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ESF-EMBO Symposium

Spatio-Temporal Radiation Biology: Transdisciplinary Advances for Biomedical Applications

Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava) • Spain
16-21 May 2009

Chair: **Yann A. Gauduel,** INSERM, Loa-CNRS,
Ecole Polytechnique – ENS Techniques Avancées, Palaiseau, FR

Co-organizers :
Laure Sabatier, CEA Fontenay aux Roses, FR
Georg Bauer, University of Freiburg, DE
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Jean Laissue, University of Bern, CH
Frank Wien, Synchrotron Soleil, FR

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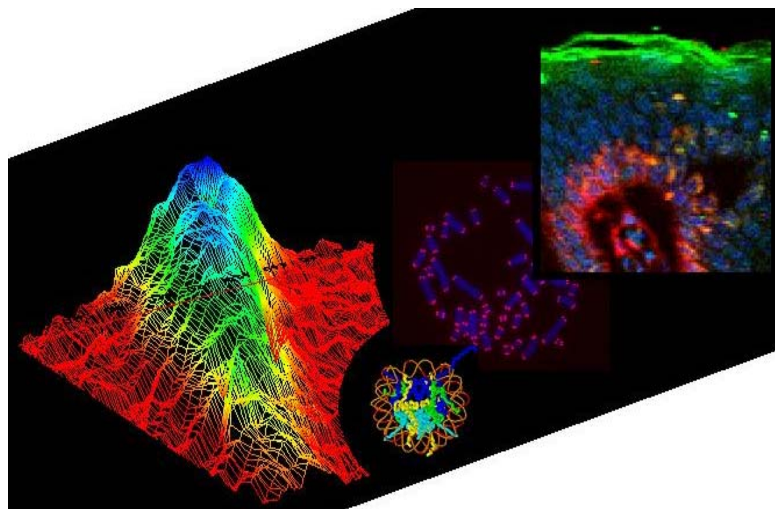
Booklet of abstracts



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Organization

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Mrs. Jean Kelly, European Science Foundation, Brussels, BE

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Mrs. Judith A. Sabaton, European Science Foundation, Brussels, BE

Presentation

This ESF-EMBO conference aims to create a timely forum for multi-disciplinary discussions of recent developments in the ionising radiation effects on integrated living systems, from molecular to tissular scales, bringing together physicists, chemists, biochemists, biologists, genetics experts as well as physicians with a common interest in using different radiation sources and advanced techniques to explore transdisciplinary aspects of modern radiation biology and related biomedical applications.

Deeply understanding the basic mechanisms of radiation damage in vitro and on living cells, starting from the early radical and molecular processes to mutagenic DNA lesions, cell signalling, genomic instability, apoptosis, microenvironment and Bystander effects, radio sensitivity should have in the near future many practical consequences like the customization of radiation therapy or radioprotection protocols. In this context, spatio-temporal radiation biology represents a newly emerging interdisciplinary field of studies driven nowadays in strong synergy with the most recent progresses of molecular biology, genomics and proteomics, biomarker detections, X-ray synchrotron micro-imaging, microbeams, pulsed relativistic particle sources, selective targeting radiopharmaceutical, advanced radiation therapies. The programme will address a number of highly topical aspects of spatio-temporal radiation biology, evolving over several orders of magnitude, typically from femtosecond and sub-micrometric scales.

The ESF-EMBO symposium would gather together actors from the academic research, applied and medical research as well as private companies and clinicians, to take advantage of the high research quality and technological environment developed in a European and international context.

Acknowledgements

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and the external sponsors for their financial supports

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Synopsis

Conference time table	*7*
Programme	*8 – 13*
Invited lectures	*14 – 34*
Short talks	*35 – 62*
Posters	*63 – 115*
List of participants	*116 – 119*

Conference time table

Saturday 16 May	Sunday 17 May	Monday 18 May	Tuesday 19 May	Wednesday 20 May	Thursday 21 May
	Breakfast	*Breakfast*	*Breakfast*	*Breakfast*	*Breakfast*
	9H00 – 13H00 Session 1 **Molecular and sub-cellular imaging of radiation effects** ***Flash poster presentations 1***	*9H00 – 13H00* Session 3 **Induction, amplification of damages** ***Flash poster presentations 3***	*9H00 – 13H00* Session 5 **Micro-environments and radiation responses**	*9H00 – 13H00* Session 6 **Cellular imaging for radiation biology**	*Departure*
	Lunch	*Lunch*	*Lunch*	*Lunch*	
Arrival ***Registration***	*15H00 -19H00* Session 2 **Pre-thermal and thermal radiation processes** ***Flash poster presentations 2***	*15H00 -19H00* Session 4 **Microbeam radiation** ***Flash poster presentations 4***	*14H00 – 19H00* **Half-day excursion**	*15H00 – 18H40* Session 7 **Innovating approaches for radiotherapies**	
Welcome Drink ***Dinner***	*Dinner*	*Dinner*	*20H00 -22H00* **Pre-Dinner Cocktail** **Conference Dinner** ***Including EMBO Poster Prize Award***	*Dinner*	
	20H30 -22H00 **Poster session I**	*20H30 -22H00* **Poster session II**		*20H45 -22H00* **Forward Look Plenary Discussion**	

Programme

Saturday 16 May

Late afternoon / Early evening	Registration at the ESF-RC desk
19.00	Welcome Drink and dinner

Sunday 17 May

08.45-09.00 Conference Opening

Yann A. Gauduel
INSERM, LOA-CNRS, Ecole Polytechnique – ENS Techniques Avancées, FR

Michel Salzet
Université des Sciences et Technologies de Lille, FR *ESF Presentation*

Anne-Marie Glynn
European Molecular Biology Organization, DE
EMBO Presentation

Session 1: Molecular and subcellular imaging of radiation events

Chairs: Elena Giulotto, Università di Pavia, IT - Darel Hunting, Faculty of Medicine, Sherbrooke, CA

09.00-09.45 Noel Lowndes *IL1- p.17*
National University of Ireland, Galway, IE
Sensing and responding to DNA damage

09.45-10.10 Marie-Pierre Gaigeot *ST1-p.38*
LAMBE, Evry University, FR
Theoretical investigation of the ultrafast dissociation of ionized biomolecules immersed in water: direct and indirect effects

10.10-10.55 Olga Sedelnikova *IL2- p.18*
NCI, NIH, Bethesda, US
γ-H2AX as a biomarker of DNA damage

10.55-11.20 Coffee break

11.20-12.05 Ennio Prosperi *IL3-p.19*
CNR, Pavia, IT
Spatio-temporal aspects of cell response to DNA damage: recruitment of checkpoint and DNA repair proteins at DNA damage sites

12.05-12.30 Burkhard Jakob *ST2-p.39*
Department of biophysics, GSI, Darmstadt, DE
Analysis of radiation-induced DNA double strand break motion and protein exchange at damaged sites after charge particle irradiation

12.30-13.00 **Flash poster presentations 1** (P1-P10) **Chairs: A. Styczynska, UK – S. Costes, US**

13.00-15.00 Lunch

Session 2: Pre-thermal and thermal radiation processes

Chairs: Andrey V. Solov'yov Goethe University Frankfurt am Mai, DE - Frank Wien, Synchrotron Soleil, FR

15.00-15.45 **Dimitra Markovitsi** *IL4-p.20*
CEA-Cnrs, Saclay, FR
Interaction of UV radiation with DNA: from photon absorption to photodamage

15.45-16.10 **Qing-Bin Lu** *ST3-p.40*
University of Waterloo, CA
Effects and applications of prehydrated electrons in radiation biology and radiotherapy

16.10-16.55 **Werner Friedland** *IL5-p.21*
GSF, Neuherberg, DE
Simulation of DNA repair via NHEJ based on track structure calculation

16.55-17.25 Coffee break

17.25-17.50 **Aidan Meade** *ST4-p.41*
Focas Research Institute, Dublin, IE
Spectroscopic and chemometric approaches to radiobiological analyses

17.50-18.15 **Vaclav Stepan** *ST5-p.42*
Nuclear Physics Institute ASCR, Prague, CZ
Importance of particle track structure in radiation DNA damage calculations

18.20-19.00 **Flash poster presentations 2** (P11-P22) **Chairs: K. Stankova, BG – F. Paris, FR**

19.00-20.30 Dinner

20.30-22.00 **Poster session I**

Monday 18 May

Session 3: Induction, amplification of damages

Chairs: Martin Falk, Institute of biophysics ASCR, CZ - Sandrine Lacombe, University Paris Sud 11, FR

09.00-09.45 **Stanley Botchway** *IL6-p.22*
Rutherford Appleton Laboratory, Didcot, UK
Femtosecond near-infrared laser microbeam technique as a sub-micron point source for high-resolution cell DNA damage, signalling and repair studies

09.45-10.10 **Katsumi Kobayashi** *ST6-p.43*
High Energy Accelerator Research Organization, Tsukuba, JP
Enhancing mechanism of radiation effect by incorporation of heavy elements

10.10-10.55 **Elena Giulotto** *IL7-p.23*
Università di Pavia, IT
Chromosomal aberrations and gene amplification

10.55-11.20 Coffee break

11.20-12.05 **Darel Hunting** IL8-p.24
Faculty of Medicine, Sherbrooke, CA
DNA interstrand crosslinks induced by ionizing radiation in the presence and absence of radiosensitizers

12.05-12.30 **Andrey V. Solov'yov** ST7-p.44
Goethe University Frankfurt am Main, DE
Physics of ion beam cancer therapy: a multiscale approach

12.30-13.00 **Flash poster presentations 3** (P23-P33) **Chairs: R.Anderson, UK - C.De Wagter, BE**

13.00 – 15.00 Lunch

Session 4: Microbeam radiation

Chairs: Jean Laissue, University of Bern, CH - Jean Doucet, CNRS, Orsay, FR

15.00-15.45 **Kevin Prise** IL9-p.25
Queens University of Belfast, UK
Microbeam technologies for probing radiation responses at the subcellular and tissue levels

15.45-16.10 **Stéphanie Blockhuys** ST8-p.45
Faculty of medicine, Ghent, BE
In vitro models for investigating spatially fractionated irradiation: physics and biological results

16.10-16.55 **Elke Bräuer-Krisch** IL10-p.26
ESRF, Grenoble, FR
Microbeam radiation therapy (MRT)and dosimetric challenges

