The role of extracellular vesicles in mediating ionizing radiation-induced bystander and systemic effects

This satellite meeting is supported by the CONCERT project that has received funding from the EURATOM research and training programme 2014-2018 under grant agreement No 662287.

http://www.concert-h2020.eu/;

https://www.researchgate.net/project/The-Role-of-Extracellular-Vesicles-in-Modulating-the-Risk-of-Low-Dose-Radiation-induced-Leukaemia-LEU-TRACK

https://sites.google.com/view/separate-project/home

Extracellular vesicles (EVs) are key mediators of intercellular communication both under physiological conditions and in a wide range of pathologies. Recent studies have shown the role of EVs in mediating radiation-induced bystander and systemic effects. The main aim of this satellite meeting is to present recent scientific data related to the impact of ionizing radiation on EV biology and function.

In the frame of the EURATOM-funded CONCERT project two research projects (LEU-TRACK and SEPARATE) specifically investigate the role of EVs in mediating radiation-induced bystander effects. LEU-TRACK focusses on the role of blood and bone marrow-derived EVs in mediating radiation-induced leukaemogenesis. SEPARATE investigates exosomes from exposed tissues, and their specific bioactive cargo, for their role in mediating out-of-target effects *in vitro* and *in vivo*. A further specific aim of this satellite meeting is to present latest findings generated in these two projects.

Sunday – 25.08.2019, 10:00 – 13:30, Room Charter 4

Chair: Soile Tapio
Co-chair: Katalin Lumniczky

10:00 – 10:10	Welcome and introduction *Soile Tapio*
10:10 – 10:20	LEU-TRACK: The Role of Extracellular Vesicles in Modulating the Risk of Low Dose Radiation-induced Leukaemia – an introduction to the project *Katalin Lumniczky*
10:20 – 10:40	The SEPARATE project: origin and perspectives *Mariateresa Mancuso*
10:40 – 11:00	Exosome signalling roles in the non-targeted effects of radiation: investigating tissue and organ dependence *Munira Kadhim*
11:00 – 11:20	Radiation-induced cargo modifications in specific bone marrow cell subpopulations *Eric Rutten*
11:20 – 11:40	Potential involvement of extracellular vesicles in ionizing radiation induced bone marrow pathologies *Katalin Lumniczky*
11:40 – 12:00	**Coffee break**
12:00 – 12:20	Exosomal secretion and cellular uptake of the inhibitor of apoptosis protein Survivin modulates DNA repair capacity and apoptosis in colorectal tumor cells *Franz Rödel*
12:20 – 12:40	Exosomes from irradiated peripheral blood mononuclear cells suppress apoptosis in recipient cells *Simone Mörtl*
12:40 – 13:00	Extracellular Vesicles are Informative of Cranial Irradiation Exposure Induced Inflammatory Phenotype *Amrita K Cheema*
13:00 – 13:20	Extracellular Vesicles as a Novel Therapy for Radiation-Induced Cognitive Dysfunction *Charles Limoli*
13:20 – 13:30	Concluding remarks *Soile Tapio, Katalin Lumniczky*

LEU-TRACK: The Role of Extracellular Vesicles in Modulating the Risk of Low Dose Radiation-induced Leukaemia

Katalin Lumniczky[1], Christophe Badie[2], Soile Tapio[3], Franz Rödel[4]

[1]National Public Health Center, Division of Radiobiology and Radiohygiene, Budapest, Hungary; [2]Public Health England, UK; [3]Helmholz Center, Neuherberg, Germany; [4]Goethe University Frankfurt, Frankfurt-am-Maine, Germany

e-mail: lumniczky.katalin@osski.hu

Basic mechanisms leading to low dose radiation-induced carcinogenesis and evaluation of health risks attributable to low dose exposures represent key research lines identified in the MELODI Strategic Research Agenda. In line with these priorities, the LEU-TRACK project proposes to study basic mechanisms in low dose radiation-induced leukaemia by focusing on the role of crosstalk between the bone marrow microenvironment and the stem cell compartment in initiating the leukemic process. Radiation-induced leukaemia at low doses most probably involves additional mechanisms distinct from those at high doses. Extracellular vesicles (EVs) are major vehicles of intercellular communication due to their complex cargo. The proposal aims to investigate mechanisms and pathways how bone marrow-derived EVs, by influencing the communication between the different cellular components can induce bone marrow damage and thus modulate low dose radiation-induced leukaemia.

