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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 9.45, 1.1.2 of the project, describes the results of in vitro experimentation on stress and communication in cellular models to date. Following detailed validation and optimisation of the experimental protocols, human lens epithelial (HLE)-B3 cells were irradiated with different doses at high (0.3 Gy/min) and low (0.065 Gy/min) dose rate using a Cs-137 gamma irradiator, as a practical onsite alternative to the Co-60 source for these initial validation experiments. Oxidative stress analysis was carried out and reactive oxygen species (ROS) levels seem to increase as dose of radiation increases, when examined at 1 hour post-irradiation. However, it appears that 24 hours after irradiation, cells treated at 0.3 Gy/min (high dose rate) have the highest levels of ROS at 0.25 Gy. Whereas, cells that have been treated at low dose rate (0.065 Gy/min), show higher ROS production than the cells treated at 0.3 Gy/min at 0.5 Gy. Senescence assay, telomerase activity analysis and telomere length measurements are under investigation with current work focusing on optimisation.

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.

CONTENT

PROGRESS SUMMARY ---------- 5

1 INITIAL CELL LINE CHARACTERIZATION ---------- 5

2 CELL CULTURE OPTIMISATION ---------- 5

3 KARYOTYPING ---------- 5

4 X-RAY PILOT STUDIES ---------- 5

5 CELL VIABILITY ---------- 5

6 ROS MEASUREMENT ---------- 6

7 COMET ASSAY ---------- 6

8 CS-137 GAMMA IRRADIATION ---------- 7

9 IN VITRO STRESS, COMMUNICATION STUDIES ---------- 7

10 ONGOING WORK ---------- 8

REFERENCES ---------- 8

Progress summary

1 Initial cell line characterization

Different cell lines received and established in OBU's lab. Cells were propagated, and some vials were stored in liquid nitrogen for future use. Initial cell line characterisation was carried out for HLE-B3, FHL124 and HLEC Donor 2 cells, including

- Cell culturing optimisation
- Karyotyping for chromosome numbers

2 Cell culture optimisation

Cell culture optimisation was carried out in terms of media requirement and estimation of their cell cycle profile.

3 Karyotyping

Chromosomal analysis was performed for aforementioned cell line and the results show that HLEC Donor 2 cells which are primary cell line contain, as expected, around 46 chromosomes, whereas HLE-B3 and FHL124 cells which are immortal, contain more than 46 chromosomes (**Figure 1**).

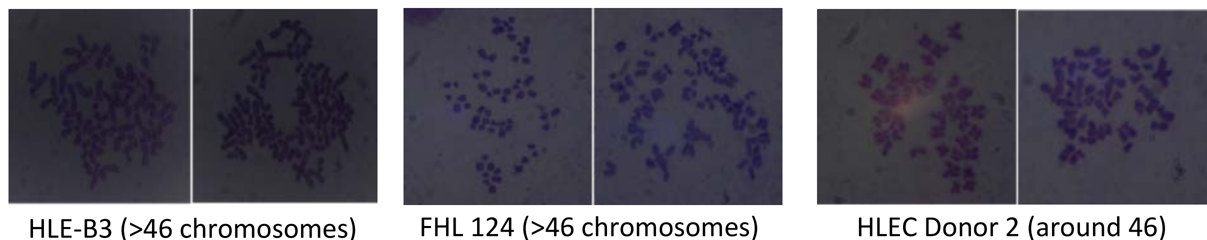

Figure 1. Karyotype of lens epithelial cells received.

4 X-Ray pilot studies

A series of optimisation and pilot studies were carried out using the OBU Cs-137 X-Ray irradiation facilities. Cells were harvested and analysed for a range of endpoints using doses of 2 and 10 Gy (at 0.3 Gy/min).

5 Cell Viability

Viability of irradiated cells were assessed 1 hour and 24 hours post-IR using a Muse® Cell Analyser. Our results show that at 1-hour post-IR in X-Ray irradiated cells, there is a slight reduction in viability of cells irradiated with 10 Gy. Whereas 24 hours post-IR the viability of cells has decreased in a dose dependent manner, which is clearly, lower than 1-hour time point (**Figure 2**).

