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Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Identifying a bystander proteom after microbeam radiation therapy	**Experiment number**: MD 491
Beamline: ID 17	**Date of experiment**: from: April 1 to: April 4, 2010	**Date of report**: Sep. 1 , 2010
Shifts: 12	**Local contact(s)**: Elke Bräuer-Krisch	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report: This was our second microbeam radiation therapy (MRT) experiment addressing a radiobiological question rather than pursuing a purely therapeutic goal. The focus was on the radiation-induced *bystander effects* (**RIBE**), which are radiation-like responses in cells which have not been directly irradiated. As with the first radiobiology study in this series, the major portion of the radiobiological expertise was contributed by our collaborators from McMaster University. In addition to repeating experiments from the first (2009) study for validation of the results (demonstrating reproducibility), we also used some of the spare animals for an extra experiment: since Prof. Mothersill's group had already proven in fish that *bystander effects* can be transmitted between irradiated and non-irradiated animals, the question had arisen whether this might also be true in mammals.

Working again in adult male Wistar rats, we conducted our experiments in one of the animal models which we had used previously for our therapeutic experiments. Since the goal of this experiment was to verify observations made in the 2009 experiments regarding the interaction of MRT with brain tissue, we worked again in healthy rather than tumour-bearing animals. A total of 67 adult male Wistar rats was irradiated. For MRT, the beam array was 10 mm wide and 14 mm high, with a beam width of 15 µm and a center-to-center distance of 200 µm. In order to assure that irradiation was exclusively applied to the right cerebral hemisphere, the beam array was offset from the midline (sagittal) by 2 mm towards the right. Unilateral MRT mode was applied at 17.5 Gy, 35 Gy or 70 Gy. Seamless irradiation was applied in corresponding groups with the same dose per volume. All irradiation experiments were conducted with the animals under general anaesthesia (Ketamine- Xylazine mixture, intraperitoneal injection). Cotrary to the 2009 study, we only sacrificed animals at 4 and 8 hrs after irradiation, because we felt that no significant additional knowledge could be gained with sacrificing animals at both 8 and 12 hrs after irradiation. Also, based on the results from

the clonogenic study from the 2009 experiments, rather than repeating the 350 Gy studies at this point, we decided to work with lower skin entry doses. Animals were assigned to one of 11 irradiated groups (n = 5 per group) and 2 non-irradiated control groups (sham irradiation and cage stay controls, n = 4).

Gafchromic Film (Nuclear Associates, NY, U.S.A.) was used to verify applied irradiation doses and irradiation patterns (MRT vs. seamless irradiation). Irradiation was given always to the right cerebral hemisphere, while the lefts cerebral hemisphere served as non-irradiated control and potential field of study for bystander effects. Animals were killed at either 4, 8 hrs after MRT.

Due to last-minute decisions based on the data analysis from the first run, not to include 12-hrs groups and use the same animals for clonogenic and proteomics studies as well as for histology, we had some spare animals available to test the hypothesis whether *bystander effects* could also be transmitted between irradiated and non-irradiated animals in mammals. Twenty-eight animals were used for this pilot experiment. Four animals each were irradiated with unilateral MRT at 350 Gy and 35 Gy, two animals each with correcponding homogeneous doses. Two irradiated animals each were placed together with two non-irradiated animals in one cage for 48 hrs. Analysis was conducted using the clonogenic assay like for the primary experiment.

RESULTS: According to the clonogenic assays from the additional pilot experiment, we can say that also in animals bystander effects are transmitted between irradiated and non-irradiated animals. Since this is a phenomenon which might have practiacl consequences on the treatment and handling of irradiated patients, we have already applied for beam time of a full follow-up experiment.

The first proteomics assays from the animals irradiated with 350 Gy MRT (the dose that we usually use for our therapeutic studies) are also very interesting, as shown in Figure 1.

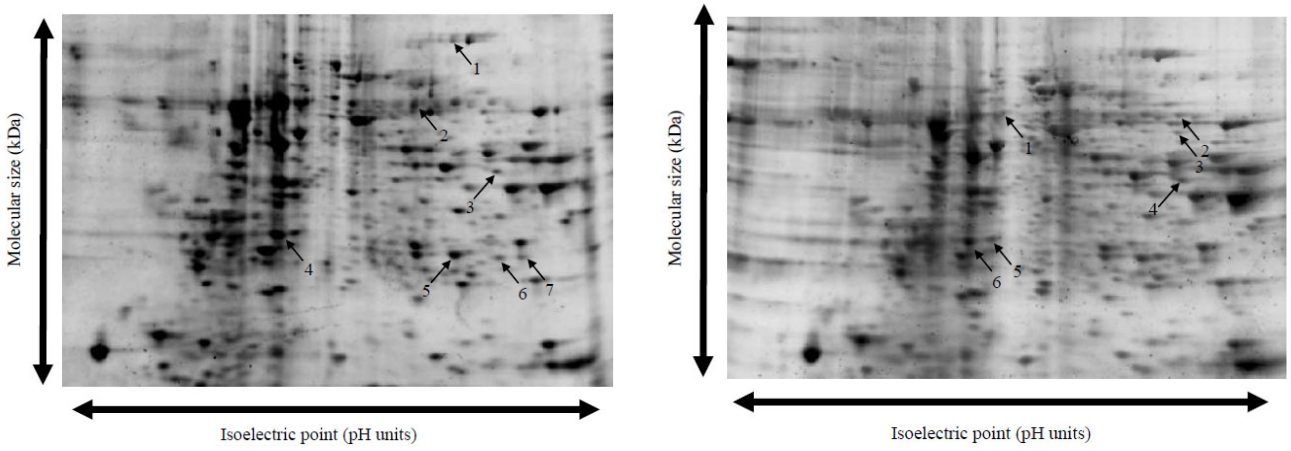

Figure 1: Proteomic analysis. A) Right (irradiated hemisphere; B) Left (non-irradiated hemisphere)
Initial 2D-gel proteomic analysis of brains, irradiated with 350Gy MRT, has revealed 7 proteins affected by directly irradiating the right brain hemisphere (5 possible increases in expression and 2 possible decreases in expression) and 6 proteins responding to a bystander effect in the left hemisphere (3 possible increases in expression and 3 possible decreases in expression). Two proteins were found to respond in both hemispheres. One exhibited an increase to both direct irradiation and the bystander effect whereas the other showed the opposite effect; direct irradiation increase and bystander effect decrease. The others were unique to direct irradiation or the bystander effect. All of these protein spots will now be excised and identified by mass spectrometry.

We wish to thank everybody at ID 17 for helping to make our experiments running as smoothly as they did. Our special thanks goes to our local contact, Elke Bräuer-Krisch, for her energetic and knowledgable support before, during and after our experiments.