Radioresistant subpopulation in a culture of glioma-initiating cells

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Introduction

According to the cancer stem cell hypothesis, a tumor comprises different cell types including tumor cells with stem cell properties. These cells, called cancer stem(-like) cells or tumor-initiating cells, are believed to be responsible for tumor initiation, progression and relapse. Moreover, it was found that these cells are more resistant to radiotherapy and chemotherapy than the bulk tumor cells. As heavy ion irradiation can effectively inactivate radioresistant cells, it was proposed that heavy ions could overcome the radioresistance of cancer stem-like cells [1]. Our irradiation experiments with glioma-initiating cells could indeed show that accelerated C- and Ti-ions reduced the neurosphere formation rate more effectively than X-rays [2]. A limiting factor in these experiments was the time-consuming counting of neurospheres, which had to be done manually using a phase-contrast microscope. In the last year, a software-based analysis system called CARL was developed at Hochschule Darmstadt (see Sonnemann et al., this report) and was now tested on glioma-initiating cells.

Materials and Methods

We used glioma-initiating cells kindly provided by Dr. E. Kim, Neurosurgery Department, University Hospital Mainz, Germany. The cells are a radioselected subpopulation of the cell line #10 described in a publication by Barrantes-Freer et al [3]. The cells were cultured as described previously [2] and irradiated with different doses of X-rays (0-12 Gy). To test the new software-based analysis-system CARL, the analysis was performed in parallel by eye under the phase-contrast microscope and with CARL.

Results and Discussion

Figure 1 shows the dose-effect curve for neurosphere formation after X-ray irradiation. The neurosphere formation rate was determined by eye under the microscope and by the CARL system. Both results are in good agreement. Similar tests were performed for other cell lines and showed good agreement between the automatic analysis and the scoring by eye (data not shown). A reference analysis by eye is of crucial importance for each cell line in order to adjust the software parameters. Once this is made, the software produces reliable results. Compared to the manual analysis, the CARL system is much faster (less than one compared to 15 minutes per sample), provides a size distribution of the neurospheres in addition to the number and the results are independent of the individual scorer.

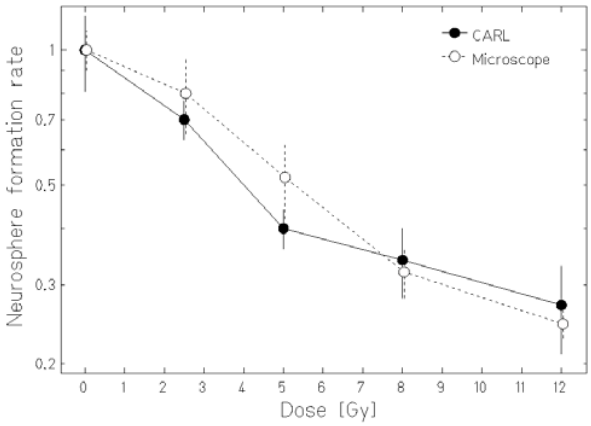

Figure 1: Neurosphere formation (normalized to unirradiated control) after different doses of X-rays. The neurosphere number was determined by eye under the microscope (open circles) or using the CARL system (closed circles). Error bars represent standard deviation of four flasks.

The curve progression in figure 1 shows the typical linear-quadratic behavior in the dose-range 0-5 Gy, but at higher doses the slope becomes shallower, indicating that the culture contains a subpopulation of radioresistant cells. This phenomenon was observed with a second line of glioma-initiating cells as well (data not shown), but not with U87-MG, an established line of glioblastoma cells (data not shown). Further experiments are necessary to characterize this radioresistant sub-group. According to the cancer stem cell hypothesis, these resistant cells could be a cell population in a more stem-like state. They could therefore be of special interest for the understanding of cancer stem-like cells, their mechanisms of radioresistance and how to successfully inactivate them.

References

[1] D. Pignalosa and M. Durante "Overcoming resistance of cancer stem cells" Lancet Oncol. 13, e187-e188 (2012)

[2] C. Hartel et. al. "First irradiation results unsing the neurosphere formation assay", GSI Scientific Report 2012 (2013) p. 443

[3] A. Barrantes-Freer et. al. "Human glioma-initiating cells show a distinct immature phenotype resembling but not identical to NG2 glia"', J Neuropathol Exp Neurol 72(4) (2013) p. 307