16.55-17.25 Coffee break

17.25-17.50 **Guido A. Drexler** ST9-p.46
Radiobiological Institute, University of Munich, DE
Sequential ion microirradiation reveals competition effect in DNA damage response

17.50-18.15 **Erik Albert Siegbahn** ST10-p.47
Karolinska hospital, Solna, SE
X-ray microbeam dosimetry

18.20-18.50 **Flash poster presentations 4** (P34-P43) **Chairs: K.Ivanova, BG - P.Lopez-Tarifa, ES**

19.00 Dinner

20.30-22.00 **Poster session II**

Tuesday 19 May

Session 5: Cellular imaging for radiation biology

Chairs: Kevin Prise, Queens University of Belfast, UK - Michèle Martin, CEA Evry, FR

09.00-09.45

Roland Kanaar *IL11-p.27*

Erasmus MC, Rotterdam, NL

Cellular metabolism of radiation-induced DNA double-strands breaks

09.45-10.10

Alberto Astolfo *ST11-p.48*

Synchrotron Trieste, IT

Long term cell tracking in small animals phase using phase contrast based micro CT and synchrotron radiation

10.10-10.35

Guanghua Du *ST12-p.50*

Technische Universität München, Garching, DE

Dynamics of DSB related protein foci: a 2D and 3D analysis

10.35-11.00 Coffee break

11.00.-11.45

Martin Falk *IL12-p.29*

Institute of biophysics ASCR, Brno, CZ

Relationship between higher-order chromatin structure, DSB induction and repair

11.45.-12.10

Igor Belyaev *ST13-p.51*

General Physics Institute, Russian Academy of Science, Moscow, RU

53BP1/γ-H2AX foci do not always co-localize and their complex kinetics may not correlate with DSB repair

12.10-12.35

Claire Heride *ST14-p.52*

CEA, Fontenay-aux-Roses, FR

Chromosome organization in human epithelial cancer cells

12.35-13.00

José Penagaricano *ST15-p.53*

Department of radiation oncology, University of Arkansas, Little Rock, US

Evaluation of spatially fractionated radiotherapy (GRID) and definitive chemo-radiotherapy with curative intent for locally advanced squamous cell carcinoma of the heart and neck

13.00-14.00 Lunch

14.00 Half-day excursion

20.00 Pre-Dinner Cocktail (Dali Bar)

20.30 Conference Dinner – including EMBO Poster Prize Award

Session 6: Microenvironments and radiation responses

Chairs: Noel Lowndes, University of Ireland, Galway, IE **- Laure Sabatier,** CEA Fontenay-aux-Roses, FR

09.00-09.45 **Sylvain Costes** *IL13-p.30*
LBNL, Berkeley, US
Quantifying and modelling cellular response to ionizing radiation: from DNA damage to phenotype

09.45-10.10 **L'emira Ghida Harfouche** *ST16-p.55*
CEA, Evry, FR
Activation of DNA double strand break repair after γ-rays is dependent on FGF2 signaling in human keratinocyte stem cells

10.10-10.55 **Marie Dutreix** *IL14-p.31*
Hospital Institut Curie, Orsay, FR
Dbait: a trick to lure the DNA damage signalling and inhibit DNA repair in tumors

10.55-11.25 Coffee break

11.25-12.10 **Hans Rabus** *IL15-p.32*
PTB, Braunschweig, DE
Recent advances in particle-track simulations with applications to nanodosimetry

12.10-12.35 **Chris Wang** *ST17-p.56*
Georgia Institute of Technology, Atlanta, US
A nanodosimetry-based linear quadratic cell survival model for radiobiology

12.35-13.00 **Chunlin Shao** *ST18-p.57*
Fudan University, Shanghai, CN
Signaling factors and regulating of irradiation induced bystander responses

13.00-15.00 Lunch

Session 7: Innovating approaches for radiotherapies

Chairs: Peter M. Corry, Arkansas University for Medical Sciences, US **- John Gueulette,** UCL, Bruxelles, BE

15.00-15.45 **Victor Malka** *IL16-p.33*
LOA-CNRS, Ecole Polytechnique – ENSTA, Palaiseau, FR
Laser plasma accelerators : innovative electron beam and potential applications for radiation biology and radiotherapy

15.45-16.10 **Robert Griffin** *ST19-p.58*
University of Arkansas for medical sciences, Little Rock, US
Precise millimetre beam positioning of a conformal radiation therapy for the optimization and study of spatially fractionated radiotherapy

16.10-16.35 **Janusz Dabrowski** *ST20-p.59*
Faculty of Biochemistry, Biophysics and Biotechnology, Krakow, PL
New opportunities of near infrared radiation for cancer diagnosis and therapy

16.35-17.05	Coffee break
17.05-17.50	Tadashi Kamada *IL17-p.34* NIRS, Chiba-Shi, JP *The past, present and future of carbon ion radiotherapy at NIRS-HIMAC*
17.50-18.15	Emanuele Scifoni *ST21-p.60* Frankfurt Institute for Advanced Studies, Frankfurt am Main, DE *Ion-beam induced damage: spectra of secondary electron generated by carbon ions in tissue-like media*
18.15-18.40	Lorenzo Manti *ST22-p.62* University of Naples Federico II, Naples, IT *Time-dependent onset of cellular senescence in response to carbon-ions: implications for hadrontherapy*
19.00-20.30	Dinner
20.45-22.00	Forward Look Plenary Discussion

Thursday 21 May

Breakfast & Departure

Invited Lectures

▪ Stanley Botchway p. 22
Rutherford Appleton Laboratory, Didcot, UK
Femtosecond near infrared laser microbeam technique for sub-micron point source for high-resolution cell DNA damage, signaling and repair studies

▪ Elke Bräuer-Krisch p. 26
ESRF, Grenoble, FR
Microbeam radiation therapy (MRT) at the ESRF

▪ Sylvain Costes p. 30
LBNL, Berkeley, US
Quantifying and modelling cellular response to ionizing radiation: from DNA damage to phenotype

▪ Marie Dutreix p. 31
Hospital Institut Curie, Orsay, FR
siDNA: a military strategy to fight radioresistance in tumors

▪ Martin Falk p. 29
Institute of biophysics ASCR, Brno, CZ
The role of higher-order chromatin structure and nuclear topography in DSB induction, repair and chromatin translocation

▪ Werner Friedland p. 21
GSF, Neuherberg, DE
Simulation of DNA repair via NHEJ based on track structure calculation

▪ Elena Giulotto p. 23
Università di Pavia, IT
Gene amplification and the other chromosomal aberrations in cancer and radiation biology

▪ Darel Hunting p. 24
Faculty of Medicine, Sherbrooke, CA
DNA interstand crosslinks induced by ionizing radiation in the presence and absence of radiosensitizers

▪ Tadashi Kamada p. 34
NIRS, Chiba-Shi, JP
The past, present and future of carbon ion radiotherapy at NIRS-HIMAC

▪ Roland Kanaar p. 27
Erasmus MC, Rotterdam, NL
Cellular metabolism of radiation-induced DNA double-strands breaks

▪ Noel Lowndes p. 17
National University of Ireland, Galway, IE
Sensing and responding to DNA damage in model systems

▪ Victor Malka — p. 33
Cnrs, Ecole Polytechnique – ENSTA, Palaiseau, FR
Laser plasma accelerators : innovative electron beam and potential applications for radiation biology

▪ Dimitra Markovitsi — p. 20
CEA-Cnrs, Saclay, FR
UV induced DNA damage studied by time-resolved spectroscopy

▪ Kevin Prise — p. 25
Queens University of Belfast, UK
Microbeam technologies for probing radiation responses at the subcellular and tissue levels

▪ Ennio Prosperi — p. 19
CNR, Pavia, IT
Spatio-temporal aspects of cell response to DNA damage: recruitment of checkpoint and DNA repair proteins at DNA damage sites

▪ Hans Rabus — p. 32
PTB, Braunschweig, DE
Recent advances in particle-track simulations with applications to nanodosimetry

▪ Olga Sedelnikova — p. 18
NCI, NIH, Bethesda, US
Gamma-H2AX as a biodosimeter in irradiated and bystander human tissues

Molecular and subcellular imaging of radiation events – IL1

Sensing and responding to DNA damage

Noel F. Lowndes

Genome Stability Laboratory, Centre for Chromosome Biology and National Centre for Biomedical Engineering Science, National University of Ireland Galway, Galway, Ireland

An effective cellular response to ionizing or ultraviolet radiation is critical to the maintenance of genome stability and tumour suppression. Central to this response are biochemical signalling pathways, collectively termed the *DNA damage response* (DDR). We study early events in the DDR, specifically, the molecular mechanisms behind the detection of, and the initial signaling from, DNA lesions. Our research focuses on two classes of DDR proteins, mediators and PI3K kinases, both with functions early in the DDR. We primarily use two eukaryotic model systems, the budding yeast, *S. cerevisiae,* and DT40 chicken B-lymphocytic cells. Both systems have the major advantage of genetic tractability, as gene targeting is straightforward. Using these model systems a major focus of our work is the function and regulation of the so-called ‘mediator’ proteins, of which budding yeast Rad9 is the prototypical example. Although there is no obvious higher eukaryotic homologue of Rad9, there are proteins that resemble Rad9 both structurally and functionally, these ‘Rad9-like’ proteins include, 53BP1/BRCA1/MDC1, and we are dissecting their function using DT40 cells. Additionally, we are performing a comparative analysis of PI3K kinases that function in the DNA damage response, particularly yeast Mec1 protein, and it orthologue in DT40 cells, Atr.

Molecular and subcellular imaging of radiation events – IL2

γ-H2AX as a biomarker of DNA damage

Olga A. Sedelnikova

Laboratory of Molecular Pharmacology, CCR, NCI, NIH Bethesda, MD 20892 U.S.A.
Email: sedelnio@mail.nih.gov

The DNA double-strand break (DSB) plays dual roles in genome integrity. On one hand DSB formation is essential to several normal cellular processes, but on the other, accidental DSB formation may lead to permanent genomic damage, tumorigenesis, and cell death. A universal cellular response to a DSB is the phosphorylation of several thousand molecules of histone H2AX to form γ-H2AX in the chromatin flanking the break site. Immunocytochemical analysis with anti-γ-H2AX reveals the number and position of each nuclear DSB as a focus of γ-H2AX.

