This project has received funding from the Euratom research and training programme 2014-2018 under grant agreement No 662287.

EJP-CONCERT

European Joint Programme for the Integration of Radiation Protection Research

H2020 – 662287

D9.125 – Progress report from 1st Periodic Meeting of SEPARATE

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Work package / Task	**WP 9** **T 9.7.3**	
Deliverable nature:	**Report**	
Dissemination level: (Confidentiality)	**Public**	
Contractual delivery date:	**CONCERT M46 (SEPARATE M18)**	
Actual delivery date:	**Month 18**	
Version:	**1**	
Total number of pages:	**6**	
Keywords:	**Progress report SEPARATE**	
Approved by the coordinator:	**M46**	
Submitted to EC by the coordinator:	**M46**	

Abstract

The risks posed by inhomogeneous exposures for cancer and non-cancer endpoints can only be evaluated for radiation protection purposes if there is a plausible and consistent mechanism for detriment, a dose-response relationship that allows risk assessment, and biomarkers of response available for molecular epidemiological analysis. The SEPARATE project was designed to study each of these points by both whole body and partial body irradiation of mice.

To date, the partners have made excellent progress in data generation and collection, and data gathering continues in earnest. Omics analyses are progressing remarkably well. The most notable result is that data obtained from miRNome analysis have thus far been mirrored in the proteomics results and supported by neurogenesis analysis. These are indeed crucial achievements, although further work will be required to identify what the central molecular mechanism might be.

We look forward to collecting results from WP3, that will encompass exosome-mediated radiation signalling between tissues exposed in different conditions and underlying mechanisms, the analysis of exosomal cargo, the search for differentially expressed miRNAs, transfer of EVs/exosomes to in vitro cultures and ultimately in vivo, by testing the potential of irradiated exosomes to transfer harmful, or even possibly protective signals in brain tissues of unirradiated recipient mice.

Progress report from 1st Periodic Meeting of SEPARATE

After the 1st Periodic Meeting of SEPARATE, held on 13th - 14th December 2018, the SEPARATE Consortium submitted a detailed summary report of the achievements between June 2018 and February 2019 (M37-M45) as part of D 1.4 to the EC. This also gave plans of SEPARATE activities for the fifth CONCERT year.
To avoid redundancy, the present deliverable will summarise the progress until February 2019 and will give details of activities during March 2019.

The scientific achievements in the period June 2018 - February 2019 were in all aspects of the SEPARATE objectives. The results to date are of very high quality:

a. Within WP1, irradiation of all mouse groups was completed (ENEA). A total of 200 mice were irradiated. Irradiation was carried out at postnatal day 80 with 0.1 or 2Gy of x-rays, with individual shields used to protect the anterior two-thirds of the body, with the hindmost part directly exposed to radiation. A duplicate experiment with no shielding in place was performed for comparison between the effects of total-body irradiation (TBI) and partial-body irradiation (PBI). Mice were sacrificed at 15 days or 6 months after irradiation. Distribution of samples from different organs of all groups to partners [(HMGU, OBU, TU Dublin (formerly DIT)] for transcriptomics, proteomics and metabolomics analyses was achieved ahead of schedule. Dosimetry has also been completed.
b. Under WP1, experiments were conducted to optimize microvesicle/exosome isolation methods (OBU). Mouse brain, heart and liver were used. The extraction method was further optimized with respect to the storing/processing of tissues prior to extraction as described in the midterm report.
c. As part of WP2, Next Generation Sequencing (NGS)-based miRNome analysis in control, irradiated and shielded hippocampi and hearts was completed for the 2 Gy groups at 15 days post irradiation (ENEA). Proteomics data have also been collected from hippocampi of 2 and 0.1 Gy groups at 15 days post irradiation (HMGU). Furthermore, an integrative analysis from proteomics and miRNA data originating from in-field and out-of-field hippocampus at 2 Gy provided preliminary data on a set of miRNAs that are potentially important central regulators of the abscopal effect in the hippocampus. Proteomics analysis has been performed from the 6-month samples (proteome changes in hippocampus being irradiated with 0.1 or 2 Gy TBI or PBI compared to sham-irradiated controls) and is now being analysed and compared to the changes at 2 weeks.
d. Methods for metabolomics / Raman spectral analysis within WP2 have been optimized at TU Dublin (former DIT). Raman spectral maps were recorded from heart tissue sections from mice 6 months post irradiation: 0Gy, 0.1 Gy (PBI and TBI) and 2 Gy (PBI and TBI). Initial analysis of the multivariate data shows clustering of the Raman spectral data into unirradiated, PBI and TBI groups. For WP3, Raman spectral analysis has been performed on isolated exosomes from brain, heart and liver from mice at 15 days post irradiation.
e. For WP3, after initial experiments with mouse bone marrow cultures, embryonic fibroblasts (MEFs) were selected for the functional assays, and interesting Initial results were obtained using increased DNA damage and increased adhesion to fibronectin as endpoints in exosome-treated MEFs. Results for both parameters point to increased effects with 2Gy TBI and PBI liver exosomes treated MEF cells (OBU). In addition, murine hippocampal neuronal cell line HT22 was sent from HMGU to OBU as a model for functional assays.

