DoReMi

Low Dose Research towards Multidisciplinary Integration

2nd Periodic Meeting

22-24 January 2013
Institut Curie, Paris, France

Book of abstracts

Student/post-doc session

Wednesday, 23 January

15h30-17h45

Constant Burg lecture theatre

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Chairs:

Siamak Haghdoost, PhD
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An Aerts, PhD
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Program

Oral presentations

The length of each oral presentation is restricted to 15 minutes, including 5 minutes of discussion

15h30: Charlotte Rombouts

Chronic low dose rate γ-irradiation of HUVECs: a high throughput gene expression analysis

Radiobiology Unit, Belgian Nuclear Research Centre, SCK•CEN, Belgium; Department of Molecular Biotechnology, Ghent University, Ghent, Belgium

15h45: Ramesh Yentrapalli

Chronic low-dose rate gamma exposure accelerates senescence in human umbilical vein endothelial cells

Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Biology, Germany; Center for Radiation Protection Research, Department of Molecular Biosciences Wenner-Gren Institute, Stockholm University, Sweden

16h00: Martin Large

Discontinuous DNA Damage Response in Endothelial Cells after Low Dose Irradiation

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

16u15: Ainars Bajinskis

Blood serum proteome assay as a tool for biological dosimetry

Centre for Radiation Protection Research, Stockholm University, Sweden

16u30: Elina Staaf

DNA lesions repaired by homologous recombination are not contributing to the synergistic effect observed after mixed beam exposure.

Centre for Radiation Protection Research, Stockholm University, Sweden; Department of Molecular Biosciences Wenner-Gren Institute, Stockholm University, Sweden

16u45 -17h00: Break

17u00: Charlot Vandevoorde

Direct assessment of low dose effects in children undergoing a computed tomography examination

University Ghent , Dept Basic Medical Sciences, Belgium

17u15: Anna Acheva

Human 3-D tissue models in radiation biology – current status and future perspectives

STUK Säteilyturvakeskus, Helsinki, Finland

17u30: Poisson Clémentine

Effects of uranium chronic exposure on oxidative stress in rat kidney

Institut de Radioprotection et de Sureté Nucléaire (IRSN), PRP-HOM, SRBE, LRTOX, France

17h45: End

Overview
Poster presentations

Laury Perriaud

Ionizing radiation dose rate dependent TP53 pre-mRNA processing

Institut Curie, Centre de Recherche, Centre Universitaire, France ; Inserm, , Centre Universitaire, France

Asal Fotouhi

Nucleotide pool is a significant target for free radical induced mutation

Centre for Radiation Protection Research, Department of Molecular Bioscience, Wennergren Institute, Stockholm University, Sweden

Marina Ilic

Cellular systems and methods for studies of epigenetic effects of γ- and UV-radiation

Center for Radiation Protection Research, Department of Molecular Bioscience, Wennergren Institute, Stockholm University, Sweden

Sara Shakeri Manesh

Mutagenic and cytogenetic effect of low dose rate gamma radiation on human lymphoblastoid cells

Centre for Radiation Protection Research, Department of Molecular Biosciences Wenner-Gren Institute, Stockholm University, Sweden

Elina Staaf

SWE-RAYS: a new Swedish network for young scientists within radiation-related sciences

Centre for Radiation Protection Research, Stockholm University, Sweden; Department of Molecular Bioscience, Wenner-Gren Institute, Stockholm University, Sweden

Nada Samari

Maturating neurons exhibits a delay in neurite outgrowth upon exposure to low and moderate doses of ionising radiation

Radiobiology Unit, Belgian Nuclear Research Centre, Mol, Belgium

Roel Quintens

Identification of novel p53 target genes in the developing mouse brain

Radiobiology Unit, Belgian Nuclear Research Centre, SCK•CEN, Boeretang 200, B-2400 Mol, BELGIUM

Roland Wunderlich

Low/intermediate dose ionising radiation induces an anti-inflammatory phenotype of activated peritoneal macrophages of BALB/c mice

Department of Radiation Oncology, University Hospital Erlangen, FAU Erlangen-Nürnberg

Clélia Le Gallic

Chronic internal 137Cs contamination on early and intermediate stages of atherosclerosis

Institut de radioprotection et de sûreté nucléaire (IRSN), PRP-HOM, SRBE, LRTOX, France

Marie Legrand

Is neurogenesis altered after chronic internal contamination of uranium during brain development?

Institut de radioprotection et de sûreté nucléaire (IRSN), PRP-HOM, SRBE, LRTOX, France

Anne Graupner

Pilot Experiment in the Figaro facility: Chronic Gamma Irradiation of Rodents

Norwegian Institute of Public Health, Oslo, Norway

Gabriele Babini

Pathway analysis techniques to study proteomic data from low-dose/low-dose-rate irradiated cells

Physics Department, University of Pavia, Italy

Luca Mariotti

Influence of oxidative stress and different radiation quality exposure on cytokine release

Dipartimento di Fisica, Università degli Studi di Pavia, Italy; 2: Istituto Nazionale di Fisica Nucleare (INFN), Sezione di Pavia, Italy

Oral presentations

Abstracts

Chronic low dose rate γ-irradiation of HUVECs: a high throughput gene expression analysis

C. Rombouts [1,2], A. Aerts [1], M. Harms-Ringdahl [3], S. Haghdoost [3], A. Janssen [1], A. Michaux [1], R. Yentrapalli[3], R. Quintens[1], M. A. Benotmane [1], S. Baatout [1,2]

1: Radiobiology Unit, Belgian Nuclear Research Centre, SCK•CEN, Mol, Belgium; 2: Department of Molecular Biotechnology, Ghent University, Ghent, Belgium; 3: Centre for Radiation Protection Research, Stockholm University, Stockholm, Sweden