Ionizing radiation (IR) leads to the formation of γ-H2AX foci which reaches a maximum at 15-30 minutes post-IR and then declines within several hours. The high resolution of the γ-H2AX focus formation assay permits single DSB detection after low dose IR, as well as in bystander cells sharing the same milieu with irradiated cells. In marked contrast to directly irradiated cells, γ-H2AX focal formation and repair in bystander cells are substantially delayed. We recently found that other stress factors, including the presence of untreated tumor cells and tumors, also induce γ-H2AX focal formation in bystander cells and tissues, indicating that the bystander effect is a general response to cellular stress.

Normal human cells accumulate DNA DSBs marked by γ-H2AX foci during both *in vitro* senescence and *in vivo* aging, as well as with oncogenic transformation. These endogenous foci related to cellular genome destabilization have multiple origins, telomeric and non-telomeric, which may result from oxidative damage. DNA DSB repair proteins accumulate at these foci, indicating that they are sites of DSBs and repair. When DSBs are generated in human cell cultures with IR, their numbers are similar in cells at different stages of senescence and at different age, but the rates of dimensional focal growth and accumulation of DSB repair proteins are substantially slower in aged cells. Thus, the ability of cells to repair DNA damage decreases with age.

The γ-H2AX focus formation assay is currently the most sensitive way to examine genome integrity. We have successfully applied this assay to many human materials (including peripheral blood mononuclear cells (PBMCs), various tissues and skin) to monitor DNA damage produced by IR or drug exposure, the bystander effect, or cancer and age-related genome instability. Being able to routinely monitor DSB levels in individuals could provide useful tools for improving human health.

Molecular and subcellular imaging of radiation events – IL3

Spatio-temporal aspects of cell response to DNA damage: recruitment of checkpoint and DNA repair proteins at DNA damage sites

Ennio Prosperi

Istituto di Genetica Molecolare del CNR (IGM-CNR), Pavia, Italy

Exposure to genotoxic agents (e.g. chemicals, ionizing radiation etc.), may induce the formation of a variety of DNA lesions that cells must remove in order to avoid genomic instability, and to prevent cancer formation. To this end, cells have developed genome surveillance and signalling pathways (checkpoints) that are flanked by DNA repair systems. These systems, collectively known as the DNA damage response, are highly conserved among organisms and consist of DNA damage signalling cascade, and of DNA repair processes able to recognize and remove a great number of DNA lesions. Recent findings have shown that cell cycle checkpoints and DNA repair systems cross-talk each other. However, the role and the molecular mechanisms underlying these connections are not yet well understood.

Among cell cycle regulatory proteins that are activated following DNA damage, the cyclin-dependent kinase inhibitor $p21^{CDKN1A}$ plays fundamental roles in the DNA damage response, by inducing cell cycle arrest, direct inhibition of DNA synthesis, as well as regulation of transcription and apoptosis. During the last years, we have provided evidence showing that p21 may be directly involved also in DNA repair. Participation of p21 in DNA repair pathways, like nucleotide excision repair (NER), and base excision repair (BER), is thought to occur thanks to its interaction with PCNA, an important protein involved both in DNA replication and repair. A common feature of checkpoint and DNA repair factors is their recruitment at nuclear sites where DNA damage has occurred. In this communication, results will be presented showing spatio-temporal dynamics of p21 protein recruitment at sites where DNA damage has been induced by UV radiation. This process is related to activation of DNA repair since protein recruitment occurs concomitantly with DNA repair factors, and is dependent on functional DNA repair pathway, e.g. NER. In addition, some technical approaches using a UV-lamp, or laser radiation to induce DNA lesions localized whithin small nuclear regions, will be described. These approaches allow the spatio-temporal investigation, both in living cells, and in fixed samples, of the recruitment of proteins participating in cell cycle checkpoints, and DNA repair, at the same sites of DNA damage.

Pre-thermal and thermal radiation processes – IL4

Interaction of UV radiation with DNA: from photon absorption to photodamage

Dimitra Markovitsi

Laboratoire Francis Perrin, CEA-CNRS URA 2453 F-91191 Gif-sur-Yvette, France *Email: dimitra.markovitsi@cea.fr*

Direct absorption of UV radiation by DNA bases is known to trigger photochemical reactions which may lead to carcinogenic mutations. Although the major lesions are well characterized, the physicochemical processes which precede their formation remain unknown. Recent experimental and theoretical studies of model DNA shed some light on the properties of the excited states populated by photon absorption and their relaxation, energy transfer among bases and their one-photon ionization as well as on the dynamics of thymine dimer formation.

These studies revealed that the DNA helices cannot be considered as the sum of their monomeric constituents. Electronic coupling induces delocalization of the Franck-Condon excited states. Consequently, energy transfer takes place among bases via intraband scattering in less than 100 fs. Moreover, organization of the bases within helices leads to a lowering of their ionization potential. Finally, it was shown that the formation of (6-4) thymine dimers in the single strand $(dT)_{20}$ takes place within 4 ms and involves a reaction intermediate.

F. Miannay, A. Banyasz, T. Gustavsson, D. Markovitsi, *J. Am. Chem. Soc.* **2007** *129* 14574-75.
D. Onidas, T. Gustavsson, E. Lazzarotto, D. Markovitsi, *J. Phys. Chem. B* **2007** *111* 9644-50.
D. Markovitsi, T. Gustavsson, F. Talbot, *Photochem. & Photobiol. Sci.* **2007** 717 - 24.
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D. Markovitsi, T. Gustavsson, A. Sharonov, *Photochem. Photobiol.* **2004** *79* 526-30.

Pre-thermal and thermal radiation processes – IL5

Simulation of DNA repair via NHEJ based on track structure calculation

Werner Friedland

Institute of Radiation Protection, Helmholtz Zentrum München, Germany

A new DNA repair simulation model describing the non-homologous end joining pathway of DSB rejoining has been developed. Track structure calculations with PARTRAC and the DNA model adopted in this code (1) provide initial boundary conditions for DNA repair simulation. Characteristics of initial DSB taken into account in the model are genomic and geometric positions of DSB as well as its complexity in terms of nearby strand breaks and base damages. DSB are represented by two independent items corresponding to the two DNA termini. Each item is primarily characterized by a state index number which corresponds to the attachment and phosphorylation of repair enzymes during the repair process. Association and reverse dissociation of repair enzymes at DSB ends are modeled as stochastic first order kinetic processes. The sequence of DSB end states involves initial mobilisation, attachment of Ku70/Ku80, attachment of DNA-PK$_{cs}$, synapsis, phosphorylation of DNA-PK, attachment and action of XRCC4/Ligase IV, processing of nearby lesions for complex DSB ends and cleaning of joined DNA. Joining of DNA termini distinguishes between correct rejoining, chromosomal exchange aberrations, rings and other incorrectly joined DNA termini. Motion of DNA termini is modeled by step-by-step displacement simulating Brownian diffusion. Movement is temporally limited to the time interval between initial mobilisation and synapsis; spatial constraints are set by assumed attachment of the chromatin fiber to a nuclear matrix according to the loop geometry of the DNA model. Parameters of the simulation model have been partly derived from experiments on enzyme attachment kinetics including fluorescence recovery after photobleaching (2); the overall DSB repair kinetics has been adapted to corresponding experimental data after irradiation of human fibroblast cells with ^{137}Cs γ-rays (3). Using the same parameter set, the DSB repair model is applied to and compared with experimental results on cell irradiated with N ions (4) in order to analyse critically how far the working hypotheses implemented in the model can withstand this stringent test.

1. Friedland et al., Radiat Environ Biophys 47, 49 (2008)
2. Uematsu et al., J Cell Biol 177, 219 (2007)
3. Karlsson et al., Radiat Res 169, 506 (2008)
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Induction, amplification of damages – IL6

Femtosecond near infrared laser microbeam technique as a sub-micron point source for high-resolution cell DNA damage, signalling and depair studies

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Radiation microbeam technology for radiobiology research is undergoing exponential and rapid growth. The use of microbeam techniques to induce sub-micrometer localised energy deposition within a region of a cell nucleus can be used to address questions relating to the effects of low doses of radiation, the propagation and treatment of DNA damage (repair) in individual cells as well as non-targeted cell to cell effects. We have pioneered a near infrared (NIR) laser microbeam to mimic ionising radiation through multiphoton absorption within the 3D femtolitre volume of a highly focused Gaussian beam. This phenomenon provides a novel optical microbeam probe for mimicking both complex ionising and UV-radiation-type cell damage such as double strand breaks (DSBs) and base damage. We have used the NIR laser microbeam probe to investigate the formation of DNA DSB and recruitment of repair proteins to the sub-micrometre size site of damage in viable cells. Using both immuno-fluorescent staining of γ-H2AX (a marker for DSBs) and real-time imaging GFP-labelled repair proteins in viable mammalian cells, we show that the co-localisation of ATM, p53 binding protein 1 (53BP1) and RAD51, an integral protein of the homologous recombination process in the DNA repair pathway and Ku-80-GFP involved in the non- homologous end joining (NHEJ) pathway, show differences in the repair kinetics of DNA DSB. We have observed persistent DSBs at later times post laser irradiation which are indicative of DSBs arising at replication presumably from UV photoproducts or clustered damage containing single strand breaks (SSBs). Cell cycle studies have shown that in G1 cells, a fraction of multi-photon laser induced DSBs persist for > 6 h in addition to those induced at replication thus further elucidating the repair of complex DNA damage revealed by the laser microbeam technique.

Induction, amplification of damages – IL7

Chromosomal aberrations and gene amplification

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A typical manifestation of chromosomal instability in tumour cells is gene amplification, the copy number increase of a DNA sequence. Gene amplification is a mechanism of oncogene activation and drug resistance in tumour cells, involving DNA breakage and repair. Similarly to other chromosomal aberrations, gene amplification can occur spontaneously or can be induced by ionizing radiation. Using cell lines, in which the expression of genes involved in the repair of DNA breaks was inhibited by "RNA interference", we demonstrated that the genetic background can affect the propensity to gene amplification. A single DNA double strand-break seems to be sufficient to trigger a cascade of fusion-bridge-breakage cycles that generates several copies of an extended chromosomal region, strongly affecting the expression of several genes. The acquisition of extra-copies of a chromosome region in cells undergoing gene amplification is paralleled by the loss of genetic material from other regions; therefore, the same initiating event can cause over-expression of some genes and inactivation of other genes. A similar series of events can be initiated by a dysfunctional telomere that can be "seen" by the cell as a DNA break. It has also been shown that the presence of a short telomere can enhance the cellular sensitivity to ionizing radiations by favouring recombination events between this telomeric end and the end of a break. In conclusion, we postulate that several illegitimate recombination events may occur during successive cell divisions as long term consequences of a single DNA break or alteration of a single telomere, causing extensive modifications of the genome and contributing to tumour progression.

