Mouse lens epithelial cells and lymphocytes exhibit similar sensitivity to γ-irradiation

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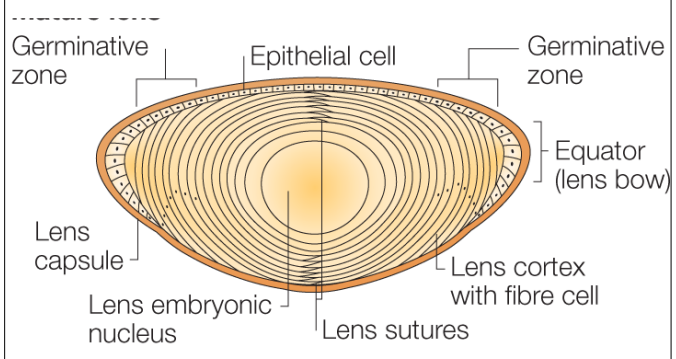
Introduction:

The eye lens is usually considered as a radiation-sensitive tissue, and it is known for a long time that ionizing radiation causes opacification of the lens (cataracts). This concept of a particular radiation sensitivity of the ocular lens is based primarily upon its unique cellular architecture: the life-long dividing lens epithelial cells (LECs) at the anterior part, and the terminally differentiated fiber cells persisting for the entire life (**Fig. 1**).

Fig. 1: The lens: cell types and differentiation.

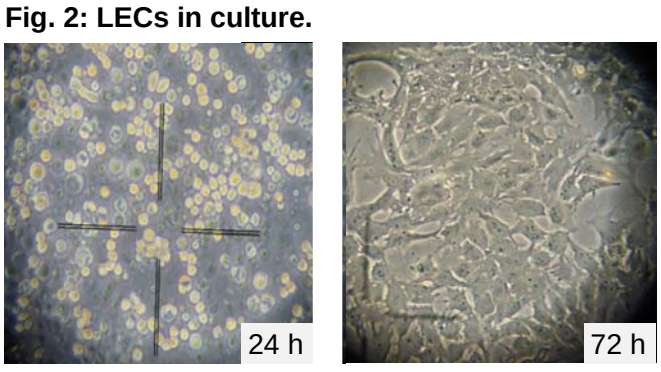


In the mature lens, epithelial cells (LECs) divide throughout entire life; they move towards the equator and elongate forming new fiber cells. During this process of terminal differentiation, they loose their nuclei and mitochondria but remain persisting in an onion-like structure for the entire life (from Graw, 2003).

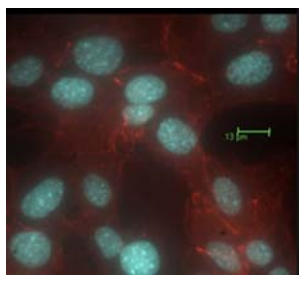
Methods

Lens epithelial cells (LECs) were prepared from adult male mice (age: 7-8 weeks). The lens capsule with the attached lens epithelium was removed from the fiber cells. After a short treatment with trypsin, the cells were re-suspended and transferred into 24-well plates (37°C with 5% CO_2). For γ-H2AX-foci assay, the LECs grew in the wells on coverslips. Cells were further processed after 3 days in culture; they still showed features of lens epithelial cells (**Fig. 2**).

Fig. 2: LECs in culture.



Lens epithelial cells (LECs) of 7-8 weeks old C57BL6/J mice in culture after 24h or 72 h (magnification 20x).



After 72 h in culture, LECs still express E-cadherin (red; indicating their epithelial characteristics) and FoxE3 (green; indicating their lens characteristics; blue: DAPI). FoxE3 is in the nuclei only.

Resting **lymphocytes** were prepared according to standard procedures from 4-5 ml heparin blood of 2-3 adult male mice; peripheral blood mononuclear cells were separated via a Biocoll gradient.