Within DoReMi WP 7 (Task 7.3), the effects of chronic low dose rate γ-radiation on Human Umbilical Vein Endothelial Cells (HUVECs) were investigated. It is hypothesized that chronic low dose γ-radiation induces premature senescence in HUVECs as supported by beta-galactosidase positive staining (S. Haghdoost, Stockholm University) as well as proteomic analysis. To complement these data, whole genome microarray analyses were performed at SCK•CEN to assess changes at the transcriptomic level. Microarrays were performed in biological triplicates using the Affymetrix Human Gene 1.0 ST Arrays. Partek Genomics Suite v6.5 was used for single gene analysis. The samples used in our experimental set-up are presented in the table:

	week 1	week 3	week 6
0 mGy/h	0 Gy	0 Gy	0 Gy
1.4 mGy/h	0.24 Gy	0.71 Gy	1.41 Gy
4.1 mGy/h	0.96 Gy	2.07 Gy	4.13 Gy

The number of differentially expressed genes ($P < 0.05$ and $FC > 1.5$; < -1.5) between control and irradiated cells was in general quite small at all time points suggesting that low dose rate exposure induces rather subtle changes in individual gene expression. Therefore, Gene Set Enrichment Analysis (GSEA) (based on Gene Ontology biological process, Kegg and Reactome databases) was used to see whether predefined gene sets, instead of individual genes, were significantly enriched (FDR < 0.05). In general, it was observed that one week of exposure to both dose rates induces a broad range of cellular pathways that point to a general stress response. Interestingly, after three weeks, most of these pathways were enriched in the control cells but no longer in the irradiated cells. In the latter only a limited number of gene sets, which were mostly related to inflammation and immune response, were enriched. After six weeks, only a very small number of gene sets were enriched in the irradiated compared to control cells. Based on our findings we hypothesize that chronic low dose rate γ-radiation induces a premature stress response in HUVECs leading to premature stress-induced senescence. Further in-depth analysis has revealed a promising gene, IGFBP5, involved in radiation-induced premature senescence.

Acknowledgements: This project is supported by the EU FP7 DoReMi Network of Excellence (grant agreement: 249689).

Chronic low-dose rate gamma exposure accelerates senescence in human umbilical vein endothelial cells

Ramesh Yentrapalli[1, 2], Omid Azimzadeh[1], Katharina Malinowsky[3], Karl-Friedrich Becker[3], Andrzej Wojcik[2], Mats Harms-Ringdahl[2], Michael J. Atkinson[1], Siamak Haghdoost[2] and Soile Tapio[1]

1: Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Radiation Biology, Neuherberg, Germany. 2: Center for Radiation Protection Research, Department of Molecular Biosciences Wenner-Gren Institute, Stockholm University, 106 91 Stockholm. 3: Institute of Pathology, Technische Universität München, Munich, Germany.

Epidemiological studies indicate a risk of cardiovascular disease among nuclear workers exposed to chronic low-dose radiation. However, the molecular mechanism behind the development of radiation-induced cardiovascular disease is not fully elucidated. Endothelial senescence is associated with cellular dysfunction leading to increased risk of age-related cardiovascular diseases such as atherosclerosis. We previously showed that chronic low-dose rate ionising radiation of 4.1 mGy/h leads to premature senescence in human umbilical vein endothelial cells (HUVECs). Yet, acceleration of premature senescence is not studied under chronic very low dose rates.

Here, we studied the dose- and dose-rate-dependent influence in the progression of cellular senescence in HUVECs. Irradiation was performed in a custom-made cell culture incubator modified to hold 137Cs-gamma source to expose cells to chronic very low dose rates (1.4 mGy/h and 2.4 mGy/h). Acceleration of senescence was assessed by quantification of cumulative population doublings and senescence associated beta-galactosidase staining (SA-β-gal). Further, we studied the expression of several proteins related to senescence process by reverse phase protein array.

Our results indicate an induction of premature senescence by chronic low-dose rate (2.4 mGy/h) irradiation verified by the loss of the growth potential and the early appearance of senescence-associated markers (SA-β-gal). However, HUVECs exposed to a dose rate of 1.4 mGy/h did not enter premature senescence in relation to cumulative population doublings. Moreover, analysis of senescence associated proteins clearly showed activation of p21 pathway. In addition, Akt activity, cytosketal proteins (Rho GDI), nuclear transport (nucleolin) and posttranslational modification (SUMO1) were also affected. Analysis of relative changes in protein expression differentiated between low -dose (1.4 mGy/h) rate-induced senescence from replicative senescence. We suggest that endothelial function altered by chronic ionising radiation may contribute to the increased risk of cardiovascular diseases observed in chronically exposed cohorts.

Discontinuous DNA Damage Response in Endothelial Cells after Low Dose Irradiation

Large M., Reichert S., Hehlgans S., Rödel C., Rödel F.

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

Purpose: A discontinuous dose-response relationship is a major characteristic of the anti-inflammatory effects of low dose radiation therapy (LD-RT), used for decades in the treatment of benign diseases. Yet irradiation induced or modulated molecular mechanisms underlying these characteristics remain elusive. In the present study, we analyzed whether DNA Damage Repair (DDR) may originate or contribute to discontinuous dose-responses in human endothelial cells (EC).

Material and Methods: Primary human umbilical vein EC (HUVEC) and HUVEC derived immortalized EA.hy926 cells were used. As a pro-inflammatory stimulus, Tumor Necrosis Factor-α (TNF-α) was added (20 ng/ml) 4 h before irradiation of the cells with a dose of 0.3 Gy; 0.5 Gy; 0.7 Gy; 1 Gy and 3 Gy, respectively. To analyze DDR, residual phospho-histone γH2AX-foci (an established marker to quantify DNA double strand breaks) were counted at 24 h after irradiation. In addition, cell cycle distribution and apoptosis induction was investigated by flow cytometry and caspase 3/7 activity assays.