Induction, amplification of damages – IL8

DNA interstrand crosslinks induced by ionizing radiation in the presence and absence of radiosensitizers

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The induction of DNA interstrand crosslinks by ionizing radiation has been largely ignored in favor of studies on double-strand break formation and repair. Indeed, the detection of interstrand crosslinks is confounded by the presence of single-strand breaks induced by ionizing radiation, since detection of crosslinks generally involves a denaturation step, as in alkaline elution or the alkaline comet assay. We have studied the induction of interstrand crosslinks involving the uracilyl radical, following the irradiation of DNA containing bromouracil at specific sites. We found that the formation of interstrand crosslinks requires the presence of a few (3-5) non-hybridized bases. In our experiments, we used short, double-stranded oligonucleotides with a region of from 0 to 5 mismatched bases in the middle. The bromouracil was located in the center of this mismatched region. In the absence of mismatched bases, no radiation-induced crosslinking was observed; however, in the absence of bromouracil, crosslinking still occurred, albeit at a lower efficiency. Our molecular modelling studies demonstrate that the mobility of the bases in the mismatched region is essential for the crosslinking process. Thus, our hypothesis is that ionizing radiation induces DNA interstrand crosslinks in non-hybridized regions of DNA. Some obvious examples are replication forks, transcription bubbles and the D-loop of telomeres. However, an abundance of studies have made it clear that there must be many single stranded regions in the genome. For example, hairpins and cruciforms (from inverted repeats) involve single strand regions and are quite common. Alpha satellite DNA, present in centromere regions of human chromosomes, forms hairpins. Inverted repeats are hotspots of genomic instability in both prokaryotes and eukaryotes, almost certainly because of their ability to form cruciforms. Thus, a variety of non-B DNA structures (hairpins, slipped DNA and tetrahelical structures) exist in the genome and should be susceptible to the formation of radiation induced interstrand crosslinks. In conclusion, although interstrand crosslinks have thus far been ignored in radiation biology, it will be worthwhile to develop methods to detect their presence following exposure of cells to biologically relevant levels of ionizing radiation, since they should be more toxic than double strand breaks.

Microbeam radiation – IL9

Microbeam technologies for probing radiation responses at the subcellular and tissue levels

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Rapid advances in our understanding of radiation responses, at the subcellular, cellular, tissue and whole body levels have been driven by the advent of new technological approaches for radiation delivery. Microbeams allow precise doses of radiation to be delivered with high spatial accuracy. They have evolved through recent advances in imaging, software and beam delivery to be used in a range of experimental studies probing spatial, temporal and low dose aspects of radiation response. A range of microbeams have been developed worldwide which include ones capable of delivering charged particles, X-rays and electrons. Localised delivery of radiation at the subcellular level is proving a powerful tool. For example, localized production of radiation-induced damage in the nucleus allows probing of the key mechanisms of DNA damage sensing, signalling and repair. Crucially this can be done under conditions where cells retain viability and where the responses to relevant environmental, occupational or clinical doses can be tested. These approaches have started to unravel some of the early events which occur after localised DNA damage within cells.

The ability to target radiation with microbeams at subcellular targets has been used to address fundamental questions related to radiosensitive sites within cells. Key evidence has now emerged for sites outside the nucleus being important. Recent studies have shown an important role for mitochondria as potential direct targets of radiation exposure leading to reactive oxygen species mediated responses. Another application has been in delineating bystander responses, where cells not directly irradiated can respond to irradiated neighbours. Although these processes have been studied using a range of experimental approaches, microbeams offer a unique route by which bystander responses and their underlying mechanisms can be elucidated. Much of this has come from charged particle microbeam studies, but increasingly, X-ray and electron microbeams are starting to contribute quantitative and mechanistic information on bystander effects. A recent development has been the move from studies with 2-D cell culture models to more complex 3-D systems where the possibilities of utilizing the unique characteristics of microbeams in terms of their spatial and temporal delivery will make a major impact. Our own studies with both charged particle and X-ray microbeam approaches are targeting 3-D skin reconstruct models to probe for the underlying signalling mechanisms which are leading to the evolution of new models of radiation response.

Microbeam radiation – IL10

Microbeam Radiation Therapy and dosimetric challenges

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Microbeam Radiation Therapy (MRT) uses highly collimated, quasi-parallel X-ray microbeams of 50-600 keV, produced by 3rd generation synchrotron sources, for example at ID17, the bio-medical beamline at the European Synchrotron Radiation Facility (ESRF), in France.

The results of preclinical trials on different animal models, including mice, rats and piglets, will be presented to illustrate the unprecedented sparing of normal radiosensitive tissues as well as the preferential damage to malignant tumor tissues. Typically, MRT uses arrays of narrow (~25-75 micron) microplanar beams separated by wider (100–400 microns centre to centre) microplanar spaces. Peak entrance doses of several hundreds of Gy are surprisingly well tolerated by normal tissues. The plans for the clinical trials with pet animal patients and the upgrade of ID 17 required for the trials will be briefly presented.

The second part of the presentation will deal with dose calculations and measurements of peak and valley doses. Quantitative experimental dosimetry for MRT has proven to be very challenging, especially at dose rates of 20000 Gy/sec and when a spatial resolution of about 5 micron is required. Over the last 10 years several dosimeters were tested. They showed either severe limitations with respect to the dose rate or in terms of spatial resolution. For the spatially non-fractionated beam several ionization chambers, TLDs, Gafchromic films and Alanine Dosimeters were compared, but do still not meet the usual standards required in conventional radiation therapy, such as absolute dose measurements within 3 % accuracy. Spatially fractionated dose profiles at different depth calculated by Monte Carlo techniques were compared to dose measurements obtained by MOSFET dosimeters, radiochromic films, TLDs and other dosimeters. An overview of the different approaches to measure absolute dose in the peak and in the valley will be presented.

Cellular imaging for radiation biology – IL11

Cellular metabolism of radiation-induced DNA double-strands breaks

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We are interested in the mechanisms through which DNA double-strand breaks (DSBs) are metabolized in cells. Understanding how cells maintain genome integrity when challenged with DNA double-strand breaks (DSBs) is of major importance, particularly since the discovery of multiple links between DSB metabolism and genome instability and cancer-predisposition disorders. We are analyzing DSB metabolism in cells and in single molecule biochemical reactions.

To study DSB metabolism in cells, ionizing radiation is the agent of choice to produce DSBs in cells, however, targeting DSBs and monitoring changes in their position over time can be difficult. We have developed a procedure for induction of easily recognizable linear arrays of DSBs in nuclei of adherent eukaryotic cells by exposing the cells to alpha particles from a small Americium source. Each alpha particle traversing the cell nucleus induces a linear array of DSBs, typically 10-20 DSBs per 10 micron track length. Using immunodetection, recruitment of repair proteins to individual DSB sites as early as 30 s after irradiation can be detected. Furthermore, combined with fluorescence live-cell microscopy of fluorescently tagged DSB-response proteins, allows spatiotemporal analysis of the DSB repair response in living cells.

At the biochemical level we analyzed the RAD51 DSB repair protein in detail. RAD51 is the central catalyst in eukaryotic ATP-dependent DSB repair through homologous recombination. To promote repair, RAD51 polymerizes around single-stranded DNA. This nucleoprotein filament recognizes and invades a homologous duplex DNA segment. After strand exchange, the nucleoprotein filament should disassemble so that the recombination process can be completed. The molecular mechanism of RAD51 filament disassembly is poorly understood. We showed, by combining optical tweezers with single-molecule fluorescence microscopy and microfluidics, that disassembly of human RAD51 nucleoprotein filaments results from the interplay between ATP hydrolysis and the release of the tension stored in the filament. By applying external tension to the DNA, we found that disassembly slows down and can even be stalled. We quantified the fluorescence of RAD51 patches and found that disassembly occurs in bursts interspersed by long pauses. After relaxation of a stalled complex,

pauses were suppressed resulting in a large burst. These results indicate that tension-dependent disassembly takes place only from filament ends, after tension-independent ATP hydrolysis. This integrative single-molecule approach allowed us to dissect the mechanism of this principal homologous recombination reaction step, which in turn clarifies how disassembly can be influenced by accessory proteins.

Cellular imaging for radiation biology – IL12

Relationship between higher-order chromatin structure, DSB induction and repair

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A growing body of evidence shows that higher-order chromatin structure and dynamic nuclear architecture play a fundamental role in regulation of all nuclear processes. Repair of DNA double strand breaks (DSBs) represents crucial and the most challenging process responsible for maintaining genome integrity and is therefore indispensable for preservation of normal cell functions. Simultaneous interruption of both DNA chains in the place of DSB induces severe disruption to primarily genetic information, local chromatin structure and nuclear architecture. Thus, this process should be studied in its whole complexity, considering not only the “biochemical” aspects but also organization in space and time.

In this lecture, fundamental spatio-temporal questions of DSB repair will be discussed, for the first time on the basis of simultaneous and direct 3D-visualization of specific proteins and genetic loci. Our experiments revealed that DSBs induced by low-LET radiation (γ-rays, ^{60}Co) are not homogeneously distributed in the cell nucleus, so we will first focus on two intensively discussed questions: 1) whether DSBs appear preferentially somewhere in the cell nucleus and 2) do some subcompartments specialized to DSB-repair exist there in the nucleus or are these lesions repaired at the places of their origin? In other words, is it a different sensitivity of structurally/functionally distinct chromatin domains to DSB induction that is responsible for the nonhomogeneous DSB distribution or does this distribution reflect the directed migration of DSBs into specific “repair factories”? Do clusters of two or more DSBs, occasionally observed, impersonate these putative factories or, conversely, congregated lesions that are repaired only with difficulty and with a high risk of chromatin translocations formation? Does DSB repair precedes with the same efficiency anywhere in the cell nucleus or does it require specific, repair-competent environment? In latter case, are these repair-competent subdomains already preformed in the cell nucleus or are they built *de novo* at the places of DSB? What changes in chromatin structure occurs during DSB repair? Could these changes be favorable for chromatin movement and allow thus interactions of originally distant chromatin loci? These and many other questions will be discussed and a new model for relations between DSB repair, chromatin translocations formation and higher-order chromatin structure will be suggested.