The project focuses on the following main research objectives:

- to investigate mechanisms and pathways how bone marrow-derived EVs, by influencing the communication between the different cellular components of the bone marrow can induce bone marrow damage and thus modulate low dose radiation-induced leukaemia
- to perform a deep and systematic analysis of EV cargo by using multiple omics techniques and complex phenotypical approaches with the aim to identify biomarkers of radiation exposure potentially indicating an increased risk for leukaemia development
- to correlate blood-derived EV markers identified in experimental animals with markers present in human leukaemia patients treated with prophylactic irradiation

Thus, it is anticipated that the project will provide a better understanding of pathways and/or mechanisms of low dose radiation carcinogenesis and will contribute to a better evaluation of the risks associated with low doses, helping to improve risk perception, disease prevention, health promotion and in the later run therapy development.

The LEU-TRACK project has received funding from the Euratom research and training programme 2014-2018 in the framework of CONCERT under grant agreement No 662287.

The SEPARATE project: origin and perspectives

Mariateresa Mancuso[1], Paola Giardullo[1,2], Barbara Tanno[1], Gabriele Babini[3], Francesca Antonelli[1], Caterina Merla[1], Soile Tapio[4], Munira Kadhim[5], Fiona Lyng[6], Simonetta Pazzaglia[1], Anna Saran[1]

[1]Laboratory of Biomedical Technologies, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile, Rome, Italy; [2]Department of Radiation Physics, Guglielmo Marconi University, Rome, Italy; [3]Department of Physics, Pavia University, Pavia, Italy; [4]Institute of Radiation Biology, Helmholtz Zentrum München, Munich, Germany; [5]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom; [6]Centre for Radiation and Environmental Science, Technological University Dublin, Dublin, Ireland

e-mail: mariateresa.mancuso@enea.it

The concept that radiation effects are not confined to directly irradiated tissues has been described in a wide variety of experimental systems (*in vitro*, cultured artificial 3-D human tissue systems, *ex vivo* models). In the attempt to fill a general lack of *in vivo* data, in the last ten years, our research activities have been focused on *in vivo* validation of non-targeted effects, with the main emphasis on implications for cancer development. Using a mouse model of radiation sensitivity, we showed that non-targeted (abscopal) effects are factual *in vivo* events with carcinogenic potential in different tissues (i.e., central nervous system and skin) and we also established that interplay between radiation dose and exposed tissue volume plays a critical role in carcinogenesis occurring in off-target tissues.

Recently, a variety of non-malignant diseases, in particular neurocognitive, cardiovascular and metabolic liver disease, have been recognized to be in association with direct radiation exposure. The CONCERT-funded SEPARATE project is designed to analyze abscopal radiation effects on brain, heart, and liver following exposures that completely spare these organs. Changes at the transcriptome, non-coding RNAs, protein, and metabolic levels in these important organs will be analyzed. We will also investigate exosomes from exposed and shielded tissues, and their specific bioactive cargo, for their role in mediating out-of-target effects *in vitro* and *in vivo*. By combining these cellular, molecular and bioinformatics data we will be able to identify the response pathways in the different tissues, and by inference, suggest the candidate signaling molecules involved. A second major outcome of this project will be the identification of candidate biomarker molecules of both whole body and partial body irradiation responses. Aims, objectives and first results will be presented and discussed.

The SEPARATE project has received funding from the Euratom research and training programme 2014-2018 in the framework of CONCERT under grant agreement No 662287.

Exosome signalling roles in the non-targeted effects of radiation: investigating tissue and organ dependence

Munira Kadhim[1], Ammar Mayah[1], Seda Tuncay Cagatay[1], Mariateresa Mancuso[2], Paola Giardullo[2,3], Simonetta Pazzaglia[2], Anna Saran[2], Fiona Lyng[4], Soile Tapio[5]

[1]Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, United Kingdom; [2]Laboratory of Biomedical Technologies, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile, Rome, Italy; [3]Department of Radiation Physics, Guglielmo Marconi University, Rome, Italy; [4]Centre for Radiation and Environmental Science, Technological University Dublin, Dublin, Ireland; [5]Institute of Radiation Biology, Helmholtz Zentrum München, Munich, Germany.

e-mail: mkadhim@brookes.ac.u

Cells and tissues are constantly subjected to various types of endogenous and exogenous stressful stimuli including radiation exposure, infection and tissue trauma, which can cause serious and even permanent damage. The ability of cells/tissues and organs to sense and adapt to environmental alterations is thus vital for maintaining tissue homeostasis during development and throughout adult life. There is emerging evidence to support the roles of microvesicles (MV), including exosomes, in these events.