Results: Irrespective of stimulation by TNF-α, EA.hy926 cells but not primary HUVEC revealed a non-linear characteristic considering the DDR with significantly elevated residual γH2AX-foci at 24 h following irradiation with a dose of 0.5 Gy. By contrast, TNF-α stimulated HUVEC displayed a discontinuous induction of apoptosis as shown by elevated levels of caspase 3/7 activity at 24 h after irradiation 0.5 Gy versus 0.3 Gy and 0.7 Gy, thereas no considerable changes in cell cycle distribution due to stimulation or irradiation were observed in the dose range between 0.3 Gy and 1 Gy.

Conclusion: Our results implicate cell type specific regulation of DDR in EC following irradiation with doses > 1Gy that may contribute to the immunomodulatory effects of LD-RT. However, the impacts of NA damage repair mechanisms (HR, NHEJ) require further investigation.

Blood serum proteome assay as a tool for biological dosimetry

Ainars Bajinskis[1], Siamak Haghdoost[1], Marc Benderitter[2], Andrzej Wojcik[1]

1: Centre for Radiation Protection Research, Stockholm University, Sweden; 2: IRSN, Fontenay-aux-Roses, France

Different assays have been tested on biological samples as tools for biological dosimetry in large scale radiological accidents. Alterations in serum proteome of mice local exposure of skin to high radiation doses allowed discriminating between unirradiated animals and animals irradiated with doses of 40 and 80 Gy. These results suggest the possibility of using the serum proteome as an indicator of radiation exposure (Chaze, Slomianny et al. 2012). Eight most abundant proteins were selected from the mouse experiments as candidates for serum proteome analysis. Blood was collected from 16 breast cancer radiotherapy patients and the expression of proteins analysed by the enzyme-linked immunosorbent assay (ELISA). Blood samples were obtained before radiotherapy, after total doses of 2, 10 or 20 Gy and 1 month after completing radiotherapy. Blood serum samples were aliquoted and stored at -80 ◦C, and used for ELISA according to manufacturers' protocols. Discrimination between samples for groups before therapy or 2 Gy exposure and 10 Gy, 20 Gy or after therapy was possible for Pantothenate Kinase 4 (PANK4) and Factor 10 (FX) protein. These proteins might be used as indicators of locally exposed individuals to high radiation doses.

Chaze, T., M. C. Slomianny, et al. (2012). "Alteration of the serum N-glycome of mice locally exposed to high doses of ionizing radiation." Mol Cell Proteomics.

DNA lesions repaired by homologous recombination are not contributing to the synergistic effect observed after mixed beam exposure.

Elina Staaf[1,2,§], Daniel Vare[2,§], Siamak Haghdoost[1,2] and Andrzej Wojcik[1,2]

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Mixed beams of ionizing radiation are present in our environment, for example when living in areas with high background radiation, when travelling by plane or in space, and for cancer patients receiving some forms of radiotherapy (BNCT or high energy photons for example) are also exposed to mixed beams of gamma radiation and neutrons. Little is known about the health effects of exposing organisms and cells to mixed beam radiation. Earlier studies have concluded additivity as well as synergism (a greater than additive effect). But up until now no efforts have been made to investigate which DNA lesions that are resulting in the synergistic effect. Therefore, we have applied a selection of repair-deficient cell lines to study this issue. The Chinese hamster ovary cell lines were derived from wild type AA8 and were deficient in base excision repair - BER, homologous recombination - HR and non-homologous end-joining – NHEJ.

The wild type and the three deficient cell lines were seeded on glass cover slips and exposed to a mixture of alpha particles and X-rays at a unique facility allowing simultaneously exposure of cells to X-rays and Am-241 alpha particles at 37 ºC (1). The cellular response was analyzed by clonogenic survival and immunofluorescent foci (53BP1 as well as gamma-H2AX). Doses applied were 0.2, 0.4 and 0.8 Gy for clonogenic survival, and 0.4 Gy for foci.

A lower than expected level of clonogenic survival (synergistic cellular toxicity) was observed for the wild type and the HR-deficient cell line. The synergistic effect was confirmed by envelopes of additivity calculations. For wild type cells, alpha particles showed a higher toxicity compared to X-rays, while there were no significant differences between the irradiation types for BER-deficient cells. In HR- or NHEJ-deficient cells alpha particles showed a lower induced toxicity than X-rays. When investigating the induction and repair of DSB indicated by 53BP1- and gamma-H2AX foci, the synergistic effect was once again observed for the wild type and HR-deficient cell lines. Significantly larger than predicted total foci areas were observed for both foci types, 3 h after exposure for wild type cells and 6 h after exposure for HR-deficient cells. For the BER-deficient and NHEJ-deficient cell lines, alpha particles were consistently more efficient in foci-induction than X-rays, but there were no significant differences between observed and predicted.

The results indicate that the DNA damage repaired by homologous recombination does not contribute to the synergistic effect of mixed beam exposure. Additionally we found that deficiency in

either BER or NHEJ abolished the synergistic effect observed in wild type cells, indicating that it is the repair of these lesions that results in the observed synergistic effects.

(1) E. Staaf et al. (2012) Characterization of a setup for mixed beam exposure of cells to 241-Am alpha particles and X-rays. Radiation Protection Dosimetry. 151(3):570-79

Direct assessment of low dose effects in children undergoing a computed tomography examination

C. Vandevoorde[1], A. Vral[1], S. Baatout[2], P. Willems[3], P. Smeets[4], N. Herregodts[4], H. Capiau[5], A. De Backer[5], C. Ernst[6], K. Van De Moortele[7], L. Breysem[8], H. Thierens[1]

1: University Ghent , Dept Basic Medical Sciences; 2: SCK-CEN, Radiobiology Unit, Lab Mol Cell Biol; 3: FANC-AFC; 4: University Ghent Hospital, Radiology Dept; 5: St Lucas Hospital Ghent, Pediatrics and Radiology Depts; 6: University Hospital Brussels, Radiology Dept; 7: St Jan Hospital Bruges, Radiology Dept; 8: University Hospital Louvain, Radiology Dept

Introduction: In view of the higher radiosensitivity of children and the annual increase in number of CT scans, a better knowledge of the x-ray dose for pediatric patients and the associated radiation risk is warranted. A study, in which gamma-H2AX foci in PBLs were used as a biomarker for radiation-induced effects, was initiated in order to evaluate the situation in Belgium and to estimate the radiation risks for pediatric patients after a CT examination.