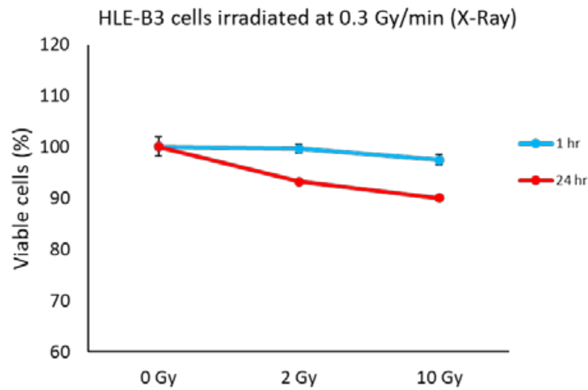

Figure 2. Viability of HLE-B3 cells irradiated with X-Ray at 0.3 Gy/min.

6 ROS measurement

The data from our x-ray experiments show that there seems to be a slight increase in ROS production in irradiated cells, which is not significant (**Figure 3**). However, technical issues mean the results need to be confirmed by repeating the experiment.

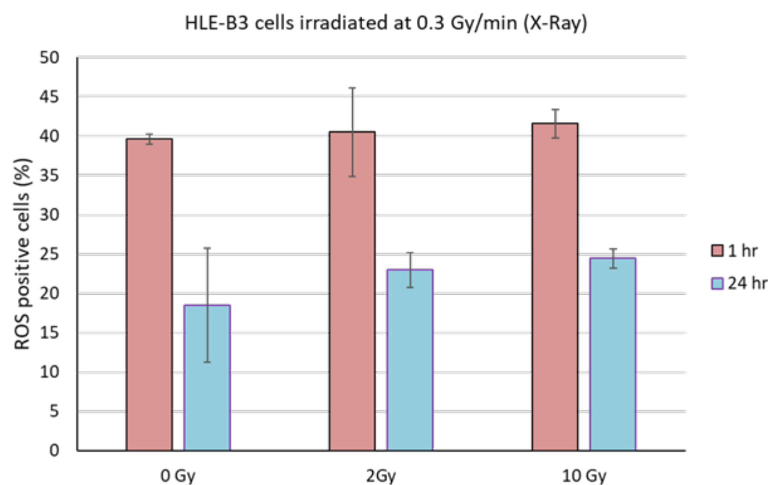

Figure 3. Percentage of ROS positive cells in X-ray irradiated HLE-B3 cells.

7 Comet Assay

Cells were harvested at 1 hour and 24 hour post-IR to determine the DNA damage using comet assay.

Our data indicate that following X-Ray irradiation there is an increase in the percentage of DNA in comet tail at both 2 and 10 Gy. However, it seems that after 24 hours the DNA damage has been partially repaired (**Figure 4**).

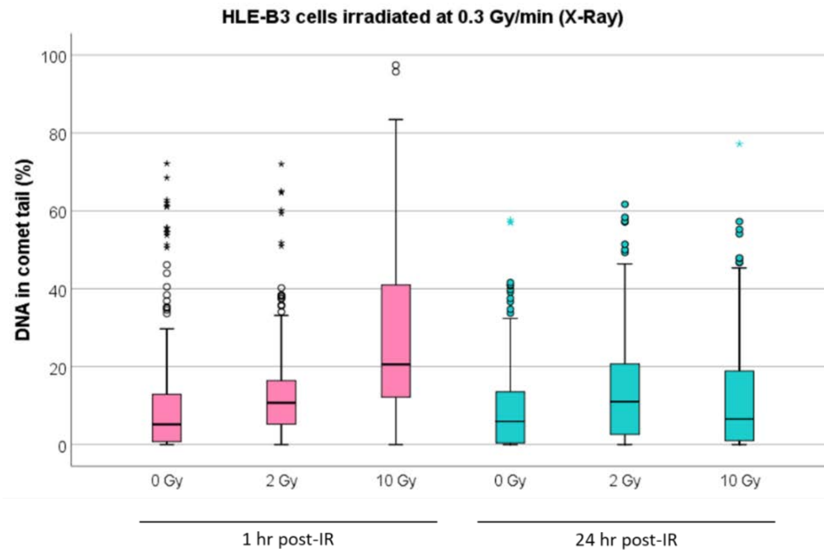

Figure 4. Comet assay results in X-Ray irradiated cells (at 0.3 Gy/min dose rate; Box and whisker plot with boxes indicating interquartile ranges around the median (solid line) and bars indicating 95% confidence limits).