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Microenvironments and radiation responses – IL13

Quantifying and modeling cellular response to ionizing radiation: from DNA damage to phenotype

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The NASA Specialized Center of Research (NSCOR) at the Berkeley lab has led to the development of new modeling tools to understand better the radiation response from DNA double strand breaks (DSB) a few minutes after exposure to cell phenotypes weeks and months later. Very distinct approaches were taken for each time scale.

1.For the early response to radiation, we introduced an imaging approach to compare the molecular response of DSB markers in real images to the pattern of DSB predicted from a biophysical model on a per nucleus basis (Costes, et al. PLoS Comp Bio, 2007). We imaged radiation-induced foci (RIF) patterns measured by light microscopy using γH2AX and 53BP1 immunofluorescence as a function of radiation quality. Modeling predicted that DSB should be more frequent in the heterochromatin. However, high content analysis revealed that RIF in human cells were underrepresented in the heterochromatin for both low and high-LET in human cells. Furthermore RIF were non-randomly associated with the euchromatin interface within a few minutes following exposure to radiation. These results were confirmed with live cell imaging of 53BP1-GFP. Overall, our analysis suggests that RIF are more readily formed upon complex DSBs whereas simple DSBs elicit a lower and slower foci response.

2.Primary human mammary epithelial cells (HMECs) typically undergo senescence within a few population doublings following extraction from human donors. However it is hypothesized that a sub-category of senescence-resistant cells (SR) is also present in primary cultures. In order to test such hypothesis, an agent-based model (ABM) was used. ABMs are computer models simulations that represent a system as collections of autonomous decision-making entities called agents. Contextual rules were imposed on agents to replicate cellular behaviors measured by time lapse imaging such as proliferation, transient growth arrest, or permanent senescence. An unknown percentage of SR agents were mixed with senescence prone (SP) agents in our simulations. Challenging the ABM with IR predicted the outgrowth of SR agents by selectively reducing the fitness and inducing the early senescence of SP agents. This result was confirmed experimentally and the model suggested that SR cells were present at a ratio of 1/200 to 1/2000 in breast tissues, depending on the donor. Finally, by identifying dose ranges with distinct phenotypes, this kind of modeling may be proven to be a useful tool to predict radiation risk in more complex systems.

Microenvironments and radiation responses – IL14

Dbait: A trick to lure the DNA damage signaling and inhibit DNA repair in tumors

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Enhanced DNA repair activity often is associated with cell resistance to irradiation. We tested a novel strategy for inhibiting DNA repair, using short DNA molecules that mimic DNA double-strand breaks (called Dbait) to lure the cells and trigger “false” damage signaling. We postulated that cells do not distinguish foreign DNA from their own DNA as they transcribe, replicate, recombine and repair incoming DNA whatever its origin is. Therefore short DNA molecules with stable blunt ends should be detected as double double-strand breaks (DSBs) by damage signaling and repair complexes.

We show that, *in vitro*, Dbaits specifically bind to the DNA-dependent protein kinase (DNA-PK) repair complex and activate damage signaling pathway. Numerous targets of the PI3K kinases (H2AX, RPA32, P53, CHEK1, CHEK2, NBS1...) are phosphorylated for several hours after Dbait transfection. Associated to the pan nuclear phosphorylation of H2AX in Dbait transfected cells, we observe a strong reduction of repair/recombination *foci* formation after irradiation. Dbait transfection inhibits homologous recombination, non-homologous recombination and DNA repair, thereby increasing cell death in response to irradiation.

In vivo, Dbait administration induces regression of radioresistant head and neck squamous cell carcinoma (Hep2), glioblastoma (U87) and melanoma (SK28 and LU1205) tumors. The combination of Dbait treatment and fractionated radiotherapy significantly enhanced the therapeutic effect. Tumor growth control by Dbait molecules depend directly on the dose and is observed with various irradiation protocols (Clinical Cancer Research, 2009). The induction of H2AX phosphorylation in tumors treated with Dbait suggests that it acts *in vivo* through the induction of “false” DNA damage signaling and repair inhibition as shown *in vitro*.

Microenvironments and radiation responses – IL15

Recent advances in particle-track simulations with applications to nanodosimetry

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The initiation of radiation-induced damage to biological cells is dominated by inelastic interactions occuring at the location or in the vicinity of DNA molecules. Hence, the pattern of inelastic interactions in sub-cellular dimensions plays a key role for the biological effectiveness of ionizing radiation. The appropriate definition of concepts like radiation quality, therefore, requires the investigation of particle-track structure in detail down to nanometric dimensions.

Inaccessible to experiment, this detailed particle-track structure is investigated by simulations based on Monte Carlo techniques. Ad-hoc codes have been developed, that overcome the restrictions in the particle-transport algorithms of general-purpose Monte Carlo tools. These advanced codes feature a step-by-step treatment of electron scattering based on the knowledge of low-energy electron scattering cross sections down to a few electron volts, which have been systematically measured for a number of materials of interest.

From the simulated particle tracks, characteristic quantities are derived, such as the frequency distribution of the number of ionizations (the ionization-cluster size) produced by the interaction of single ionizing particles within target volumes of nanometric dimensions in condensed matter. In nanodosimetric simulations, the target size chosen correponds to the diameter of DNA strands and the typical length of strand segments within which clustered multiple single strand breaks lead to a double strand break.

In a twofold way, ionization cluster size distributions play a central role in the field of nanodosimetry: On the one hand, the formation of ionization clusters within targets of macroscopic dimensions in dilute gases can be studied by simulation and experiment, such that the latter can be deployed for benchmarking the Monte Carlo codes. This validation is a prerequisite for the paramount aim of nanodosimetry, i.e. to establish a correlation between the cross sections for the induction of biological end points and the statistical moments or particular values of the ionization cluster size distribution in nanometric volumes of condensed matter, on the other hand.

Innovating approaches for radiotherapies – IL16

Laser plasma accelerators: innovative electron beam and potential applications for radiation biology and radiotherapy

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Laser plasma accelerators provide electron beams with parameters of interest in many fields such as material science and medical physics. A short review of progress achieved recently including bubble [1] and colliding [2] schemes will be presented. It has been shown that the electron beam properties such as duration (few fs), charge (100pC), energy in the range 5 MeV- few 100 MeV are relevant for innovative developments in high energy bioradical femtochemistry, radiation biology and radiotherapy.

Regarding radiation biology, one major challenge concerns the complete understanding of spatio-temporal events triggered by an initial energy deposition inside confined clusters of ionization and evolving over several orders of magnitude, typically from femtosecond (10^{-15} s) and sub-micrometric scales. In this way, high energy radiation femtochemistry (HERF) performed with very short pulsed relativistic particles bunches would foreshadow the development of new applications in radiation biology such as real-time nanodosimetry or selective anticancer radiotherapy using quantum states of very short-lived radicals [3, 4].

The innovating advent of powerful laser sources and laser plasma interactions also open exciting opportunities in medical physics and radiotherapy. Considering the last improvements of laser-plasma accelerators, dose deposition simulations have been performed using a quasi-monoenergetic electron beam in the 200 MeV range [4]. It is shown that electron beam properties offer advantageous dosimetric characteristics compare to those calculated with high energy photons. The dose curve shows a broad maximum at large depths (> 20 cm). The lateral penumbra of treatment fields for focused electron beams is smaller compared to 6 MeV photons at depths smaller than 10 cm. These advantages result in an improvement of the quality of a clinically approved prostate treatment plan. While the target coverage is the same or even slightly better for 250 MeV electrons compared to photons the dose sparing of sensitive structures is improved. E.g. the dose to the rectum is reduced by 19% for 250 MeV, focused electrons [5, 6]. These findings agree with previous results regarding very high energy electrons as a treatment modality. The lack of compact and cost-efficient electron accelerators could be overcome by laser-plasma systems.

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[6] T. Fuchs et al., Submitted to Med Bio.

Innovating approaches for radiotherapies – IL17

The past, present, and future of carbon ion radiotherapy at NIRS-HIMAC

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Carbon ion radiotherapy (CIRT) is a unique radiotherapy, which possesses well localized, and superior depth dose distribution in addition to uniform, less repairable radiobiological effects. The use of CIRT for various diseases has been explored as clinical trials at the Heavy Ion Medical Accelerator in Chiba (HIMAC), Japan, since 1994. From June 1994 to February 2009, a total of 4,493 patients were enrolled into clinical studies using carbon beams. We treated almost 700 new patients/ year in 2008. Our experience to date is summarized as follows: CIRT is effective especially (1) in regions such as the head and neck, skull base, lung, liver, prostate, bone and soft tissue, and the pelvic recurrence of rectal cancer and (2) for histological types such as non–squamous cell cancers and sarcomas. It is emphasized that (3) the short course hypo-fractionated regimen was effective against various tumors by using the advantages of biological dose distribution. In the lung and liver, very short-course irradiation allowing treatment to be completed in 1 or 2 fractions has been made possible. In addition, the short-course therapy that is about half as short as the treatment time of conventional radiotherapy has become possible: e.g., 16-20 fractions/4-5 weeks for tumors of the prostate and uterus and 16 fractions/4 weeks for tumors of the head and neck and bone/soft tissue. For some tumors in the head and neck and pancreas, the prevention and treatment of distant metastasis are important to further improve the survival, and we have just started combination treatment with CIRT and Chemotherapy. It is necessary to further improve the treatment results for malignant glioma, pancreatic cancer, uterine cancer, and esophageal cancer, for which clinical studies will be continued. In the early stages of the clinical trials developing treatments for tumors in the lower abdomen, some patients developed severe intestinal complications at high doses and required surgery, but similar complications were no longer observed after improvement in the irradiation techniques. The number of patients treated with radiotherapy in Japan is about 160,000 per year and is expected to further increase. Since not only the number of patients treated but also the number of patients requiring sophisticated and advanced treatment may increase in the future, it is necessary to secure human resources and a wider range of physical resources to provide radiotherapy of higher quality. In this regard, the expectations for CIRT, promising better outcomes including a high QOL, will be correspondingly higher.

