control
4. Immunocytochemical staining: γH2AX and 53BP1 primary antibody
5. Fluorescent microscopy and quantitative analysis

NHDF — Normal human dermal fibroblasts
Normal skin cells

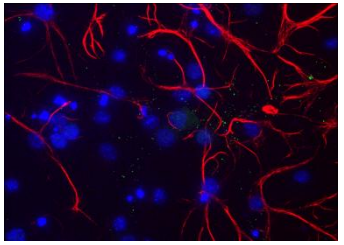
B16 — Mouse melanoma cell line
Malignant skin cancer

U87 — Glioblastoma cell line
Malignant brain cancer

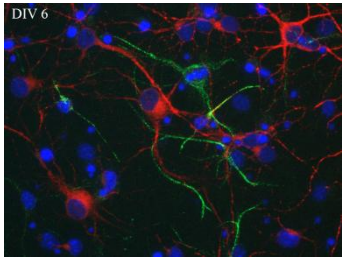
HCC — Rat primary hippocampal cell culture
Cell line extracted from rat hippocampus during first days after birth (P0-P1)



Astrocytes



Mature neurons and NSC

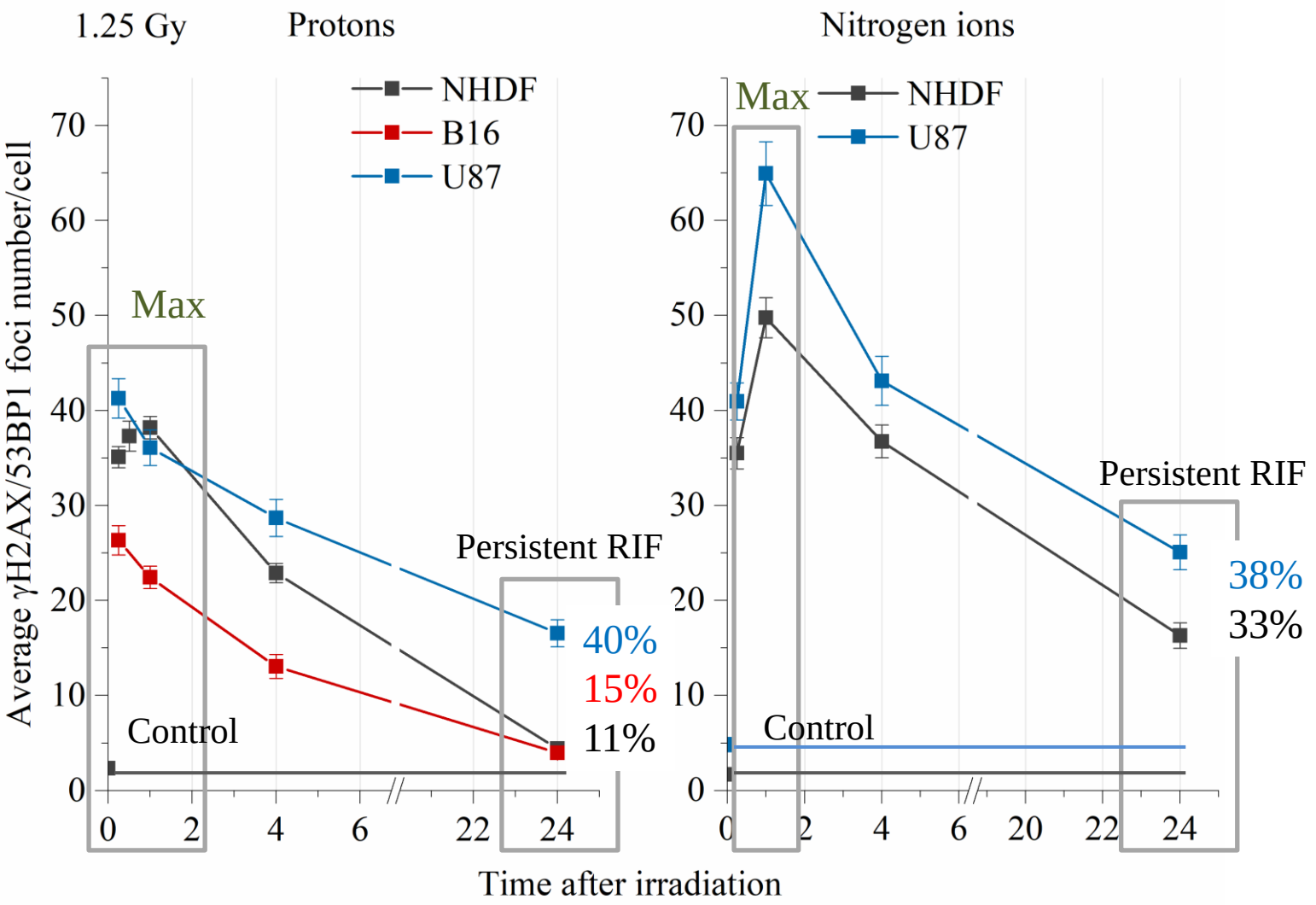


Aims and goals

To analyze the DNA DSB induction and repair kinetics *in vitro* in normal (normal fibroblasts and hippocampal cells) and cancer (U87 and B16) cell lines exposed to particle irradiation with different linear energy transfer (LET) using the immunostaining method and fluorescent microscopy

