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D9.46 – In vitro DNA damage studies

Lead Author: OBU

With contributions from: All project partners (DH-PHE, HMGU, ENEA, DU, OBU) and Advisory Board members

Reviewer(s): CONCERT coordination team

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Abstract

The lens of the eye is known to be more radiosensitive than previously thought but, despite a substantial reduction in occupational dose limits based on recent epidemiological information and reanalyses, the mechanisms of low dose radiation cataract induction are still unclear. This is an important current public health issue, for instance for medical radiation workers, many of whom will need to amend their working practices despite a clear understanding of the underlying process and ultimate effects of chronic, low dose, ionising radiation exposure.

The LDLensRad project aims to bring together experts from across Europe to answer a number of key research questions on this topic, including: how does low dose radiation cause cataracts; is there a dose rate effect, and how does genetic background influence cataract development after radiation exposure. CONCERT Deliverable 9.46, 1.1.2, describes progress to date on DNA damage in cellular models. Note that detailed validation and optimisation of the experimental protocols is described in Deliverable 9.45.

Thus far, Comet assay data indicates that Cs-137 irradiated cells exhibit DNA damage at 1 hour post irradiation which is partially repaired after 24 hours. γH2AX staining studies as a second means of assessing DNA damage are currently in progress. Also, viability of irradiated cells demonstrated a dose dependent decrease 24 hours after irradiation.

Further data will be collected during the following months with the results of both D9.45 and D.46 expected to be submitted for publication together.

Progress summary

Note: Methods are described in D9.45.

1 Cellular viability assay

As proof principle of radiation effects, cell viability measurement after irradiation was carried out. Our data indicate a slight decrease in cell viability at 1 hour post irradiation at both high (0.3 Gy/min) and low (0.065 Gy/min) dose rates. However, 24 hours after irradiation a significant and dose dependent decrease in cell viability can be observed at both dose rates (except from 0.1 Gy at 0.3 Gy/min, in which there is no decrease in cell viability) (**Figure 1**).

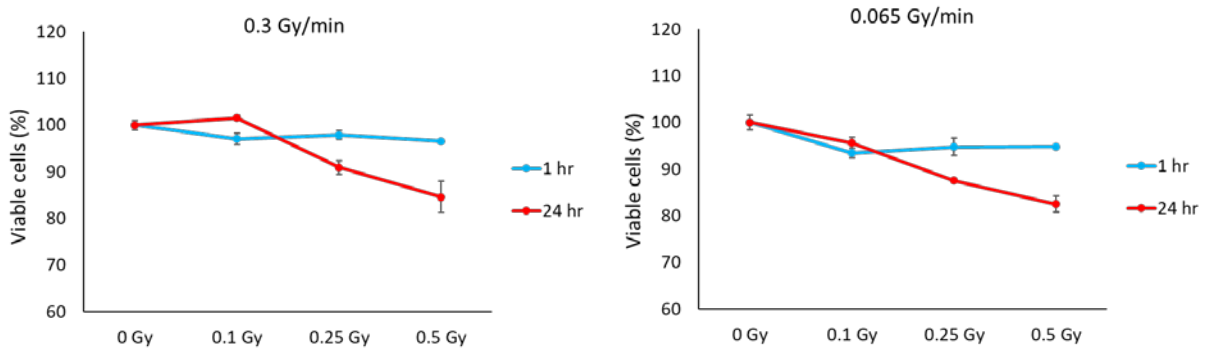

Figure 1. HLE-B3 cells analysed for cell viability after treating with different doses of Cs-137 irradiation at high and low dose rates.

2 Comet assay

Under task 2.2 (DNA damage responses), cells were harvested at 1 hour and 24 hours after irradiation and analysed by comet assay. DNA damage increases dose dependently 1 hour post irradiation at both high and low dose rates. However, most of the damage appears to be repaired after 24 hours of irradiation (**figure 2**).

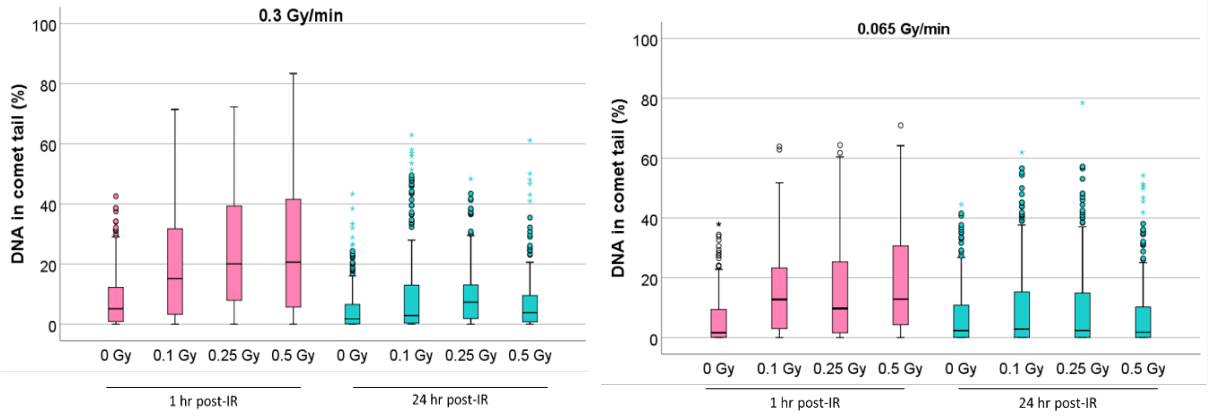

Figure 2. HLE-B3 cells treated with high and low dose rates of Cs-137 irradiation and analysed by comet assay (Box plot with boxes showing interquartile ranges and bars 95% confidence intervals).

3 Ongoing work

Immunofluorescence (γH2AX staining) has been performed as a second method to determine DNA damage. Cells are awaiting microscopy and analysis at the moment.

Discussion and conclusions will be carried out once the data collection and analysis are complete.