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Synchrotron Trieste, IT
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Georgia Institute of Technology, Atlanta, US
A nanodosimetry-based linear quadratic cell survival model for radiobiology

Molecular and subcellular imaging of radiation events – ST1

Theoretical investigation of the ultrafast dissociation of ionized biomolecules immersed in water: direct and indirect effects

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Theoretical simulations are particularly well suited to investigate, at a molecular level, direct and indirect effects of ionizing radiations in DNA, as in the particular case of irradiation by heavy ions, such as those used in hadron therapy.

We have modelled the production of HO_2 radical in the ion track generated in liquid water irradiated by swift Argon ions with first principle simulations combining Monte Carlo and Car Parrinello Molecular Dynamics simulations [1], and shown a very good agreement with the experimental measurement [2]. In order to complete this study, the early stages of the Coulomb explosion of a doubly ionized water molecule immersed in liquid water has been investigated with Time-Dependent Density Functional Theory molecular dynamics simulations (TD-DFT MD). Our aim was to verify that the double ionization of one target water molecule immersed in liquid water leads to the formation of an atomic oxygen as a direct consequence of the molecule coulomb explosion. To that end, we used TD-DFT MD in which effective molecular orbitals are propagated in time. We have investigated the double ionization of one target water molecule immersed in bulk water following the removal of two electrons from one of its four molecular orbitals, i.e. 1B1, 3A1, 1B2 and 2A1, in turn. We show that the doubly charged water molecule explodes into its three atomic fragments in less than 4 femtoseconds, leading to the formation of one isolated oxygen atom, while we observe also an ultra fast transfer of electron to the ionized molecule in the first femtosecond. A faster dissociation pattern can be observed when the electrons are removed from the most inner shell molecular orbitals [3].

In parallel, we have studied the dissociation of ionized biomolecules in the gas phase and in the liquid phase. We are able to simulate the evolution of chemical primary event, in a few tens of femtoseconds, either when the holes are created in water or in the biomolecule. Some examples are shown in the case of the Uracil molecule, focusing on the role of the surrounding water molecules.

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Molecular and subcellular imaging of radiation events – ST2

Analysis of radiation-induced DNA double-strand break motion and protein exchange at damaged dites after dharged particle irradiation

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Exposure of cell nuclei with charged particles leads to spatially defined damaged chromatin domains. Analysis of track morphology facilitates studies on the dynamics of IRIF associated with lesion processing and repair. Irradiation induces discrete formation of γ-H2AX foci colocalizing with other proteins involved in DNA repair like 53BP1 or RPA. RPA stains only micro-compartments, probably more directly related to the site of ongoing repair. Interestingly, the repair proteins, as well as γ-H2AX can be either detected at chromatin peaks or, more often adjacent to the DNA maxima.

After carbon-ion irradiation, repair of DSBs was evident, indicated by loss of foci at later times. Repair was less pronounced after higher LET. DSBs mobility pointed to a relatively stable positioning of the damaged chromatin domains during repair, especially in human fibroblasts. In a small fraction of HeLa-cells significant changes in the streak patterns during incubation could be observed, suggesting slightly higher mobility during local processing of DSBs in this tumor cell line. To circumvent the limitations of fixed samples, we studied the spatiotemporal organization of DNA damage processing by live cell microscopy analysis. In unirradiated U2OS-cells, a fast confined Brownian-like motion of DNA repair protein foci was observed, which was not altered by radiation. By analyzing the motion of GFP-53BP1 foci in live cells up to 12 hours post-irradiation, we detected an additional slower mobility of damaged chromatin sites showing a mean square displacement of around 0.6 $\mu m^2/h$. This motion is most likely driven by normal diffusion of chromatin. Only occasionally, larger translational motion connected to morphological changes of the nucleus or the formation of repair clusters could be observed.

In addition to the evaluation of motional aspects of DNA DSBs we started to examine the binding characteristics and exchange of repair proteins recruited to the ion traversal induced DNA lesions in relation to the damage density and complexity. To achieve this goal, we developed a fast FRAP setup using a 473nm diode laser. First results will be presented showing a fast exchange of NBS1 and MDC1 at ion induced DSBs, as well as a more tightly bound fraction.

Our data indicate that the repair of high-LET radiation-induced DSBs in mammalian cells is not coupled to an increased motional activity of lesions enhancing the probability of translocations. Long range displacements of DSBs seem not to occur generally during DSB repair. The occasional appearance of cluster formation of radiation-induced foci may represent a higher mobility of chromatin along the ion trajectory. These observations support the hypothesis that spatial proximity of DNA breaks is required for the formation of radiation-induced chromosomal exchanges.

Pre-thermal and thermal radiation processes – ST3

Effects and applications of prehydrated electrons in radiation biology and radiotherapy

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Since the pioneering observation of its existence by Migus et al. in 1987 [A. Migus, Y. Gauduel, J. L. Martin and A. Antonetti, Phys. Rev. Lett. **58**, 1559(1987)], the prehydrated electron has been a fascinating species because of its fundamental importance in radiation chemistry and biology. This species had been studied intensely both in experiments and in theoretical simulations, but its lifetime and physical nature were the subjects of significant controversies until very recently. We found that the controversies are, to a great extent, caused by a coherence "spike" effect on electron hydration dynamics in femtosecond time-resolved measurements. With careful measurements and this spike effect removed, we have recently resolved that the prehydrated states are electronically excited states and have lifetimes of ~200 fs and ~500 fs [Phys. Chem. Chem. Phys. **10**, 4463 (2008)]. Furthermore, we have observed that prehydrated electrons play a very important role in causing biological damage under ionizing radiation, and in activating anticancer drugs used in radiotherapy. In this talk, I'll address these issues.

Pre-thermal and thermal radiation processes – ST4

Spectroscopic and Chemometric Approaches to Radiobiological Analyses

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Vibrational spectroscopy (Raman and FTIR microscpectroscopy) provides a non-invasive acquisition of the complete biochemical fingerprint of the sample. Our current research has developed these techniques with associated analytical methodologies for use in modeling and prediction of radiobiological effects from the direct and bystander irradiation of human keratinocytes (HaCaT). In the direct-irradiation study, human skin cells were exposed to gamma radiation (at ten dose points from 0 Gy to 5 Gy) and assayed using fourier-transform infrared and Raman microspectroscopy, with parallel measurements of physiological function, at times ranging from 6 hours to 96 hours post-irradiation. Further studies were conducted with these analytical techniques (and other unsupervised classification algorithms) to examine their performance in predicting the fate of cellular samples after exposure to bystander medium, with inhibition of bystander effect mediated via the MAP kinase transduction pathway, relative to measurements of apoptosis via flow cytometry. Multivariate regression and analysis was conducted using principal components analysis (PCA), partial least squares modelling (PLS), generalized regression neural networks (GRNN), and support vector machines (SVM), together with heuristic techniques (such as genetic algorithms (GA)), for variable selection and selection of processing parameters. The results demonstrate the power of vibrational spectroscopy in providing non-invasive insights into the molecular processes occurring in the cell as a result of radiobiological damage.

Research interests currently focus on the application of vibrational spectroscopy to the analysis of radiobiological effect, nanotoxicological effects, chemotherapeutic effects, and cytological studies. In radiobiology efforts focus on the development of measurement and analytical methodologies for the modeling and analysis of radiobiological effect. Much of the work focuses on the construction of analytical algorithms that allow the translation of the measurement of the biochemical fingerprint of the sample that the spectroscopy provides into metrics of radiobiological and biological significance, and ultimately the validation of the techniques for application in biological and clinical settings.

Pre-thermal and thermal radiation processes – ST5

Importance of particle track structure in radiation DNA damage calculations

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It is well known that damage to DNA by ionizing radiation is not governed only by the amount of energy deposited, but also by the non-homogeneous spatial distribution of energy by the incident particles. Multiple detailed Monte Carlo codes were developed to model and study radiation action on biomolecules. As detailed simulations of the particle track structure and its consequent development in time and space are computationally expensive, several authors have proposed simplifying procedures based on the assumption that for low-LET radiation the initial spatial distribution of energy is random.

Aim of this work is to show how detailed information about the track structure influences the time-space development of the track and consequent DNA damage in a detailed computer simulation. Detailed structures of tracks of ionizing particles were obtained using TRIOL Monte Carlo code. Time and space development of the tracks in water and the resulting DNA damage were followed using RADAMOL Monte Carlo code. DNA damage was evaluated for a 100 bp long linear double helix segment.

Results of calculations will be presented for selected energies of electrons, protons and alphas as primary ionizing particles. Three approaches will be compared for each particle/energy combination: i) a full simulation with track structure information taken into account, ii) simulation with initial random distribution of radicals, their numbers corresponding to the same deposited energy as within particle track segment (i.e. radical yields after prechemical stage of water radiolysis) and finally iii) simulation using random initial distribution of e_{aq}^-, $H^{\cdot}$ and $OH^{\cdot}$ radical species, their number corresponding to escape yields of radicals from non-homogenous radiation chemistry within track.

Spectra of DNA damage and yield kinetics of radical species will be compared and discussed with regard to the importance of particle track structure.

Induction, amplification of damages – ST6

Enhancing Mechanism of Radiation Effect by Incorporation of Heavy Elements

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Enhancement of radiation effect by heavy atoms has attracted much attention from therapeutic viewpoint. A hypothesis has been proposed that inner shell electrons of heavy elements are excited by energetic electrons produced in the radiation field, leading to Auger effects. Auger effects are known to release many Auger electrons in vicinity of the heavy atoms, causing dense ionization to produce non-reparable clustered damage. Auger effects can be induced efficiently with monochromatic synchrotron X-rays tuning at absorption edges of the elements. We first studied induction of strand breaks in plasmid DNA loaded with chloro-terpyridine-platinum (PtTC) and found that X-ray induced Auger effects at platinum atom showed enhancement in the induction of strand breaks. From study of X-ray energy dependence of the enhancement, it was suggested that Auger effects can also be induced with secondary or photo- electrons in the radiation field. This hypothesis was substantiated with experiments using heavy particle radiation. Participation of OH radicals has also been demonstrated in the enhancement.