Methods and Results: In 28 pediatric patients (mean age, 4 years) who underwent a CT abdomen, chest or neck; blood samples were taken before and shortly after the CT procedure using the catheter for contrast agent. After Rosette-Sep blood separation, gamma-H2AX foci were determined in peripheral blood T lymphocytes. The induced number of gamma-H2AX foci, representing DNA double-strand breaks as a result of the exposure to x-rays, was scored. The most frequent type of examination was CT of the chest (19), followed by CT scans of the neck region (5) and CT abdomen (4). Although in each hospital low dose state-of-the-art CT equipment and adjusted CT dose parameters are used for imaging of pediatric patients, a low but statistically significant increase in foci number was observed ($p<0.001$). An average increase of 0.220 foci/cell was observed for an average DLP of 77.35 mGy.cm.

Conclusion: This study is still ongoing, but the first results show that in all pediatric radiology departments involved in the study large efforts are made to reduce CT doses and resulting effects for children in Belgium. All hospitals use CT scan equipment with state-of-the-art dose reduction techniques. Consequently it is not surprising that the X-ray induced foci yields obtained in present study (0.22 foci/cell) are significantly less than observed in a previous study of DNA damage resulting from CT examinations in adult patients (0.79 foci/cell).

Human 3-D tissue models in radiation biology – current status and future perspectives

Acheva Anna[1]*, Baatout Sarah[2], Aerts An[2], Launonen Virpi[1], Salomaa Sisko[1], Kämäräinen Meerit[1]

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The overall objective of DoReMi Task 5.2. is assessing the relative contribution of targeted, (DNA), non-targeted and systemic processes to radiation carcinogenesis. The aim of the current work was to summarize and discuss the methodological aspects related to the 3-D tissue model utilization as a test system in the radiation biology. Special emphasis had been put on the most widely exploited 3-D models like skin, lung and endothelial models. We also review the use of a variety of 3-D models in radiobiological research with specific highlights on endpoints studied and the differences in responses compared to 2-D culturing conditions (monolayers). Furthermore, we present common problems as distance determination, primary cells source, oxygenation levels etc., encountered by us and other groups. We also propose solutions to overcome some of the issues. In conclusion, we summarize that the 3-D models could be applied in the radiation biology as suitable tissue in vitro model and they have several advantages over the 2-D monolayer cultures traditionally applied in radiobiology. They allow studying the radiation response at tissue level on humanized models, which give numerous advantages over the genetically and radiosensitively different animals. The models can be maintained in laboratory conditions for up to six months, which is of considerable benefit for studying long term or late effects of ionizing radiation.

Effects of uranium chronic exposure on oxidative stress in rat kidney

Poisson Clémentine[1,*], Manens Line[1], Delissen Olivia[1], Dublineau Isabelle[1], Gueguen Yann[1]

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Objectives: Uranium is a heavy metal and a radioelement which can be found in the environment due to its natural presence in earth crust and human activities. Uranium accumulates preferentially in the kidney, in tubular proximal cells of the renal cortex [1]. Oxidative stress is one of mechanisms involved during uranium exposure. It is defined as an equilibrium between the product of reactive species and the antioxidative defense system which include endogenous antioxidants as enzymes and exogenous antioxidant as glutathione. Acute studies showed that uranium exposure induces an oxidative stress [2, 3] but there is a lack of data after chronic exposure to low doses of uranium. Our objective is then to evaluate the disturbance of pro/antioxidative balance in the renal cortex of rats exposed to different uranium concentrations during nine months.

Methods: Rats were chronically exposed (9 months) to natural uranium at environmental dose (1 mg/L), supra environmental dose (40 mg/L) or high doses (120 or 600 mg/L) and were compared to non-exposed group. Samples were collected: urine, liver, renal cortex or plasma to evaluate kidney function during the exposure by measurement of urinary parameters such as creatinine and some parameters of oxidative stress such as TBARS (Thiobarbituric Acid Reactive Substances) in plasma, glutathione pool and glutathione enzymes in the renal cortex.

Results: During the exposure, a monitoring was performed with monthly urinary samples and weekly weighing: no disturbance was observed. After nine months of exposure, plasma parameters as sodium or chloride were not altered. The measurements of TGO and TGP did not show any variation. Integrity of kidney was evaluated by creatinine clearance: kidney was not altered by the exposure whatever the dose of uranium. A decrease of TBARS was observed ($p < 0.05$) in the plasma for the animals which received 120 mg/L of uranium. The increase of glutathione level in renal cortex was dose-dependent: the glutathione pool was increased threefold for the 1 mg/L group ($p<0.01$) and up to 10-fold for the highest dose ($p<0.001$). The activity of glutathione reductase did not change after the uranium exposure whereas glutathione peroxidase activity was altered only for the 40 mg/L group.

Conclusion: After chronic exposure, uranium induced a disturbance of pro/antioxidative balance with an increase of glutathione and a decrease of TBARS. Complementary measurements are in process to explain these effects but our results suggest that adaptive mechanisms of antioxidative system could be set up after uranium chronic exposure.

1. Leggett, R.W. Health Phys, 1989. 57(3):365. 2. Priyamvada, S. et al. Prostaglandins Leukot Essent Fatty Acids, 2010. 82(1): 35. 3. Barber, D.S et al. Neurotoxicology, 2007. 28(6): 1110.