LECs and lymphocytes were **irradiated** with 0 Gy, 0.25Gy, 0.5 Gy, 1Gy and 2 Gy (137Cs-source HWM-2000; Markdorf, Germany) at a dose rate of 0.5 Gy/min. For the comet assay, the radiation was performed in suspension (10 µl suspension of LECs or 100 µl suspension of lymphocytes) and cells were kept on ice. For the γH2AX-assay, cells were irradiated as adherent monolayers at room temperature. DNA repair kinetics were measured by incubation of the irradiated samples at 37°C for defined time intervals (Comet assays: 0; 5; 15; 60 min; γH2AX assay: 1h; 4 h; 24 h) to allow DNA repair.

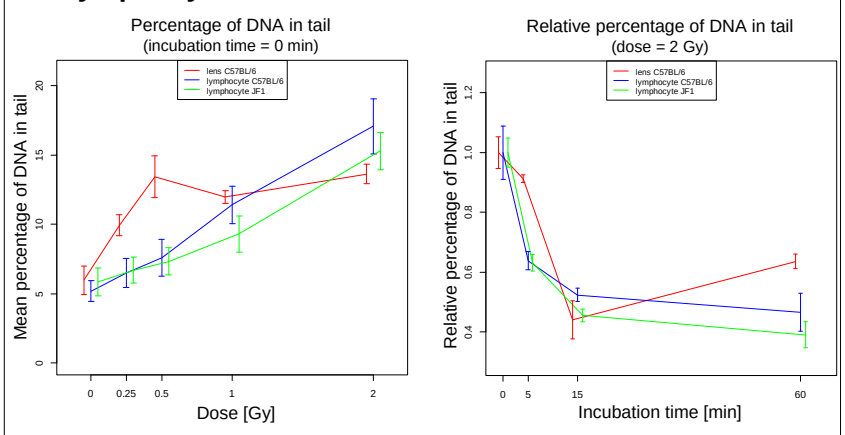
The **comet assay** and the **γH2AX** experiments were performed according to standard procedures (Rössler et al., 2006; Gomolka et al., 2005).

Aim of this study

In this pilot study we ask the question, whether the radiation sensitivity of LECs is comparable to that of lymphocytes. In both cell types, radiation damage was tested using comet assay and γH2AX foci. In a second step, we compared the radiation sensitivity of both cell types in two different mouse strains: C57BL/6J mice as a well-known reference strain, and Japanese Fancy mice (strain designation: JF1) as far different strain. γH2AX foci formation was used again as parameter for radiation damage. Within a dose range of 0.25 Gy – 2 Gy, our results indicate cell-type specific differences between both strains. Furthermore, we will be able to unravel cell-type specific differences and the underlying molecular mechanisms. This will help to better understand the variation in the frequencies of radiation-induced cataracts among humans.

Results:

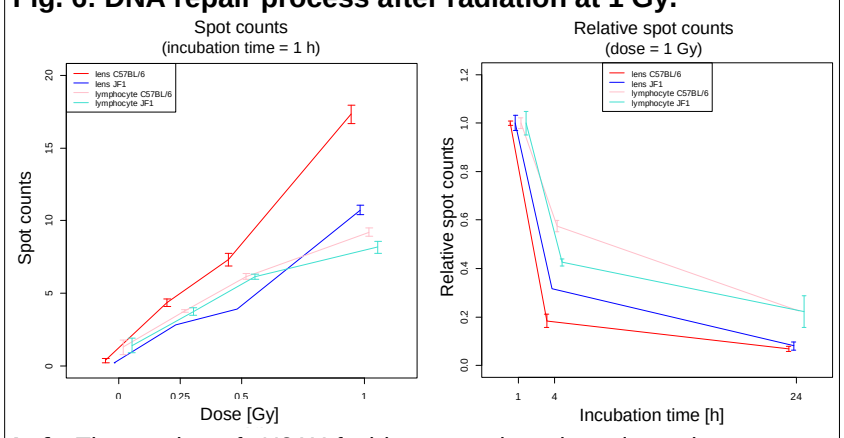
Fig. 4: Comet Assay: DNA damage in lens epithelial cells and lymphocytes after irradiation.



Left: The damage of DNA is given as tail length in the comet assay; LECs from C57BL/6J mice show a faster increase than lymphocytes from both strains. Data are presented ± SEM.

