Effects of X-rays and carbon ions on pluripotency maintenance and differentiation capacity of mouse embryonic stem cells

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Embryonic Stem (ES) cells have the ability to self-renew as well as to differentiate into cells of the three germ layers (ectoderm, endoderm and mesoderm) due to their pluripotency, giving rise to a variety of differentiated cells, such as cardiomyocytes [1]. They constitute an excellent model system to study the effects of ionizing radiation on early embryonic development that are still poorly understood [2]. The model system used for the present work is the mouse ES-D3 cell line, and the radiation effects on both the pluripotency maintenance and the ability to develop into cardiomyocytes was assessed.

The cells were exposed to X-rays (250 kV, 16 mA) or carbon ions (C-ions, 25-mm extended Bragg peak, energy range: 106-147 MeV/u with a mean LET of 75 keV/µm at sample position). Irradiated cells were grown under conditions that maintain pluripotency (i.e. cultured in the presence of leukaemia inhibitory factor, LIF) or directed to differentiate by embryoid body (EB) formation in the absence of LIF as previously described [3-4]. Ten days after differentiation initiation, beating clusters of cells can be observed by bright field microscopy. RNA from EBs on days 0, 4, 6 and 10 of differentiation was extracted and pluripotency markers’ gene expression was measured by quantitative reverse transcriptase PCR (RT-PCR).

Pluripotency of the progeny of irradiated cells was examined by performing western blot (WB) analysis. Pluripotent cells express a complex combination of transcription factors and epigenetic regulators [5]. WB analysis of the transcription factors OCT3/4 and SOX2, two of the key players in ES cell pluripotency maintenance, showed that the protein amount of both was comparable to the non-irradiated control sample about 2 weeks after exposure to X-ray and C-ions (Figure 1).

To examine the differentiation potential of irradiated ES-D3 cells, they were differentiated via EB formation. Following C-ion exposure, the fraction of beating EBs was lower than in the control, while isodoses of X-rays exerted no effect (for more details see [4]).

The low fraction of beating EBs observed after C-ion exposure might result from a delayed differentiation of the cells. Exposed cells undergo a cell cycle delay [4]. Additionally, cells are removed by apoptosis. This may impair EB formation and differentiation into cardiomyocytes, which depend on the initial cell number [4,6]. To investigate the pluripotency marker expression during the differentiation process, the expression of the genes coding for OCT3/4 (designated as POU5F1) and for SOX2 were assessed by quantitative RT-PCR. In the differentiating control cells POU5F1 gene expression decreased with time. The same effect was observed after X-ray irradiation, whereas this decrease was delayed in the C-ion exposed samples (Figure 2). Due to high inter-experimental variations in the gene expression of SOX2, it is difficult to draw firm conclusions (data not shown). Investigation of cardiac-specific gene expression is now underway.

References

[6] Preda et al., Tissue Cell, 2013, 45, 54-60

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