Influence of Ionizing radiation on the potency state of murine embryonic stem cells*

S. Luft[1], A. Helm[1], P. Hesse1[1], D. Pignalosa[1], S. Brons[2], P.G. Layer[3], M. Durante[1,3], S. Ritter[1,#]

[1]GSI, Darmstadt, Germany, [2]HIT, Heidelberg, Germany, [3]Technische Universität Darmstadt, Germany.

The cell potency describes the differentiation potential of a cell. There are different levels of cell potency, ranging from the ability to differentiate into any cell type and therefore being able to create a whole organism (totipotency) to the ability to differentiate into only one cell type (unipotency). Embryonic stem cells (ESCs) are the most potent cells that can be cultured *in vitro*. They can give rise to cells of all the three germ layers of an organism and are therefore called pluripotent (overview in [1]). Under appropriate culture conditions, ESCs closely resemble the embryonic development *in vitro*, providing a model system to investigate the effects of external factors like ionizing radiation on the early mammalian development.

To investigate whether ionizing radiation affects the potency state of ESCs, the murine ESC line D3 was chosen as a model. Cells were exposed to X-rays or C-ions. C-ion irradiation was performed at SIS, GSI and at HIT, Heidelberg (25 mm extended Bragg peak with energies ranging from 106 to 147 MeV/u, dose averaged LET=75 keV/µm), D3 cells were harvested at 10 h and 8 d after exposure and chromosome aberrations were analysed by means of the mFISH technique. Furthermore, at 0, 8 and 16 days after exposure the presence of pluripotency markers was examined by flow cytometry. In first experiments cells were stained with fluorescent labelled antibodies against Oct3/4 and SOX2, both being transcription factors that play a key role in the complex interactive network establishing pluripotency.

Analysis of chromosome aberrations in first cycle cells (at 10h) and in the progeny of cells which survived the exposure (at 8 d) demonstrates that damaged cells are efficiently removed from the culture after X-ray irradiation, while in the offspring of C-ion exposed cells the number of aberrant cells was still significantly higher than in the control sample (data not shown).

The flow cytometric quantification of the Oct3/4 presence over time is shown in figure 1A. The fluorescence signal intensity shows a noticeable inter-experimental variation. For example, between control samples there is a 2.5-fold difference. Nevertheless, in the same experiment the signal intensity of irradiated and corresponding control cells is similar indicating that the presence of the pluripotency marker Oct3/4 in the surviving cells is not affected by ionizing radiation up to16 d after exposure. This has also been shown for the presence of a second pluripotency marker, SOX2 (see figure 1B), even though its relative median fluorescence intensity was much lower compared to Oct3/4.

To verify the data, the activity of alkaline phosphatase, an enzyme which is highly expressed in pluripotent cells, was examined by performing a colour reaction assay. As shown in figure 2, D3 cells exhibit the typical red stain indicating the pluripotent state. In between the colonies, single unstained cells appear, demonstrating minor differentiation processes in the culture.

However, drawing conclusions from the expression of only few pluripotency markers is questionable, since pluripotency is established by a complex network of proteins and molecules, influencing and interacting with each other. Furthermore, Oct3/4 plays a role not only in relation to pluripotency, but is also known to accumulate at chromatin lesions, like double strand breaks [2].

Therefore, in subsequent studies we will examine the expression of a larger number of pluripotency markers by both flow cytometry and a specific stem cell signalling rtPCR array (see also [3]). The latter technique allows quantifying the expression of 96 different stem cell-characteristic genes.

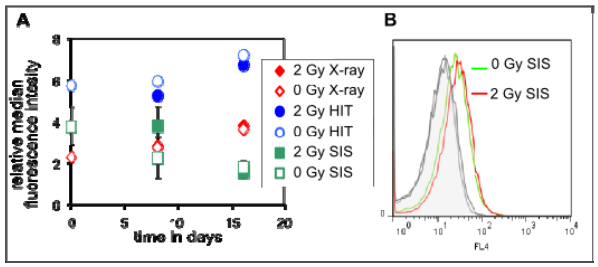

Figure 1: Immunochemical detection of Oct3/4 and Sox2 measured by flow cytometry. A: Median fluorescence intensity for Oct3/4, normalized to isotype control over time. B: One-parameter-histograms showing the fluorescence intensity for SOX2 in control (green) and 2-Gy C-ions exposed cells (red) 16 d after exposure; tinted grey: isotype control.

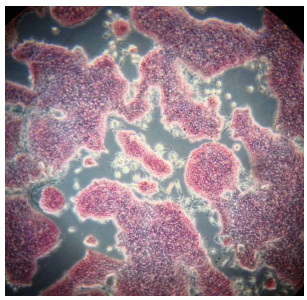

Figure 2: Alkaline Phosphatase Staining (red) of murine ESC colonies confirms the pluripotent state.

References

[1] S. Yamanaka et al., Cell and tissue research 331, 5 (2008)

[2] E. Bartova et al.,. PloS one 6, e27281 (2011)

[3] Pignalosa et al. GSI scientific report 2012 (this issue)

*Work supported by BMBF grant 02NUK025A and HGS-HIRe.

s.ritter@gsi.de