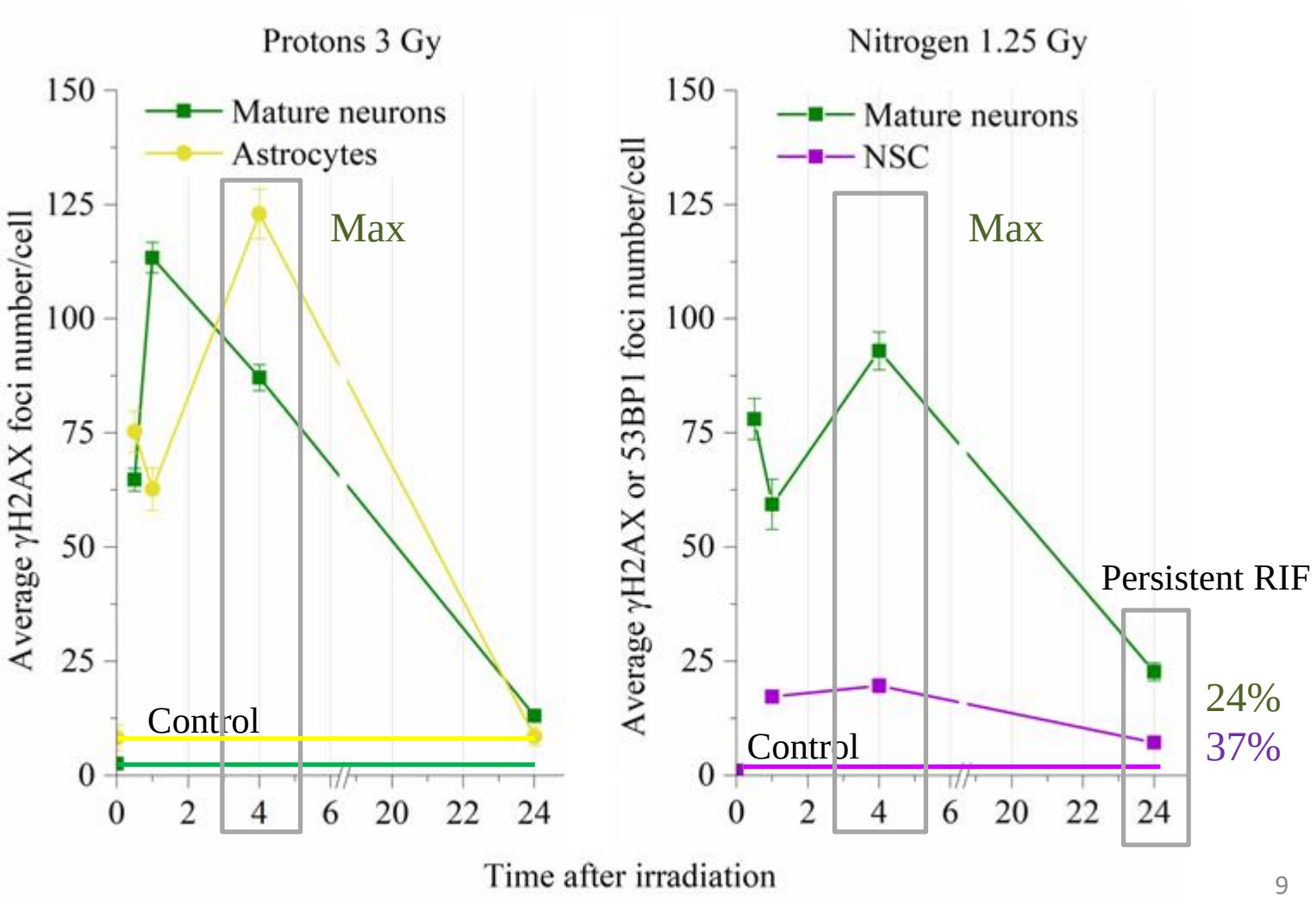
RIF elimination in normal and cancer cells after proton or nitrogen irradiation

RIF - radiation induced γH2AX/53BP1 foci



RIF elimination in normal and cancer cells after proton or nitrogen irradiation

RIF - radiation induced γH2AX or 53BP1 foci



Conclusions

• Under the action of charged particle irradiation with different LET, the higher number is generated by N-ions in all cell types revealing, also, the longer formation stage and delayed start of the DNA DSBs repair.

•Elimination kinetics of RIF demonstrated to be dependent on the cell type that is especially noticeable in primary cell culture cells with its shift to the maximum foci number at 4h post-irradiation.

• Obtained differences in foci elimination in brain cells can be explained by the influence of additional factors like DNA molecular condensation (packaging), restricting an access for repair proteins to DNA lesions, or by functional features in the case of neural stem cells

Thank you for your attention!