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Ionizing radiation dose rate dependent TP53 pre-mRNA processing

Laury Perriaud[1,2], Vincent Favaudon[1,2], Virginie Marcel[1,2*], Charlotte Sagne[1,2], Marie-Paule Teulade-Fichou[1,3], Stephan Vagner[4], Janet Hall[1,2].

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G-quadruplexes structures (G4) in the *TP53* gene have been implicated in *TP53*'s pre-mRNA 3'-end processing in response to DNA damage induced either by UV irradiation or doxorubicin exposure (Decorsière *et al*., 2011). Little is known however on how exposure to ionising radiation influences these processes. In order to investigate this and to assess the impact of the exposure dose-rate on this endpoint we have irradiated lymphoblastoid cells using a linear electron accelerator (LINAC) ($2x10^6$ Gy/s) in the presence or not of a G-quadruplex RNA binding ligand, 360A and examined 3'-end processing of the *TP53 vs TBP* pre-mRNAs. After treatment with 360A the 3'-end processing of the *TP53* pre-mRNA was increased providing evidence of the involvement of a G4 structure in this process. After treatment of the cells with the LINAC with doses ranging from 0.1 to 2 Gy *TBP* pre-mRNA 3'-end processing was halted or even decreased compared to the levels seen in untreated cell whilst the *TP53* pre-messenger RNA was increased at all doses examined compared to untreated cells. The molecular basis of these differences in 3'-end processing between genes and any cellular consequences remains to be determined but could have important implications as therapies using high dose rates, up to 10 Gy/s or more are being introduced into the clinic.

Financial support from the EU FP7 program (Grant Number 249689 for the network of excellence DoReMi (low dose research towards multidisciplinary integration)) is gratefully acknowledged.

Nucleotide pool is a significant target for free radical induced mutation

Asal Fotouhi[1], Winta Woldai Hagos[2], Jaap Jansen[2], Andrzej Wojcik[1], Leon Mullenders[2], Mats Harms-Ringdahl[1] and Siamak Haghdoost[1]

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Intra cellular nucleic acid is constantly exposed to reactive oxygen species (ROS), produced either endogenously or by exogenous agents. It is well known that ionizing radiation as well as UV radiation is amongst the exogenous agents producing ROS. While ionizing radiation ionizes molecules leading to the formation of ROS, UVR excites photosensitizers leading to the formation of ROS. ROS in turn exerts its effect by modifying DNA base in the DNA and nucleotide pool (dNTP), which could lead to mutations. The dominant forms of ROS induced base modifications in the nucleotide pool include 8-oxo-dGTP and 8-oxo-dATP. Different types of mutations (transversions or transitions) can arise depending on which dNTP has been modified in the nucleotide pool. hMTH1 is able to dephosphorylate 8-oxo-dGTP and 8-oxo-dATP and preventing their incorporation into the DNA. The aim of this project is to investigate types of mutations induced by oxidative stress in the cells exposed to UVA radiation. The cells used in this study have lower level of hMTH1 (transfected) thus more sensitive to mutagenic effect of free radicals compared to non-transfected TK6 cells. Transfected and non-transfected cells were exposed to oxidative stress induced by UVA. Mutants were selected by treating the exposed cells with triflourothymidine. To find out the exact mutation induced, the Thymidine Kinase locus was sequenced.

The most prominent type of mutation found was base pair substitutions, more precisely GC>AT transitions in non-transfected exposed TK6 cells whereas AT>GC transitions dominated in transfected TK6 cells. The results imply that oxidative stress induces mutations primarily by oxidizing 8-oxodATP in the nucleotide pool

Cellular systems and methods for studies of epigenetic effects of γ- and UV-radiation

Marina Ilic, Asal Fotouhi, Andrzej Wojcik, Mats Harms-Ringahl and Siamak Haghdoost

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Humans are constantly exposed to γ-and UV-radiation from the natural environment as well as from medical or cosmetic applications. Low doses of low linear energy transfer (LET) ionizing radiation, in addition to UV-radiation have been shown to trigger production of reactive oxygen species (ROS) and consequently raise the stress response in cells. ROS react with nucleic acids in the DNA or nucleotides in the nucleotide pool which may cause mutations, and lead to late effects such as cancer and other age related diseases.

Whilst most studies focus on genetic events post γ-and UV-radiation, the studies of epigenetic effects are recently coming into focus. Epigenetic effects are mechanisms that can regulate the gene expression. One mechanism involved in epigenetic events is DNA base methylation (gene silencing). DNA base methylation is the addition of a methyl group on a cytosine converting it to 5-methylcytosine. The cytosines that are methylated are mostly a part of cytosine phosphodiester guanine (CpG) sites found in regions known as CpG islands which are usually localized to promoter regions of the gene. There are specific proteins binding to methylated CpGs leading to repression of transcription. Thus, when CpG islands are hyper methylated the gene may be repressed while when hypo methylated the gene may be expressed. Methylations are catalyzed by DNA Methyltransferases; DNMT1 is responsible for the maintenance DNA methylation while DNMT3A and DNMT3B play their main role in de novo methylation.

Epigenetics are suggested to have a higher impact than the genetic modifications in the development of malignant melanoma and other cancers. This project aims to investigate the epigenetic effects of γ-and UV-radiation on human cells by looking at DNA methylation in the DNA and the DNMT1 activity. The different methods for studying the epigenetic effects of γ- and UV-radiation will be discussed and preliminary results will be presented.

Mutagenic and cytogenetic effect of low dose rate gamma radiation on human lymphoblastoid cells

Sara Shakeri Manesh, Marta Deperas-Kaminska, Andrzej Wojcik, Mats Harms-Ringdahl and Siamak Haghdoost

Centre for Radiation Protection Research, Department of Molecular Biosciences Wenner-Gren Institute, Stockholm University, SE-106 91, Stockholm, Sweden

We have previously shown that low doses of ionizing radiation increase the endogenous production of reactive oxygen species (ROS) which leads to an oxidative stress condition. ROS react with different cellular components (proteins, lipid and nucleic acid) and give rise to a range of different intra cellular modifications.