This presentation, which is part of the EU SEPARATE CONCERT-funded project, focuses on the role of ionizing radiation in modulating the release of exosomes from different mouse organs and the cargo they contain after total-body (TB) or partial-body (PB) irradiation, which is carried out with the targeted organs shielded. The functional effects of exosomes derived from these tissues/organs on recipient mouse embryonic fibroblast cells for several relevant endpoints will be discussed, and we will present novel data that could help our current understanding of the contribution of systemic “out of target” effects to the risks of long-term health detriment by radiation.

The SEPARATE project has received funding from the Euratom research and training programme 2014-2018 in the framework of the CONCERT [grant agreement No 662287].

Radiation-induced cargo modifications in specific bone marrow cell subpopulations

Eric Rutten, Grainne Manning, Lourdes Cruz-Garcia, Natalie Brown, Rosemary Finnon, Christophe Badie

Centre for Radiation, Chemical & Environmental Hazards, Public Health England, Chilton, Oxfordshire, UK

e-mail: Eric.AndreasRutten@phe.gov.uk

Understanding of extracellular vesicles (EV) has come a long way since initial studies, and they are now known to form part of an important intercellular communication network. In recent times, particular emphasis has been put on EVs for being a potential source of biomarkers, obtained in non-invasive assays. Furthermore, EVs have been implicated in both therapeutic and disease roles, making study of EVs particularly important.

Ionising radiation (IR) is known to cause the differential expression of miRNA transcripts in extracellular vesicles secreted by the bone marrow (BM). Previous studies have focused on the miRNA expression patterns of the total EV population, produced by heterogeneous cell types, and mostly derived from in vivo sources. EVs have also been implicated in being able to reproduce the effects of direct radiation damage in radiation-naïve cells.

This study shows the impact of ionising radiation on the miRNA expression profiles in EVs produced by cultured primary bone marrow cells: macrophages, lineage progenitors, mesenchymal stem cells and osteoclasts. Here, we report preliminary results for the specific changes incurred in EVs produced in monocultures after IR exposure, gaining a better idea of which cell subpopulations primarily contribute to biological activity at an EV level within the BM, and thus which cell subpopulations are potentially responsible for driving EV-mediated bystander effect associated damage propagation and modification of signalling pathways.

Potential involvement of extracellular vesicles in ionizing radiation induced bone marrow pathologies

Katalin Lumniczky, Dávid Kis, Tünde Szatmári, Eszter Persa, Rita Hargitai, Nikolett Sándor, Enikő Kis, Géza Sáfrány

National Public Health Center, Division of Radiobiology and Radiohygiene, Budapest, Hungary

e-mail: lumniczky.katalin@osski.hu

Bone marrow (BM) is a particularly radiosensitive organ; hematological malignancies, myelodysplastic syndrome and chronic bone marrow insufficiency are considered long-term consequences of bone marrow irradiation. Ionizing radiation (IR) damages the stem and progenitor cells and alters signaling between the stem cell compartment and the BM stroma. The major objective of our work was to investigate extracellular vesicles (EVs) mediated IR effects in the BM and stroma at low and high irradiation doses and to study possible underlying mechanisms using an in vivo murine model.

C57Bl/6 mice were irradiated with 0. 1 Gy or 2 Gy and EVs isolated from the BM supernatant were injected systemically into naive animals. EV-mediated phenotypical changes were determined by flow cytometry in the stem and progenitor cell compartment and in the BM stroma. Apoptosis in various cellular subpopulations was measured by Tunnel assay, DNA damage by immunostaining using the γH2AX assay, senescence by β-gal staining. Oxidative damage was evaluated in the BM cells by measuring protein oxidation and lipid peroxidation.

Treatment of naïve mice with BM-derived EVs from irradiated animals induced apoptosis in certain cellular subpopulations, decreased the number of hematopoietic and mesenchymal stem cells and of lymphoid progenitors, changed the ratio between the long term and short term stem cells, increased systemic release of immature progenitors into the circulation. Stroma was less affected; endothelial cells were the most sensitive. No significant oxidative damage was transmitted by EVs.