Enhancement of biological responses by Auger effects is known to depend upon the localization of the atom in relation to DNA molecule. Due to this reason, chemical nature of the drug containing heavy elements is important from viewpoint of interaction with DNA and of incorporation into living cells. PtTC, which can bind to DNA but not incorporated into cell nucleus, showed some enhancement of cell killing of mammalian cells, which indicates heavy atoms staying in cytoplasm may act as a sensitizer for cell killing and probably for radiotherapy of cancer. Considering above observation we have studied enhancing effect of two heavy element-containing molecules of different chemical nature on the induction of strand breaks in plasmid DNA. One is auro-thioglucose, which is frequently used as a medicine for rheumatism. The other is Pt nanoparticles which have been attracting much attention due to its chemical and biological nature. In order to extract the enhancement by Auger effects, we have irradiated plasmid DNA mixed with above chemicals with soft X-rays corresponding to M-III shell edge using synchrotron radiation as light source at the Photon Factory, Tsukuba, Japan. The results will be compared with those of PtTC and discussed from viewpoint of Auger effects and low energy electrons.

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Induction, amplification of damages – ST7

Physics of ion beam cancer therapy: a multiscale approach

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Ion beams are becoming more commonly used for cancer therapy as a favourable alternative to conventional radiotherapy, using photons. From the physical point of view, their advantages are related to the fundamental difference in the linear energy transfer (LET) by a massive charged particle as compared with massless photons, namely by the presence of a Bragg peak in the depth-dose distribution for ions. It is due to this peak that the effect of irradiation on deep tissue is more localized, thus increasing the efficiency of the treatment and reducing side effects.

The optimization of treatment effects requires understanding of microscopic phenomena, which take place on time scales ranging from 10−22 s to days or even longer times. Many of these processes are not sufficiently studied, also because a high interdisciplinarity is mandatory for any successful approach, but on the other hand a reconstruction of the whole sequence of events explaining, qualitatively and quantitatively, the leading effects on each structural level, presents a formidable task for physics, chemistry, biology, and medicine.

After a fast ion enters the tissue, many processes take place on different temporal and spatial scales until tumour cells die. The goal of our approach is to analyze these processes and identify the main effects which are responsible for the success of the ion-beam therapy. It turns out that many important features should be considered in such an analysis, including the interaction of ions with molecules, transport, thermodynamics, intracellular molecular events, etc.

In the first part of the talk we will introduce the various phenomena defining scales involved in the process, ion stopping, propagation of secondary electrons, and damage to DNA. This approach covers different scales, starting from the large one, defined by the ion stopping. Then we treat every different step with different methods, getting a distribution of secondary species and propagating them in the medium through a random walk distribution and evaluating their impact on a sample piece of DNA. That allows us to get the probabilities of DNA Single and Double Strand Breaks (SSB's and DSB's) as a result of irradiation of tissues with energetic ions, up to 430 MeV/u. Finally we make some estimates of DNA damage caused by ions passing through selected types of realistic cells using the results obtained in the previous steps of our calculations and biological data, achieving a remarkable agreement with experimental data.

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Microbeam radiation – ST8

In vitro models for investigating spatially fractionated irradiation: physics and biological results

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We need more *in vitro* radiobiological models to study the effect of spatially fractionated irradiation on metabolic cell activity. Recent literature describes that ionizing radiation affect the mitochondrial activity, and that sublethal doses of ionizing radiation are associated with increased migratory and invasive capacity. More, the radiobiological response may be affected by intercellular communication. Therefore, the question arises whether the commonly accepted hypothesis that cell response is solely governed by individual cell radiation sensitivity, is still valid in spatially fractionated dose distributions.

We developed three different *in vitro* experimental models which allow us to evaluate the effect of spatially fractionated dose distributions on metabolic activity. For these models, a monolayer of MCF-7/6 human breast cancer cells is irradiated with an x-ray dose gradient. In Model 1, namely the steep dose gradient model, the cells are irradiated with three separate small fields. In the Models 2 and 3, the cell monolayers are irradiated with a smooth dose gradient. In Model 2, the cells are cultured in a T25 flask and irradiated with a smooth dose gradient over the length of the flask, while in Model 3, the cells are cultured in a 96-well plate and also irradiated over the length of the plate. In an attempt to correlate the spatially fractionated dose distributions with metabolic activity we used the MTT assay. In this assay the metabolic activity correlates with the amount of formazan formed after the conversion of MTT by cellular dehydrogenases. The results of the MTT assay obtained with our three different models suggest a dose-specific effect on metabolic activity. An increased formazan optical density is measured for the doses between 1.0 and 4.0 Gy on days 5 to 7 in the steep dose gradient model and for the doses between 4.2 and 6.5 Gy on days 9 to 13, and between 2.3 and 5.1 Gy on days 9 to 11 respectively in the two smooth dose gradient. Altogether, our results suggest that the MTT assay may be used as a radiobiological dose-response meter, measuring the cell's capacity for invasion and metastasis.

Microbeam radiation – ST9

Sequencial ion microirradiation reveals competition effect in DNA damage response

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Microbeam experiments with SNAKE, the Superconducting Nanoscope for Applied Nuclear (=Kern-) Physics Experiments at the Munich 14 MV tandem accelerator, allow targeted application of single ions in distinct patterns to living cells. Here we apply targeted sequential ion microirradiation followed by indirect immunofluorescence techniques to distinguish in cell nuclei between responses to a first and a second irradiation. We show that phospho-ATM, γ-H2AX and MDC1 accumulate and form foci efficiently at both earlier (e) and later (l) microirradiated sites. In contrast, accumulation of 53BP1 and the recombination protein Rad51 were strongly inhibited at l-sites. We called the observed difference in the average signal intensity ratios between the e sites and the l sites competition effect. This competition effect for the two proteins is accompanied by a reduced amount of 53BP1 in undamaged areas of the irradiated nuclei. We suggest that a critically limited pool size combined with strong binding affinity at e-sites lead to the exhaustion of unbound factors freely roaming the nuclear space and consequently to an undersupply of these factors at l-sites. This effect suggests that DNA damage repair at individual nuclear sites depends on the time course of damage load.

Microbeam radiation – ST10

X-ray microbeam dosimetry

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In MRT, micrometer-wide, centimeter-high and vertically-oriented slices of tissue are irradiated by arrays of rectangular x-ray microbeams produced by a multi-slit collimator (MSC). Various dosimeters (GafChromic films, MOSFETs) have been tested for assessing absorbed doses with microscopic spatial resolution in targets irradiated by high-flux synchrotron-generated low-energy (~30-300 keV) x-ray microbeams. Here measured dose distributions, produced by arrays of x-ray microbeams shaped by two different MSCs, in a tissue-equivalent phantom will be presented. The detectors used for the MRT dosimetry have been chosen since they have a radiosensitive volume which is extraordinarily narrow (~ 1µm). Doses have been measured near the center of the arrays and maximum/minimum (peak/valley) dose ratios (PVDRs) have been calculated to determine how variations in heights, widths and in separation of the microbeams influence the PVDRs. Monte Carlo (MC) simulations of the absorbed dose distribution in the phantom have also been performed. The results show that the MC simulations produce estimates of PVDRs that are up to a factor of three higher than the measured values. Sources of discrepancies between measured and simulated dose depositions are discussed, of which the energy dependent response of the detectors is shown to be among the most important; calculated correction factors to take this dependency into account are as high as a factor two.

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Cellular imaging for radiation biology – ST11

Long term cell tracking in small animals using phase contrast based micro CT and synchrotron radiation

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Nowadays the *in-vivo* behavior of cells is not well understood because of the large number of physical and biological parameters that play crucial roles in *in-vivo* experiments. *In-vitro* measurements help the scientific community to better understand the main aspects of the fate of cells, but cannot explain grand parts of phenomena of the *in-vivo* reality. Such ability would enable a better understanding of cancer metastasis, migration of activated leukocytes or pleuripotent stem cells administered into host organism.

Currently there are only scarce methods to locate and track *in-vivo* clusters of cells continually in any given organisms larger than a few millimeters in diameter. To date there are different kinds of imaging techniques relying on specific intracellular markers that can perform cell tracking. To mention are here optical bioluminescence, optical fluorescence, ultrasound, SPECT, PET and MRI, using different detecting principles and different contrast agents. Each of these techniques are characterized by different advantages and disadvantages but so far none of them fulfills what can be defined as the main requests of an ideal cell-tracking tool providing: high spatial resolution (at least about tens of µm), the capability to detect single cells at each anatomic location, non invasive detection of labeled cells, the safety of the host during examination, the utilization of biocompatible contrast agents, long term contrast agent efficiency and insignificant transfer of contrast agent to non–labeled cells.

Elemental gold nano particles feature a number of properties, which render an excellent long-term cellular marker: they are chemically inert, they become sequestered as any non-digestible material into lysosomes non interfering with cellular functions, and because of their high density they can be visualized with x-rays. In this contribution we demonstrate the feasibility to locate and track tumor cells and pleuripotent cells, respectively, laden with gold nano particles and implanted at different anatomical locations in mammals utilizing advanced x-ray imaging modalities. We used micro CT systems based on a synchrotron radiation source and on a micro focus x-ray tube both operating in the edge enhancement regime yielding high-resolution 3D renderings. Cells can be laden with sufficient gold nano particles so that cell cluster comprising about 10 cells can be visualized in both cases.

We conclude our approach will extremely interesting for cell tracking in mammals in near future in the field of cancer research and in the field of cell therapies based on the implantation of pleuripotent cells.

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Cellular imaging for radiation biology – ST12

Dynamics of DSB Related Protein Foci-- a 2D and 3D analysis

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The generation of double strand breaks (DSBs) initiates the cellular response to DNA damage, and in the vicinity of DSBs foci of some activated proteins for DSB recognition and signaling, DNA repair and cell cycle checkpoints are formed. The kinetic and interdependency of these proteins foci are presently not fully understood. With a home developed image processing method we studied the uncovering features of g-H2AX, 53BP1 and NBS1 protein foci as a response to ion irradiation. In this work, we will present the development and morphology change in 2D and 3D of these DSB-related protein foci along the track of carbon ions over a long time period.

Cellular imaging for radiation biology – ST13

53BP1/gamma-H2AX foci do not always co-localize and their complex kinetics may not correlate with DSB repair

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Although it is admitted that the number of 53BP1/gamma-H2AX foci reflect that of radiation-induced DSB, contradictory reports leave open the question of a link between the disappearance of gamma-H2AX foci and DSB repair. We studied dose-responses and kinetics for radiation-induced 53BP1/gamma-H2AX foci formation in relation to their size, co-localization, DSB repair and cell survival. Cell survival, DSB and foci were analyzed by clonogenic assay, pulsed field gel electrophoresis (PFGE), and confocal laser microscopy, respectively, in normal human fibroblasts (VH-10), lymphocytes and in a cancer cell line (HeLa). Computer analysis was used to determine both the number and the area of foci.