We have also shown that low doses and dose rates of gamma radiation can modify the expression of several proteins involved in cellular protection against oxidative stress including up-regulation of hMTH1 (nucleotide sanitization enzyme) which inhibits incorporation of oxidized nucleotides (8-oxo-dGTP) into DNA during replication.

In the present study we have stably transfected human lymphoblastoid cells, TK6, using shRNA towards hMTH1. Both transfected and non-transfected cells were exposed to 0, 0.5 or 1 Gy gamma radiation either acutely or chronically. The dose rates used were 1.4, 5, 15 or 30 mGy/h for chronic and 0.41 Gy/min for acute exposure. Clonogenic survival was assessed after acute exposure in both cells. Mutant frequency in the thymidine kinase locus was studied using trifluorothymidine (TFT). Growth rate was measured during chronic exposures for 23 days and finally chromosomal aberrations were assessed after different low dose rates at the total dose of 1Gy.

The frequency of mutants, the level of chromosomal aberrations and the growth rate in our experiments did not seem to vary significantly between exposed transfected and non-transfected cells. Our data suggest that dose rather than dose rate has a significant effect on the level of mutation as well as on the cytogenetic level.

SWE-RAYS: a new Swedish network for young scientists within radiation-related sciences

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The Swedish Radiation Research Association for Young Scientist (SWE-RAYS) is a new network / society, for young scientists who are at the beginning of their careers. The aims of the society are to promote collaboration between the research groups active in Sweden, and to promote career finding and career development for young scientists (focusing on PhD students and post-docs). We welcome established researchers with experience from research, industry and government authorities as "supporting members", and Master students on suitable programs are also welcome to join. The main activity of SWE-RAYS is the organization of an annual workshop, where young researchers are given the opportunity to present their research in an interdisciplinary environment.

SWE-RAYS is a very young society and is still in the process of forming. The first initiative to this network was taken in late 2011, when the Centre for Radiation Protection Research (CRPR) at Stockholm University provided funding for the first ever SWE-RAYS workshop. This was held at Stockholm University, Sweden 30-31st of August 2012 and gathered 46 participants of which 29 were PhD students or post-docs. The participants were from different branches of the radiation-related sciences such as medical radiation physics to radiation protection, radioecology, radiation physics, radiation biology and nuclear chemistry, and were also geographically dispersed. During these two days the participants held 9 oral and 12 poster-presentations, while invited speakers had two lectures and a panel discussion about the labor market for Swedish Radiation Research. The language of the meeting was English, to accommodate people from all backgrounds.

The outcome of the workshop was very successful, and the decision of forming a network was made. A first SWE-RAYS board of mainly PhD students (see authors' list) was chosen which began working towards forming the society. This activity has been very favorably received by other radiation-related societies, as well as relevant companies and agencies. Currently the paperwork for registration of the society is being processed, while the planning for the second workshop in Uppsala, Sweden (August 2013) is well under way, We are taking into account the feedback from 2012, and the number of workshop participants are expected to increase significantly during 2013.

We are looking forward to ideas from the DoReMi partners on how to extend the network to our European colleagues.

Maturating neurons exhibits a delay in neurite outgrowth upon exposure to low and moderate doses of ionising radiation

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In human, the period of fetal development (8 to 15 weeks of gestation) is highly sensitive to ionizing radiation. Epidemiological data have reported cases of mental retardation and decrease in school performance within victims of Hiroshima and Nagazaki A-bombing, irradiated *in utero* during the fetal period. Several studies have been performed to assess the risk of ionizing radiation on fetal and children health but a lot is still to be done in order to understand the radiation-induced brain damage, especially following exposure to low doses of radiation.

In this study, we aimed at observing morphological changes in neurons following exposure to low and moderate doses of X-rays during their early maturation period, followed by gene expression analysis. For this purpose we have used nearly pure primary cultures of neurons extracted from E17 mouse fetus in order to reveal specific response of neurons to ionizing radiation. This study revealed a reduction in neurite length, neurite number and branching points number following exposure to doses of 0.1, 0.2 and 0.5 Gy of X-rays. A 3 days follow-up of these cultures after irradiation showed a resuming of neurite outgrowth, which remained however insuficient to overcome the inhibition induced by radiation. Microarray analysis using GSEA indicated a significant downregulation of pathways involved in cytoskeleton remodeling and synaptogenesis. This study showed that a dose as low as 0.1 Gy was enough to induce neurite outgrowth inhibition which may ultimatly lead to a defect in neuronal network connection and to improper neuronal communication.

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Identification of novel p53 target genes in the developing mouse brain

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Epidemiological data from atomic bomb survivors have shown that *in utero* exposure to low radiation doses especially between weeks 8-15 of pregnancy led to an increased incidence of severe mental retardation among these subjects later in life. Similar effects can be seen in mice by irradiating pregnant ones at day 12 of gestation (E12) with 1 Gy of X-rays. To identify possible molecular mechanisms which are responsible for this effect, we analysed gene expression in the fetal brains after 2 h following irradiation. Results showed a dose-dependent increase in genes involved in cell cycle arrest, DNA repair and apoptosis, which are known to be regulated by the tumour suppressor p53. However, a number of upregulated genes were so far not known to be involved in p53-mediated pathways.