BM-derived EVs mediated IR-induced damage in the hematopoietic system, which raise the role of BM-derived EVs in the development of IR-induced late hematologic pathologies.

Funding: Euratom research and training programme 2014-2018 under grant agreement No 662287 (CONCERT).

Exosomal secretion and cellular uptake of the inhibitor of apoptosis protein Survivin modulates DNA repair capacity and apoptosis in colorectal tumor cells

Franz Rödel, Stephanie Hehlgans

Department of Radiotherapy and Oncology, Goethe-University Frankfurt am Main, Germany

e-mail: Franz.Roedel@kgu.de

Purpose: The inhibitor of apoptosis protein (IAP) family member Survivin has come into focus as a prognostic/predictive marker and as a promising target for a molecular anti-cancer therapy. Following irradiation, a nuclear accumulation of the multifunctional protein has mechanistically been linked to DNA double-strand break (DNA DSB) repair. Recent data further indicate that Survivin is released into the extracellular space. In the present study we aimed to analyze an exosomal secretion of Survivin following irradiation, its uptake and functional impact on DNA damage repair and apoptosis induction in colorectal tumor cells.

Methods: HCT-15 and SW480 tumor cells were irradiated with a dose of 4 Gy and exosomes were prepared from the culture supernatant at 24 h after treatment and intracellular expression and exosomal Survivin load were assessed by Western immunoblotting. In addition, exosomes of cancer cells stably transfected with different Survivin-EGFP mutants including deletion of its X-linked IAP (XIAP) binding domain were added to recipient cells with a knockdown of endogenous Survivin by siRNA. Uptake of recombinant protein and a functional impact on DNADSB repair and apoptosis were monitored by Western Blot, residual γH2AX/53BP1 foci detection and cytofluorometry.

Result: While irradiation with a dose of 4 Gy results in an increased intracellular Survivin expression, we observed a reduced exosomal secretion at 24 h after irradiation. The cellular uptake of exosomal Survivin by recipient tumor cells could be observed by incubation with conditioned supernatant and with exosomes containing Survivin-EGFP constructs by detection of the recombinant protein in the cell lysates. On a functional level, exosomal wildtype Survivin-EGFP, but not its □XIAPmutant results in decreased residual radiation-induced γH2AX/53BP1-positive foci and apoptosis detection following endogenous Survivin knockdown and irradiation.

Conclusion: These findings indicate that Survivin secreted by exosomes is taken up by adjacent tumor cells and contributes to an increased radiation resistance mediated by modulating DNA damage response and apoptosis.

Exosomes from irradiated peripheral blood mononuclear cells suppress apoptosis in recipient cells

Simone Moertl[1], Dominik Buschmann[2], Lisa Mutschelknaus[1], Michael Schneider[1], Soile Tapio[1], Omid Azimzadeh[1], Michael W. Pfaffl[2], Michael J. Atkinson[1]

[1]Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Biology, Neuherberg, Germany; [2]Division of Animal Physiology and Immunology, TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany

e-mail: moertl@helmholtz-muenchen.de

Exosomes are small extracellular vesicles that are released from cells into all known body fluids. Once released by their donor cells exosomes are readily taken up by other (recipient) cells, where they may have an influence on cellular functionality. However, neither the composition of exosomes, nor their biological functions are fully understood. Our aim was to investigate if ionizing radiation altered the protein and microRNA cargo of exosomes from peripheral blood mononuclear cells (PBMC) and whether these exosomes were able to modify the response of recipient cells.

Whole blood samples from healthy donors were irradiated with 0 Gy, 0.1 Gy, 2 Gy or 6 Gy gamma rays. Human mononuclear cells (PBMCs) were then isolated and cultivated for 72 h. The released exosomes were collected and either analyzed for their cargo or co-cultivated with potential recipient cells (endothelial or PBMC from the same donor). Label-free proteomic analysis of the exosomes identified 602 ± 7 proteins, of which 129 had a significantly changed abundance after irradiation. Small RNA sequencing identified 335 exosomal canonical microRNAs, of which 43 displayed significantly changed expression levels after irradiation. For both, the proteome and microRNA data sets the principal component analysis showed a dose-dependent separation of control and radiation exposed groups.