f. Also in WP3, initial data on neurogenesis and neuroinflammation in the hippocampus (2 Gy - 15 days post-irradiation) have been collected (ENEA). Effects of PBI and TBI on hippocampal neurogenesis were very similar in terms of depletion of neural stem cell compartments of the subgranular zone, as detected by stage-specific markers of adult neurogenesis.
g. Within WP4, the SEPARATE project was professionally and effectively managed throughout the reporting period. The project is running very well with no major modification in the work plan, and with many potential outcomes. The level of participation by core project partners has been high and project communication has worked well. Risk management has been careful and efficient. The present stage of achievements is very good, with all current deliverables of a high quality and no modifications required.
h. WP5 - The project has successfully disseminated its results through diverse channels. Scientific dissemination and exploitation was very good considering the number presentations at various conferences, workshops, and other outreach activities targeting the general public, e.g., dissemination via the project website. A manuscript is in preparation on miRNome and proteome analysis of hippocampi from mice irradiated with 2 Gy (TBI and PBI 15 days postirradiation and unirradiated).

The SEPARATE project is committed to E&T activities and supports initiatives for students, including participation in at least one national and one international conference where they can present project data. A 'Students day' was organised during the RPW2018 meeting, and young researchers from the SEPARATE teams had the opportunity to present results of project activities. Similarly, students at partner's institutes travelled to Dublin for the 1st SEPARATE Annual Meeting in December 2018 and took active part in discussions, besides giving formal presentations of their results. Initiatives for students' support were shared by all partners.
Similar initiatives will be supported in occasion of the ICRR 2019 in Manchester, 25 - 29 August 2019, where a satellite meeting on radiation-induced changes in exosomes is being planned. Junior staff will be invited to attend and present their results.

Current activities

Following an intense programme of preparatory work, experimental set up and optimisation of experimental protocols during year 1 of the project, the work planned during year 2 is consolidating findings across the range of different experimental endpoints and will, through the analysis of in vivo irradiations, molecular/cellular biology, omics and bioinformatics results, enable concrete conclusions to be drawn as to the mechanisms of risks posed by PBI after low doses. On the whole, we definitely expect to advance the state of the art in this somewhat underdeveloped radiological area. There are no major deviations from the original work plan for the coming project period.
Several activities are ongoing since beginning of 2019, including:

- WP1 – All tasks completed.
- WP2 – Validation of differentially expressed miRNAs by home-made custom plate arrays in control, irradiated and shielded tissues for the 2 Gy group (6 months) and for 0.1 Gy groups (15 days and 6 months) post-irradiation (ENEA); completion of proteomics tasks and integrative bioinformatics analysis (HMGU); Raman spectral analysis of the brain tissue sections from mice 15 days post irradiation: 0Gy, 0.1 Gy (PBI and TBI) and 2 Gy (PBI and TBI) (TU Dublin).

- WP3: i) Western Blot for Exosome Markers (CD63, TSG101) and contaminants (GM130); ii) RNA isolations from exosomes; iii) MEF cells treatment with exosome samples from liver, brain and heart (15 days and 24 hours after irradiation) for functional assays; iv) distribution of exosomes extracts from organs and plasma collected at 24 hours after irradiation to all partners; v) addressing the impact of exosomes treatment of MEF cells effects before *vs* after 2 Gy irradiation in order to detect whether such treatments increase or protect radiation-induced changes at the DNA level (i - iv OBU); vi) neuroinflammation analysis in the different experimental groups using CD68 antibody (marker for activated microglia) at 24 hours post-irradiation (ENEA), vii) Raman analysis of isolated exosomes from brain, heart and liver from mice at 24 hours post irradiation (TU Dublin).
- WP4 - Continuing as planned.
- WP5 - Continuing as planned.

Milestones and Deliverables

The project has fully achieved its objectives and milestones for the period. All expected Deliverables were submitted without delays and approved by the EC. The due Milestones were achieved, as confirmed during the first annual meeting in Dublin, 13-14 December 2018.