Therefore, we analysed several of these radiation-induced genes for putative p53 binding sites using a matrix scanning algorithm. For all of the tested genes, we identified at least one predicted p53 binding site ($p < 10^{-4}$) within the investigated genomic sequences. Chromatin immunoprecipitation with an antibody against phosphorylated p53 showed that these genes are indeed *in vivo* targets of p53 in response to X-irrradiation of the E12 mouse brain. To further confirm the involvement of p53 in the transcriptional activation of these genes upon irradiation, we analyzed mRNA expression in irradiated primary cultures of cortical neurons from wild-type ($p53^{+/+}$) *versus* p53-deficient ($p53^{-/-}$) mice. Expression of most of these genes was significantly induced in cells from $p53^{+/+}$ mice, whereas no difference in expression could be observed in cells from $p53^{-/-}$ mice. *In silico* functional prediction analysis, suggested that these genes may be important for embryonic brain development and are implicated in neurodegenerative diseases such as Huntington's disease.

Our data shed new light on the early molecular effects of ionizing radiation in the developing mouse brain and may provide a rationale for cognitive defects seen in *in utero* irradiated mice.

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Low/intermediate dose ionising radiation induces an anti-inflammatory phenotype of activated peritoneal macrophages of BALB/c mice

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Low and intermediate dose ionising radiation (LD-X-ray) is applied in treatment of painful and degenerative inflammatory diseases. The resulting anti-inflammatory effects are based on the one hand on alterations of chemokine profiles and adhesion molecules on immune cells. On the other hand, despite lowering the amount of inflammatory immune cells, a LD-X-ray induced change of the phenotype of activated immune cells is also assumed. Key players in regulation of inflammation are macrophages that are cells of the innate immunity. They secrete inflammatory cytokines such as Interleukin (IL)-1ß or tumour necrosis factor (TNF)-α upon stimulation. In terms of medical application of LD-X-ray, but also of radiation protection issues, it is reasonable to expand our knowledge on how key immune cells involved in inflammation get modulated by LD-X-ray. Of special interest is how varying radiosensitivities due to the different genetic backgrounds influence this modulation.

We have previously shown that 0.5 or 0.7 Gy of LD-X-ray significantly reduces the amount of secreted IL-1ß of human THP-1 macrophages that have been activated with lipopolysaccharide (LPS) and co-activated with monosodium urate crystals (MSU). Using now an *ex-vivo* model of peritoneal mouse macrophages obtained from mouse strains differing in their basal radiosensitivity, we also observed a significantly reduced secretion of IL-1ß and a slight, but not significant, reduced secretion of TNF-α of activated (LPS plus MSU) macrophages of BALB/c mice after LD-X-ray treatment with 0.5 or 0.7 Gy. In contrast, the secretion of IL-1ß and TNF-α of activated peritoneal macrophages from the less radiosensitive C57BL/6 mice was not influenced by LD-X-rays.

We conclude that only the inflammatory phenotype of more radiosensitive macrophages is reduced by LD-X-ray of an intermediate single dose and that *ex vivo* and *in vivo* models with primary cells should be increasingly applied in the future to examine how the immune system is modulated by LD-X-ray. Future research will also reveal which inflammatory pathways such as the NFκB p65 and p-p38 MAPK get modulated by LD-X-rays.

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Chronic internal 137Cs contamination on early and intermediate stages of atherosclerosis

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Background: High doses of ionising radiation damage the vasculature by as yet unknown mechanisms. A concern for radiological protection is the recent epidemiological and experimental data indicating increased cardiovascular risks and damages associated with exposure to low doses such as 100-500 mGy. However, given the multifactorial origin of cardiovascular diseases and the lack of clear pathophysiologic mechanism, epidemiological results have to be carefully interpreted. In fact, the possible effects of low dose exposure on atherosclerosis development which is the first cause of morbidity and mortality in industrialized countries is unknown. We evaluated the effect of different concentrations of internal chronic ^{137}Cs contamination on early and intermediate stages of atherosclerosis.

Methods and results: C57Bl6 and genetically predisposed C57Bl6 (ApoE-/-) mice were contaminated by daily intake of 4 KBq/L, 20 KBq/L and 100 KBq/L of ^{137}Cs in drinking water for 3 or 6 months. There was no linear dose-effect response in all conditions. At 4 or 20 KBq/L of ^{137}Cs we did not observe any effect in any group. However with 100 KBq/L there was a significant increase in aortic VCAM-1 mRNA levels in ApoE$^{-/-}$ mice as compared to untreated ApoE$^{-/-}$ animals. This was associated with an increase in aortic intimal thickness in ApoE$^{-/-}$ mice which is an index of early stages of atherosclerosis. In contrast, there was no effect on reactive oxygen species levels, measured by dihydroethidium staining, even with the highest dose of ^{137}Cs. Moreover, there was no effect or a decrease on the expression of other aortic pro-inflammatory factors, such as ICAM-1, MCP-1, IFN-γ or TNF-α in all the groups. Six months after internal contamination we observed a significant decrease of adhesion molecules (E-selectin and VCAM-1) and pro-inflammatory cytokines (TNF-α and INF-γ) specifically in animals treated with 100 KBq/L of ^{137}Cs. The quantification of plaque size in different ApoE-/- mice groups did not show any differences in lesions size.

Conclusions: We conclude that exposure to chronic low dose of ^{137}Cs increases the expression of some vascular pro-inflammatory molecules and intimal thickness involved in early stages of atherosclerosis, only in predisposed animals to this disease. These effects are only transient and do not have any incidence in plaque size after 6 months contamination. ApoE$^{-/-}$ mice seem to be more susceptible to internal low dose of chronic exposure to ^{137} Cs at early stages of atherosclerosis. Since the observed effects do not persist over the time, we hypothesise that in intermediate stages an adaptative response occurs that counteracts and abolishes early stages observed- responses. It is now important to evaluate the atherosclerotic plaque composition in different groups to determine if an internal contamination of ^{137}Cs influences plaque vulnerability.

Is neurogenesis altered after chronic internal contamination of uranium during brain development?

