

New challenges in radiobiology research with microbeams

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Abstract There is a continuing interest in the use of microbeam systems designed to deliver ionizing radiation (both photons and particles) with a resolution of a few micrometers or less in biological targets. With more than 30 facilities currently in operation, several new research topics can be explored. In the 9th International Microbeam Workshop held in Darmstadt, Germany, in July 2010, several new ideas and results have been presented, indicating that microbeams will be increasingly important in radiobiology. Subnuclear targeting of single cells for DNA repair studies and microirradiation of 3D or small animal models are among the most promising new research perspectives.

Keywords Microbeam · Damage response · Tissue model

Introduction

Microbeams were originally designed to overcome the problem of the Poisson distribution of traversals in radiobiology cellular experiments. Obviously, if a given dose of

charged particles is delivered to a cell population, the number of traversals will not be the same, and consequently, the dose will vary from cell to cell. The problem is particularly serious for high-LET ions, such as α -particles and heavy ions, where at low doses the fraction of cells receiving no traversals at all can be very high. The effectiveness of single particle traversals is one of the main issues of radiation protection both on Earth (Brenner and Sachs 2003) and in space (Durante 2009).

The possibility of targeting single cells with a predefined number of particles motivated the construction of the first microbeams in the last decade of the past century (reviewed in Gerardi 2009). This soon made possible the direct study of effects of single traversals through the cell nucleus (Miller et al. 1999) or cytoplasm (Wu et al. 1999) and evidence for the bystander effect (Zhou et al. 2000). However, several other applications were soon introduced and technologies rapidly improved. With the introduction of soft X-ray microbeams (Folkard et al. 2001), it became possible to compare low- and high-LET radiation and to improve the subcellular resolution. Some of these new topics and their current status are summarized below.

DNA damage

Use of microbeams to visualize recruitment of proteins involved in damage detection, signaling, and repair began early after the turn of the century (Tartier et al. 2003). Hauptner et al. (2004) introduced use of geometric irradiation patterns and demonstrated that the pattern of emerging protein foci reflects the irradiation pattern. Irradiation in geometric patterns also allowed the analysis of the effects of sequential ion microirradiation and the detection of the competition effect, which highlights the

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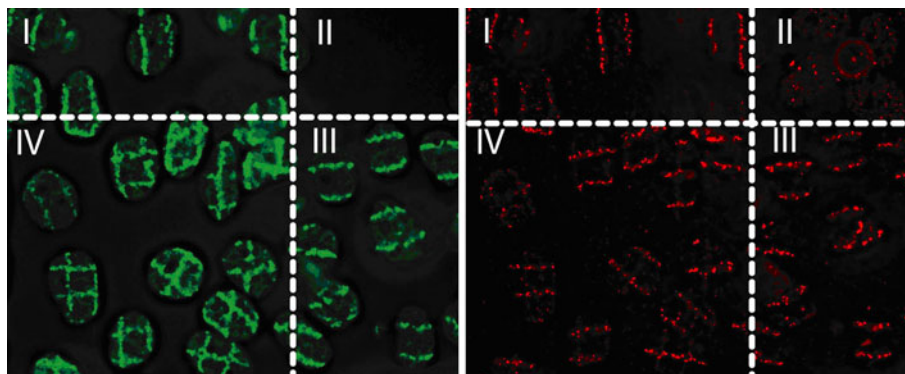


Fig. 1 Competition effect revealed by sequential irradiation with single oxygen ions delivered in line-wise patterns at the Munich microbeam SNAKE. HeLa cell monolayer was first irradiated in horizontal line pattern and after 45 min re-irradiated in vertical line pattern. Immunofluorescence detection of gamma-H2AX (*green*) reflects the irradiation pattern (first irradiation only in *quadrant III*,

second irradiation only in *quadrant I*, both irradiations in *quadrant IV*, no irradiation in *quadrant II*). In contrast, in cells irradiated twice, 53BP1 foci (*red*) develop only at damage sites induced during the first irradiation, but not in response to the second irradiation (see *quadrant IV*), while they develop readily in cells that received only the second irradiation (see *quadrant I*). From (Greubel et al. 2008); modified

turnover and binding characteristics of the different DNA repair molecules (Greubel et al. 2008, Fig. 1).

Visualization of DNA damage in subnuclear targets was demonstrated initially at GSI (Heiss et al. 2006) and is today one of the main research topics. Moreover, the subnuclear targeting with heavy ions makes possible to study heterochromatic and euchromatic compartments directly (Jakob et al. 2011, Fig. 2).

