First irradiation results using the neurosphere formation assay

C. Hartel[1], Z. Yu[1,2,3], W. Weyrather[1], E. Kim[4], A. Giese[4] and M. Durante[1,5]

[1]GSI, Darmstadt, Germany; [2]Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai, China; [3]Department of Oncology, Shanghai Medical School, Fudan University, Shanghai,China;, [4]Uniklinikum Mainz, Germany, [5]Condensed Matter Physics, TU Darmstadt, Darmstadt, Germany;

Introduction

The neurosphere formation assay is used to culture neural stem cells *in vitro*. The cells grow in suspension into round cell clusters, so-called neurospheres. The neurosphere assay is used to investigate the stemness properties of the neural stem cells. One criterion for stemness is the ability of the cells to proliferate and self-renew. This property of the cells is promoted by the neurosphere culture conditions.

In the last decade, cancer stem cells were found in many different types of cancer. These cancer stem cells share some characteristic properties with normal stem cells, including their ability for unlimited growth and self-renewal, as well as the ability to differentiate into all the types of cancer cells found in the specific cancer type. It is assumed that cancer stem cells are responsible for the formation of metastasis and for failure of therapy and tumor relapse. Moreover, it was fond that tumor stem cells are more resistant to radiotherapy and chemotherapy than the bulk tumor cells. The relative biological effectiveness RBE of high-LET radiation is generally high for radioresistant cells, thus accelerated ions should be very effective in killing radioresistant cancer stem cells[1].

At GSI, the neurosphere assay was established using glioma stem cells kindly provided by Dr. E. Kim, Neurosurgery Department, University Hospital Mainz, Germany. First irradiation experiments were carried out using accelerated Carbon-ions, Titanium-ions and X-rays.

Materials and Methods

A glioma stem cell line (#10) was cultured in serum-free neurobasal A medium supplemented with B27, EGF and FGF. Before irradiation, cells were typsinized and counted. A defined number of cells was irradiated with X-rays, Carbon-ions (extended Bragg peak, LET=60-85 keV/µm) or Titanium-ions (energy = 1 GeV/u, LET=107 keV/µm) and seeded in T25 tissue culture flasks for neurosphere formation test. From each irradiated sample, four tissue culture flasks were prepared. After two weeks of incubation the number of neurospheres was determined in each flask. Examples of neurospheres are shown in Figure 1.

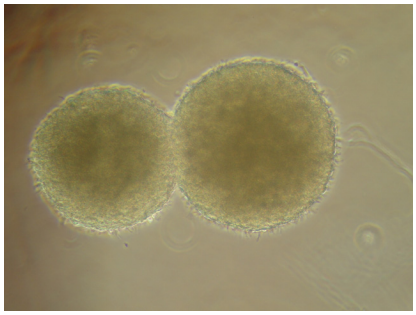

Figure 1: Microscope image of neurospheres.

Results

The fraction of neurospheres which formed after doses of 1 and 2,5 Gy X-rays, Carbon-ions and Titanium-ions is shown in Figure 2. Titanium-ions and Carbon-ions were more effective in reducing the neurosphere formation rate than X-rays, indicating that accelerated heavy ions can indeed inactivate glioma stem cells effectively. To further investigate the effects of heavy ion irradiation on glioma stem cells, experiments using additional cell lines, a wider dose-range and other biological endpoints are planned.

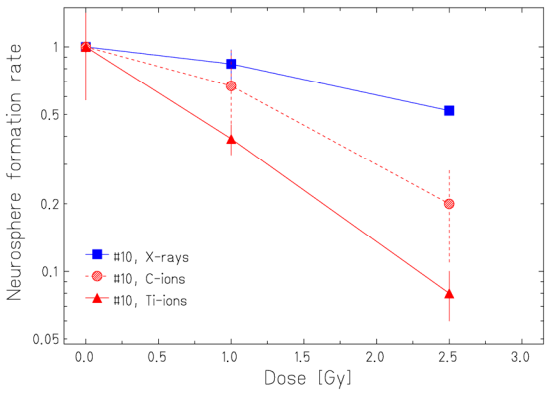

Figure 2: Fraction of neurospheres (normalized to unirradiated control) which formed after 1 and 2,5 Gy of X-rays (squares), C-ions (circles) and Ti-ions (triangels).

References

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