An IPA network analysis of the radiation-regulated exosomal proteins and microRNAs consistently predicted an association of the altered cargo components with apoptosis, cell death and survival. In line with this prediction irradiated endothelial cells showed a lower rate of apoptosis after co-cultivation with exosomes from irradiated PBMC donors, compared to cultures treated with exosomes from non-irradiated cells.

In summary, our study demonstrates that ionizing radiation of PBMCs is reflected by changes in the exosomal protein and miRNA cargo and in their function on irradiated recipient cells. Thus, our data may lead to the discovery of biomarkers for radiation dosimetry and even more importantly, they suggest exosomes as a novel systemic communication vessels between irradiated and non-irradiated cells and tissues.

Extracellular Vesicles are Informative of Cranial Irradiation Exposure Induced Inflammatory Phenotype

Charles P Hinzman, Janet E Baulch, Khyati Y Mehta, Michael Girgis, Shivani Bansal, Yaoxiang Li, Charles L Limoli and **Amrita K Cheema**

Department of Oncology, Georgetown University, Washington, D.C., United States

e-mail: akc27@georgetown.edu

Ionizing radiation exposure to the brain is common for patients receiving radiation therapy for a variety of CNS related malignancies as well as tumors that metastasize to the brain. This exposure induces gross structural and functional changes, impacting dendritic complexity, spine density and inflammation that are associated with cognitive decline. The appearance of clinical symptoms however, is observable long after irradiation protocols are complete. As such, there are no biomarkers that could predict potential CNS damage at earlier time points. Extracellular vesicles (EVs) are gaining credence as an information rich matrix to delineate biomarkers and gain insights into mediation of radiation response. We exposed mice to a clinically relevant cranial (head only) irradiation paradigm. We isolated EVs from mouse plasma 2 days and 2 weeks post irradiation. Using UPLC-QToF-MS and GC-MS, we detected several markers associated with inflammation that were up-regulated in EVs 2 days and 2 weeks post irradiation. These included triglycerides, platelet activating factor, carnitine and C16 sphinganine. These data suggest that the biomarkers are EV-cargo specific, as they were not significantly dysregulated in the total plasma profiles. Importantly, total plasma profiling confirmed inflammation with significant down-regulation of β-hydroxybutyric acid, a neuroprotectant. These findings demonstrate that metabolomic and lipidomic profiling of plasma-derived EVs can be used to study markers potentially functionally relevant to the observed deleterious effects of cranial irradiation on brain structure and function. Studies to understand the modulation of EV-mediated response by FLASH raditotherapy (FLASH-RT) are ongoing.

Extracellular Vesicles as a Novel Therapy for Radiation-Induced Cognitive Dysfunction

Ron J. Leavitt, Munjal M. Acharya, Janet E. Baulch and **Charles L. Limoli**

Department of Radiation Oncology, University of California, Irvine, CA 92697

e-mail: climoli@uci.edu

Radiation-induced cognitive dysfunction (RICD) is a progressive and debilitating health issue facing patients following cranial radiotherapy for controlling CNS cancers. There has been some success treating RICD in rodents using human neural stem cell (hNSC) transplantation, but the procedure is invasive, requires immunosuppression, and could cause other complications such as teratoma formation. Extracellular vesicles (EV) are nano-scale membrane-bound structures that contain biological contents including mRNA, microRNA, proteins, and lipids that can be readily isolated from conditioned culture media. It was previously shown that hNSC-derived EV resolved RICD following cranial radiation in an immunocompromised rodent model. We now show that hNSC-derived EV treatment is also effective in resolving performance-based deficits in wild-type mice following 9 Gy head-only irradiation without the need for immunosuppression. Cognitive function was not only improved at 5 weeks, but also at 6 months post-irradiation with just a single EV treatment. Further, we were able to administer the EV intravenously via a retro-orbital sinus injection, rather than via intracranial transplantation surgery, without any reduction in therapeutic efficacy. EV injected retro-orbitally and tracked using a fluorescent dye were found in the hippocampus confirming that these EV can cross the blood-brain barrier and target neural cells. Improvement in behavioral testing outcomes was also associated with reduced neuroinflammation, as measured by staining for activated (phagocytic) microglia. These data are the first to show that systemic administration of hNSC-derived EV are able to abrogate RICD and neuroinflammation in wild-type rodents following cranial irradiation, thereby enhancing the translational relevance of our findings.