The possibility of live cell imaging on the microbeam line will further enhance these possibilities (Hable et al. 2009). For example, the kinetics of recruitment of different proteins can be investigated in individual cells and compared (Mosconi et al. 2011). The DNA damage/repair studies will certainly in future represent one of the main

focuses of the microbeams with several different options. Given that the vast majority of data on kinetics and mutual dependence of protein recruitment to damage sites gathered so far has been obtained using laser microirradiation (Bekker-Jensen and Mailand 2010), it will be particularly important to systematically investigate whether the data obtained after laser irradiation hold after ionizing irradiation.

Tissue and animal models

The studies of bystander effect have dominated for many years the applications of microbeams (e.g. Zhou et al.

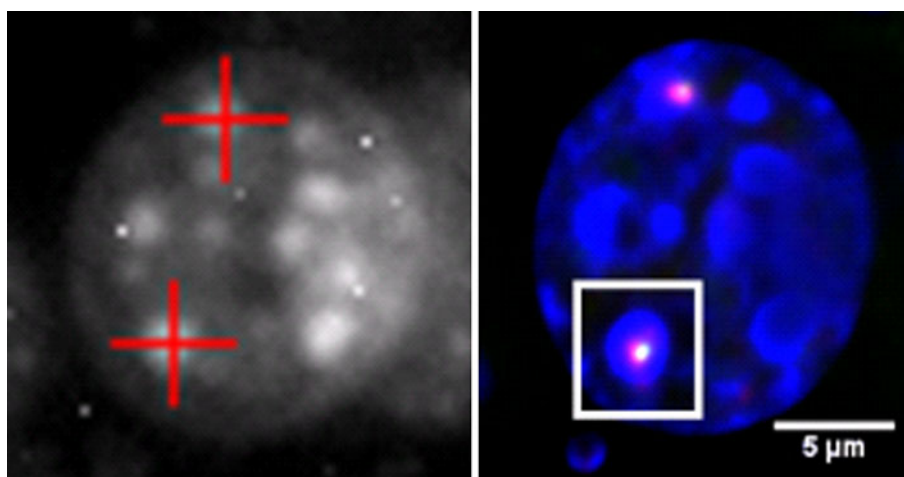


Fig. 2 Microbeam irradiation of subnuclear (heterochromatic) compartments in mouse embryo fibroblasts (MEF) with heavy ions at the GSI microbeam. The left image shows the aimed targeting of chromocenters (*red crosses*) for single ion irradiation using Hoechst 33342 (*gray scale*) as a marker in nuclei of living MEF cells. The

right-hand image shows the same nucleus after fixation at 5 min post-irradiation. DNA damage-induced foci of the repair factor XRCC1 (*green*) and gH2AX (*red*) are clearly visualized at the sites of ion traversal. Both proteins co-localize within each of the targeted chromocenters (blue: DAPI DNA staining). From (Jakob et al. 2011)

2000; Prise et al. 2005). However, experiments with cell monolayers are limited and do not take into account the complex tissue responses. Three-dimensional models can reproduce many of the tissue characteristics in vivo (Griffith and Swartz 2006) and are therefore ideal targets in microbeams for studying non-targeted effects. So far, mostly skin constructs have been used at microbeams (Belyakov et al. 2005; Sedelnikova et al. 2007; Schmid et al. 2010; Miller et al. 2011), but new organotypic slice culture methods offer the possibility of irradiating parts from all kinds of human tissues and to study their late response (Merz et al. 2010). The tissue models at microbeams can be very useful to clarify the role of cell signaling and tissue remodeling in radiation response.

Although traditionally hampered by the limited range of particles and photons used at microbeams, animal models are now also used. So far, these studies focused on very small animals, such as silkworms (Fukamoto et al. 2007) and nematodes (Bertucci et al. 2009), but can provide important insights on long-range non-targeted effects, beyond the possibility of 3D tissue targets.

Generation of ultra-high dose rates

A rather recent application of microbeams is to produce very short pulses (a few Gy in ~ 1 ns), similar to the conditions expected to occur in particle irradiation setups with laser-driven accelerators (Dollinger et al. 2009). Studies on radiobiological effects of ultra-high dose rates are a prerequisite for potential future applications of laser-driven particle acceleration in radiotherapy (Schmid et al. 2009, 2011). Recently, it was shown that irradiation of mouse tumor models at microbeams can be useful in preparing this new therapy modality (Greubel et al. 2011).

Conclusions

Microbeams started operations about 30 years ago and have decisively contributed to fundamental new discoveries in radiobiology. The microbeam community is now rapidly expanding and so are the research topics and ideas. In the near future, it can be foreseen that studies on DNA damage and repair, and new biological targets (tissues and animal) will further corroborate the importance of these devices in radiobiology and their applications in therapy and protection.

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