Right: After radiation with 2 Gy, cells are allowed to repair DNA up to 60 min. The initial DNA damage is set to 1; the two cell types have a similar repair capacity. Data are presented ± SEM.

Fig. 6: DNA repair process after radiation at 1 Gy.



Left: The number of γH2AX foci increases in a dose-dependent manner. The LECs from C57BL/6J mice show more foci than the other cells.

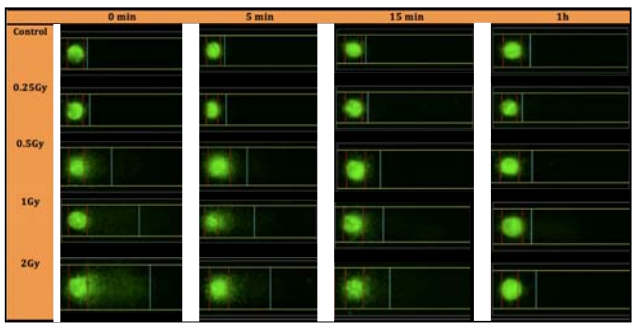
Right: The number of γH2AX foci after irradiation at 1 Gy and incubation time of 1 h was set to 1 (relative spot counts); it decreases with increasing incubation time, which was allowed for 24 h. Repair proceeds faster in the LECs compared to lymphocytes; the strain effect on repair is different between the cell types (p-value for interaction effect in the three-way ANOVA p=0.0096).

Conclusion

Our results demonstrate that on the DNA level, lens epithelial cells and lymphocytes exhibit a similar sensitivity to ionizing radiation. Moreover, the observed difference in DNA repair between the LECs from C57BL/6J mice compared to the LECs from JF1 mice and to the lymphocytes of both strains as indicated by the data of the γH2AX assay needs further experiments to identify the underlying molecular mechanisms.

Results:

Fig. 3: Comet Assay
Examples of DNA damage in LECs at different time points after irradiation.



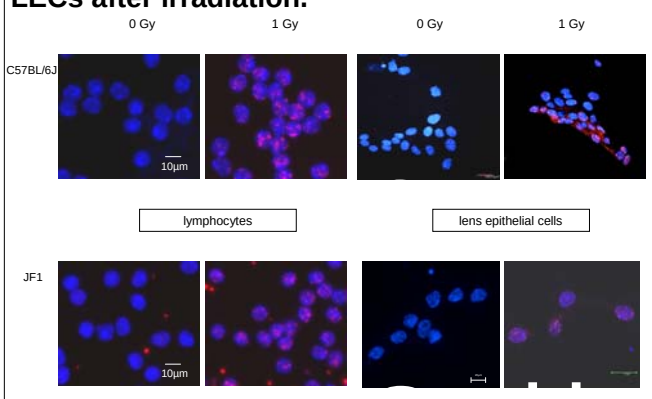
Examples of DNA damage after Comet technique in mouse lens epithelial cell nuclei after irradiation with ^{137}Cs (0.25 – 2.00 Gy) and repair time of 0 - 60 min. Bars describe the amount of DNA in head (red) and DNA in tail (blue).

Main results from the Comet-Assay:

1.The DNA damage is dose-dependent in LECs and lymphocytes;

2.Below 1 Gy, increase in DNA damage in LECs is larger than in lymphocytes.

Fig. 5: γH2AX foci in mouse lymphocytes and LECs after irradiation.



Examples of immunofluorescence staining of γH2AX in mouse cells without (0 Gy) and after 1 Gy irradiation and repair time of 1 hour. Blue: staining of cell nuclei with DAPI. Red: staining with antibody against γH2AX (coupled with Cy3). Magnification 40x; bars indicate size. Upper row: cells from C57BL/6J mice, lower row: cells from JF1 mice; left, lymphocytes; right, LECs.

Main results from the γH2AX-Assay:

1.The DNA damage is dose-dependent in LECs and lymphocytes;

2.DNA damage in LECs of C57BL/6J mice is larger than in LECs of JF1 mice and of lymphocytes;

3.DNA repair is faster in LECs of C57BL/6J than in LECs from JF1 mice and of lymphocytes.

References

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