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Uranium is a heavy metal naturally found in the environment. Its many uses in civil or military technologies give cause of concern about human health risks. A lot of studies highlight the effects of uranium on cerebral functions such as cognitive tasks. The objective of this study was to analyze the effects of depleted uranium (DU) on neurogenesis at different stages of brain development in an *in utero* model of internal contamination. Female rats have been exposed to DU (40 or 120mg/L) during gestation and lactation. Two time points of analysis have been chosen: embryonic day 13 (E13) and post natal day 21 (PND 21). In this work, we examined :
1) if there were morphological modification after contamination using Nissl staining,
2) if contamination modified proliferation of cells in neurogenic zones using BrdU immunostaining,
3) if contamination altered cell differentiation on neurogenic zones using Nestin and Doublecortin immunostaining,
4) if neurite growth was affected by DU contamination using Golgi staining.

Our results show that uranium does not generate organogenesis deficits in the CNS. The proliferation studies suggest a modification in cell proliferation at the embryonic and the postnatal stages. For example, at PND 21, the BrdU positive cells tend to decrease in the dentate gyrus of hippocampus after contamination with a number of 127±48 for the control group, 74±30 for DU at 40mg/L and 65±17 for DU at 120mg/L. In contrast, neuronal cell differentiation does not seem to be affected by uranium ingestion. Finally, no macroscopic morphological differences were observed on neurites of granular cells after contamination.

These first data suggest that chronic contamination with low dose of uranium may influence neuronal cell proliferation.

To complete these results, we will study more stages of brain development and others markers of cell differentiation and survival. We are also going to perform behavioral tests on animals exposed to DU to correlate modification of neurogenesis with cognitive changes.

Pilot Experiment in the Figaro facility: Chronic Gamma Irradiation of Rodents

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To investigate the effects of chronic ionizing irradiation on experimental animals, the Norwegian Institute of Public Health (NIPH, Oslo) and the Norwegian University of Life Sciences (UMB, Ås) recently established an animal facility which is part of a Co^{60} irradiation unit allowing low dose-rate exposures. By today the unit has been approved by the Norwegian Animal Research Authority (FDU) for experiments with rodents (inclusive TGR), fish and earth worms. Temperature (10-25 °C) and light are adjustable within the animal facility. Long term exposures lasting from days to several weeks with continuous irradiation are hence possible. In addition, the unit is planned to include light sources allowing exposure at defined spectra of ultraviolet light or solar simulated light. In total, the unit offers the opportunity to perform experiments comprising combinations of various environmental stressors.

As for the rodent experiments, the unit consists of three Scantainer (ventilated cabinets with negative pressure in relation to the surroundings) connected by a climate control system (ScanClime; Scanbur Technology, Denmark) assuring constant temperature (20-24 °C), relative humidity (45-65%) and 15-20 air shifts per hour, in harmony with Appendix A (The European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes). A maximum of 20 disposable cages fit in one Scantainer, allowing experiments with 20 cages x 3 Scantainers x 4 mice/cage, i.e. a maximum of 240 mice.

Currently, we are performing an experiment to investigate effects of chronic gamma-irradiation on male fertility. C57BL/6J mice will be exposed for 45 days (timespan of spermatogenesis in mice) to a total dose of 2 Gy, i.e. dose rate of 1.85 mGy/h. In addition to wildtype mice, we will also use Ogg1-knockout mice which lack an important pathway for the repair of oxidative DNA damage (Olsen et al., Nucleic Acids Research, 2003). This phenotype mimics male germ cells in humans, which have a much lower capacity to repair certain oxidative damages compared to mice. We will study the effects on spermatogenesis expressed as changes in reproduction, sperm quality, mutation frequency, DNA damage in somatic cells and measure oxidative stress via glutathione peroxidase.

The animal facility which has now been established is unique in providing opportunities for continuous irradiation of vertebrates. Hence, the unit provides a novel opportunity in Europe to perform experiments under controlled dosimetric and environmental conditions. This is a new possibility for collaboration on research into effects of environmentally relevant exposure situations.

Pathway analysis techniques to study proteomic data from low-dose/low-dose-rate irradiated cells

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The objective of DoReMi task 5.1 is to identify any 'phase-shifts', i.e. any biological response to radiation which is dependent on radiation dose, dose rate and quality in the shape of the dose-response curve at low dose. In this task the dose-response relationships for cellular stress response has been explored on human fibroblasts through the investigation of protein expression levels by the collaboration between BfS (Munchen) and SU (Sweden).

In particular the proteomic study carried out by the BfS' group (see also poster by M. Hauptmann et al.) produced a new data set of the proteins profiles on the experimental model perturbed by radiation. This experimental data were analyzed with the current (and newly implemented) pathway analysis techniques and approaches in order to highlight the biological pathways which have been either activated or inhibited following different irradiation exposure setup.

In this work, different pathway analysis techniques (R-based "SPIA" package, Ingenuity Pathway Analysis and Cytoscape plugin' "Reactome FI") applied to this data set, and the corresponding results, will be shown. These have been compared also in order to try to evaluate the differentially regulated pathways and if the different radiation exposures are the cause of the de-regulation of different biological pathways, showing any adaptive-like response dependence.

Influence of oxidative stress and different radiation quality exposure on cytokine release

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Aim of this work was to investigate the cell signalling - Interleukines and Reactive Oxygen Species [1,2,3] - perturbed by radiation of different qualities.

We evaluated experimentally the cytokine levels in the medium of irradiated cells, observing an effective role of radiation in modulating the release of these signals. The kinetics of the secreted cytokines (e.g. IL-6) were evaluated after deliveries (0.1 to 1 Gy) of radiation with different LETs (gamma rays vs alpha particles). To test the role of oxidative stress in the cytokine release process, this investigation took place also in combination with the treatment of specific RNS and ROS scavengers (e.g. c-PTIO and DMSO) [4]. To complete the study, the experimental data were analyzed and coupled with a phenomenological model based on differential equations to evaluate the single-cell response mechanisms. This activity quantified the single cell release rate as a function of the dose and the quality of the incoming radiation.

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