8 Cs-137 gamma irradiation

After confirming the validation of our techniques, Cs-137 gamma irradiation was carried out.

HLE-B3 cells were chosen to start our investigations. Irradiation was carried out at the Gray institution, University of Oxford. Cells were irradiated when they were around 70% confluent. Cells were irradiated at 0.1, 0.25 and 0.5 Gy at dose rates of 0.3 and 0.065 Gy/min corresponding to high and low dose rates respectively. Un-irradiated cells were used as a control.

9 In vitro stress, communication studies

OBU is involved in work package 2 (in vitro experimentation). To assess the in vitro stress after irradiation, ROS measurement was performed using Muse® cell analyser. Analysis of the data shows that 1 hour after irradiation, ROS levels increase as dose of IR increases. 24 hours after irradiation, it appears that cells treated with 0.25 Gy at 0.3 Gy/min have the highest level of ROS. Whereas, cells that have been treated at low dose rate (0.065 Gy/min), show higher ROS production at 0.5 Gy (**Figure 5**).

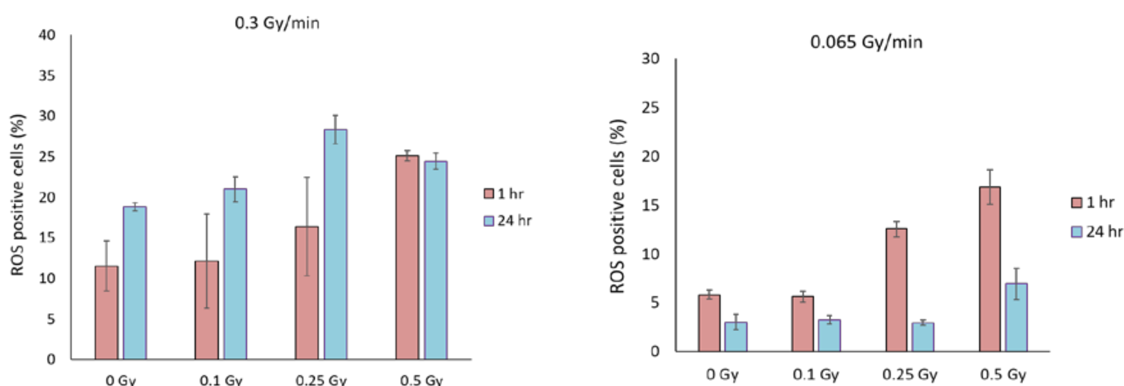

Figure 5. HLE-B3 cells irradiated at 0.3 Gy/min (high dose rate) and 0.065 Gy/min (low dose rate) and analysed for oxidative stress measurement.

10 Ongoing work

Senescence studies: It has been well documented that senescence can be associated with age-related cataract (Fu et al., 2016; Yan et al., 2019). Thus, a senescence assay was also carried out in the HLE-B3 cells following high and low dose rates of Cs-137 irradiation. The data collection and analysis are currently in progress.

Telomerase activity measurement: Currently we are in the process of optimising our methods to measure the telomerase activity using Telomere Repeat Amplification Protocol (TRAP) assay.

Telomere length measurement: We have extracted DNA from HLE-B3 cells and some other cell lines (U2OS, Hela, GM08399) in which their telomere length is known. The next step would be to perform telomere length measurement on these cells to validate and optimise the existing protocol.

Once optimization is complete, the optimized protocols will also be carried out on primary HLECs.

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

References

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Yan Y, Yu H, Sun L, Liu H, Wang C, Wei X, Song F, Li H, Ge H, Qian H, Li X, Tang X, Liu P. (2019) 'Laminin α4 overexpression in the anterior lens capsule may contribute to the senescence of human lens epithelial cells in age-related cataract'. *Aging (Albany NY)*. 11(9):2699-2723.