EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

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 399
Beamline: ID 17	**Date of experiment**: from: April 23 to: April 27, 2009	**Date of report**: July 25, 2009
Shifts: 12	**Local contact(s)**: Elke Bräuer-Krisch	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Elisabeth SCHÜLTKE*, Walton Medical Centre, University of Liverpool, U.K. and University of Saskatchewan, Saskatoon, Canada

Drs. Carmel MOTHERSILL*, Colin SEYMOUR* and Richard SMITH*, McMaster University, Hamilton, ON, Canada

Drs. Jean Laissue* and Hans Blattmann*, University of Bern, Switzerland

Dipl-Ing. Elke BRAEUER-KRISCH*, ESRF

Report:

This was our first 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. The major portion of the radiobiological expertise for these experiments was contributed by our new collaborators from McMaster University.

Working 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 first experiment was to study the interaction of MRT with brain tissue, the presence of a brain tumour was not required at this stage. A total of 61 adult male Wistar rats were used. All experiments were conducted with the animals under general anaesthesia (Ketamine- Xylazine mixture, intraperitoneal injection). Animals were assigned to one of 10 treatment groups (n = 5 per group) and 2 control groups (n = 4 per group). 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. **MRT:** MRT was administered at skin entry doses of either 35 or 350 Gy in one single treatment session, unilateral mode in anterior-posterior direction, with the animal prone on the goniometer. 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. Animals were killed at either 4, 8 or 12 hrs after MRT.

Seamless irradiation: Seamless synchrotron radiation was administered in one single treatment session, at the same skin entry doses as MRT (i.e. at 35 and 350 Gy), using the same size and location for the irradiation field as described for MRT. Animals were killed at either 4 or 12 hrs after radiotherapy.
Non-irradiated controls: 1) Sham irradiation: anaesthesia and movememnt on goniometer, but no irradiation. 2) Cage controls (never left their cage). 3) To show that none of the parameters studied was affected by the expected scattering dose, an X-ray generator was used to generate low X-ray doses at an energy of 200 keV. The whole body of one animal each was exposed to 1, 2 and 3 mGy (Sc 1-3 group).
Tissue harvest: The animals were beheaded in deep anaesthesia. The brains were quickly isolated from the skull and cut in the median dorsal sagittal plane, to separate the right and left hemispheres. Two blocks of brain tissue, cut mid-way along the right and left cerebral hemispheres and extending from the dorsal to the ventral surfaces, were acquired. Part of this brain tissue and the urinary bladders were used to set up explant cultures. The growth medium of both brain and bladder cultures was harvested about 48 hrs later. It was then shipped to the Mothersill lab in Canada, where reporter cells were exposed to establish clonogenic assays (Fig.1). The remaining brain tissue was snap frozen in liquid nitrogen and stored at – 80 º until processed for proteomics studies, which are presently under way.

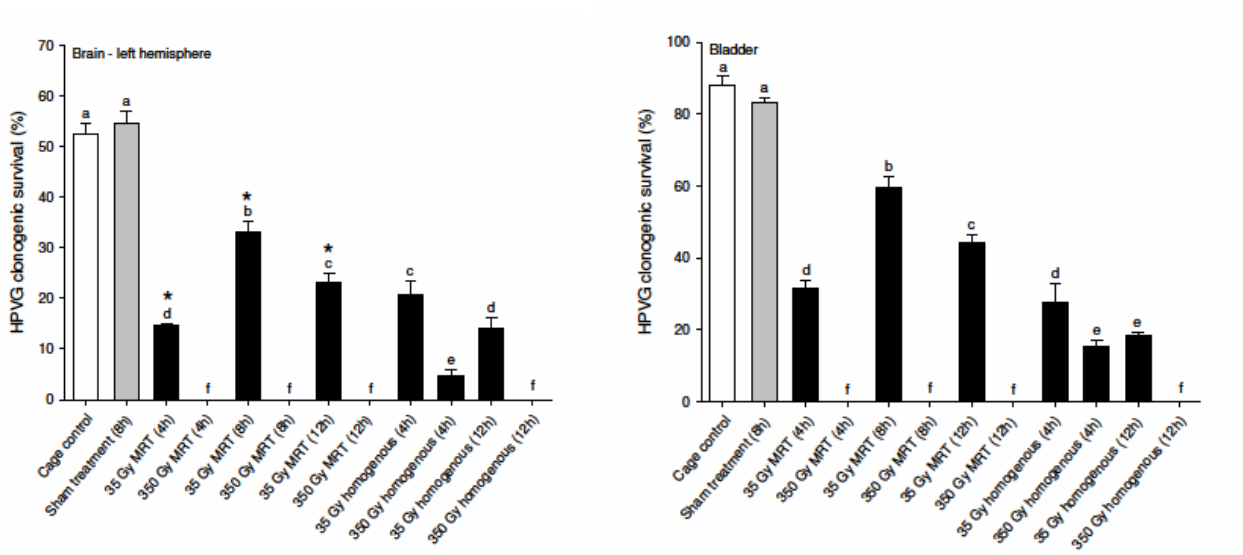

Figure 1: Clonogenic survival of reporter cells, fed with growth medium collected 48 hrs after organ explants from irradiated animals and non-irradiated controls. Except in one experimental group, reporter cells did not survive when exposed to growth medium collected from bladder or left brain (non-irradiated brain) explants from animals irradiated with 350 Gy. After irradiation with 35 Gy, clonogenic survival from both left brain and bladder explants was higher after MRT than after broad beam irradiation.

We wish to thank Mr. Dominique Dallery for the preparation of our experiments and for his dedicated care to our animals, as well as Ms. Catherine Massart for her kind support in all matters concerning the cell culture facility. All members of our research team felt well supported and taken care of by the friendly reception at the ESRF and our new team members were delighted with their stay in Grenoble. Our special thanks goes to our local contact, Elke Bräuer-Krisch, for her energetic and knowledgable support before, during and after our experiments. **A first presentation of results from this experiment is planned for the upcoming radiotherapy meeting of the British Institut of Radiology.**