We show that even at doses down to 1 cGy a statistically significant induction of 53BP1 foci is observed. Despite apparent nonlinearity at 1 – 5 cGy, the response showed a linear dependence on dose over the range of 1 – 100 cGy.

We did not observe differences in repair of radiation-induced DSB between cell types. However, foci disappeared faster in HeLa than in VH-10. In general, the kinetics of foci disappearance was slower than kinetics of DSB repair. The process of focus formation was significantly inhibited if cells were incubated on ice following irradiation. This significant decrease in foci counts did not correlate with persistent amount of DSB in cells incubated on ice. Altogether, our data are consistent with those studies that report a lack of correlation between gamma-H2AX foci and DSB and suggest that some part of gamma-H2AX foci do not contain DSBs and deal with changes in chromatin structure.

We observed that foci became larger with time after irradiation. Measurements of focus area confirmed this observation. Despite a significant decrease in the number of foci during the interval of 0.5 – 4 h after irradiation, the total area of 53BP1 foci per cell did not change. This result suggests that primary foci may cluster to produce larger secondary foci. Alternatively, some foci may disappear while others grow larger due to re-localization of 53BP1 molecules from the lost foci.

A lower fraction (10 – 30%) of gamma-H2AX foci co-localized with 53BP1 foci at 24 h as compared to 12 h post-irradiation, indicating that co-localization of these foci depends on post-irradiation time. We found that co-localization was also dose-dependent and was minimal in control unirradiated cells. No clear correlations were established between cell survival and foci formation because the dose response for 53BP1/gamma-H2AX foci may depend on time after irradiation and duration of the cell cycle.

Cellular imaging for radiation biology – ST14

Chromosome organization in human epithelial cancer cells

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Chromosomes organization seems to be important for various biological processes and appeared to be involved in the formation of rearrangements often observed in cancer cells. In mammals, chromosomes are organized in territory, i.e. in discrete sub nuclear region in the nucleus. Various studies, mainly carried out in fibroblasts and in lymphocytes, highlighted that these chromosome territories are radially positioned in the nucleus and that their position is correlated with gene density and / or with chromosome size. Here we examine the positioning and the organization of chromosome territories relative to each other in human epithelial cancer cells.

First, using 3D-FISH technique we observed that the chromosomes territories are radially organized relative to the gene density-to-chromosome size ratio in epithelial cancer cells. Moreover we observed an important modification of the radial position of an acrocentric chromosome that has lost its nucleolar organizer region. This leads us to propose that these regions might be important for the radial positioning of some internal chromosomes.

We observed another level of organization between chromosome territories measuring the distance either between their edges or between their gravity centres: a chromosome is generally closer to a heterolog than its homolog. Homologs are maintained distant, probably to disfavour homologous recombination and thus, to avoid potentially deleterious loss of heterozygosity. To determine whether this observation is correlated with a specific organization, we ran mathematical simulations positioning chromosome territories either randomly or with a constraint on their radial position. Preliminary results show that the distribution of distances between homologs is not just dependent on their radial position. Thus other constraints could be implicated in the distance between homologs.

Precise localizations of chromosome territories relative to each other will permit to study their role in the occurrence of radiation-induced rearrangements and long term chromosomal instability.

Cellular imaging for radiation biology – ST15

Evaluation of spatially fractionated radiotherapy (GRID) and definitive chemo-radiotherapy with curative intent for locally advanced squamous cell carcinoma of the head and neck

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Local control of bulky disease from SQCCHN is poor, even when hyperfractionated schemes with or without acceleration is used. Spatially fractionated radiation therapy (GRID) is being used to deliver a high, single fraction radiation dose to these large tumors. Only the volume under open areas of the grid receives irradiation from the primary beam thus creating a peak-and- valley dose distribution. Traditionally GRID therapy has been delivered by the construction of a 7-cm thick Cerrobend block with divergent holes. More recently, the feasibility of delivering GRID therapy using a MLC has been described. Clinical data suggest that GRID treatment can be safely combined with full-dose conventionally fractionated radiation therapy to achieve substantial tumor debulking that may improve local control. In this study, we present 14 subjects with bulky (≥ 6cm) disease from locally-advanced SQCCHN who received GRID therapy to the bulky mass followed by definitive concomitant chemo-radiotherapy using simultaneous-integrated boost (SIB) IMRT.

GRID therapy was delivered by creating a single treatment field with a checker-board pattern composed of open and closed areas using MLC. The open areas and center-to-center separation between open areas was $1cm^2$ and 1 cm at isocenter, respectively. The obtained grid pattern was manually modified to eliminate open areas outside of the GRID-target volume. Grid prescription was 20Gy in one fraction to the maximum dose utilizing 6MV X-rays. Quality assurance analysis was performed in all 14 plans with a difference between calculated and measured dose of less than 5% in all cases. Subjects began chemotherapy and GRID on day 0 with fractionated SIB-IMRT radiotherapy starting on day 1. Thus, from day 1 and on the subjects received definitive chemo-radiation with SIB-IMRT. The SIB-IMRT prescription was 66Gy to the high risk planning target volume (PTV), 60Gy to the intermediate risk PTV and 54Gy to the low risk PTV in 30 equal fractions. Neck dissection (ND) or guided biopsies of the primary tumor were done in 10 of the 14 patients.

Follow-up ranged from 2 to 38 months with a median of 19.5 months. Ten patients had either planned neck dissection or primary tumor biopsies of the GRID area with 8/10 (80%) achieving a pathological complete response. Two patients had a positive neck dissection with only one lymph node containing squamous cell carcinoma in the GRID volume but were clinically with no evidence of disease prior to surgery. Four patients did not have either planned neck dissection or primary tumor biopsies of the GRID area of which 3/4 remain with no evidence of disease in the treatment area at 18, 19, and 33 months with a clinical complete response rate of 3/4 (75%). The fourth, non-compliant, patient with recurrent disease died at 13 months. In these series the most

common skin and mucosal toxicities were RTOG grade 1 and grade 2, respectively.

Ten of 14 patients had follow-up exceeding 12 months. Late toxicity attributed to treatment in these patients was limited to RTOG grade 1 late mucosal toxicity (2 patients) and late skin fibrosis grade 1 (3 patients), grade 2 (3 patients) and grade 3 (1 patient).

GRID followed by chemo+SIB-IMRT is well tolerated in subjects with bulky locally-advanced SQCCHN disease yielding excellent pathological and clinical responses. Toxicity seems similar to that of chemo-radiation without GRID.

Microenvironments and radiation responses – ST16

Activation of DNA double strand break repair after γ-rays is dependent on FGF2 signaling in human keratinocyte stem cells

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We previously reported that keratinocyte progenitors isolated from human epidermis are radiosensitive, whereas keratinocyte stem cells are more radioresistant. In the present study, we addressed the mechanisms of this radioresistance. Gene profiling was performed on keratinocytes sorted on α6 integrin and CD71 labeling and revealed that radiation exposure induced specifically five DNA repair genes in the stem cells, suggesting activated repair in these cells. Thus basal DNA repair was assessed using microarrays designed to determine BER and NER activities. We found that both activities were increased in the stem cells as compared to the progenitors. Then repair of DNA double strand breaks was characterized. Initially, the same number of γH2AX foci per cell was found in both irradiated populations. However, that number decreased much more rapidly in the stem cells. Moreover, at 24 hours, unrepaired foci were found only in the progenitors. Using gene profiling, we also found that radiation exposure induced specifically the FGF2 pathway in the keratinocyte stem cells. To address the possible relationships between autocrine FGF2 and DNA repair, we inhibited this pathway at the level of both the FGF2 receptor and the MAP Kinase MEK1. We show that blocking FGF2 signaling inhibited the rapid decrease of γH2AX foci in the stem cells, suggesting a direct role for this growth factor in DNA repair. In summary, we demonstrate that keratinocyte stem cells exhibit a globally activated DNA repair, and that induction of autocrine FGF2 signalling is critical for the repair of radiation-induced double strand breaks.

Microenvironments and radiation responses – ST17

A nanodosimetry-based linear quadratic cell survival model for radiobiology

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A new nanodosimetry-based linear-quadratic (LQ) model of cell survival for mixed-LET radiations has been developed. The new model employs three physical quantities and three biological quantities. The three physical quantities are related to energy depositions at two nanometer scales, 5 nm and 25 nm. The three biological quantities are related to the lesion production and interaction probabilities and the lesion repair rate. The coefficients α and β of the LQ formula ($\alpha D + \beta D^2$) are explicitly expressed in terms of the three physical quantities and the three biological quantities. The new model is shown to be consistent with the previously published cell survival curves of V-79 cells. The advantage of this new model is that it can be conveniently adopted to estimate the iso-effect for radiotherapy modalities that use ionizing radiation of mixed LET. These modalities include heavy ion therapy, fast neutron therapy, and mixed neutron and gamma-ray brachytherapy of a ^{252}Cf source.

Microenvironments and radiation responses – ST18

Signaling factors and regulation of irradiation induced bystander responses

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Growing effort has been attracted to investigate the mechanisms of radiation-induced bystander response (RIBE) where cells respond to their neighbours being irradiated. Using different irradiation sources including conventional gamma-rays, x-ray microprobe, and particle microbeam, the present study found that bystander responses could be induced by irradiated HepG2 cells (wp53), HSG cells (mp53), and T98G cells (mp53) but not by irradiated PLC/PRF/5 cells (mp53) and Hep3B cells (p53 deficient), and these bystander responses were diminished by either pifithrin-alpha (inhibitor of p53) or cyclosporine A (inhibitor of cytochrome-c release). When the activities of cellular PI3K and MEK1/2 were inhibited by wortmannin and PD98059 respectively, the RIBEs were increased significantly. Accordingly, RIBE could be regulated by different signal pathways. We further found that nitric oxide (NO) was a key bystander signalling factor and the percentage of NO-positive cells exceeded markedly to the small fraction of irradiated cells. This percent of NO-positive cells in the bystander cell population was partly reduced by PFT but unexpectedly it was not increased by wortmannin, indicating other signalling factors being involved in RIBE. In fact, TGF-beta1 was found to be another bystander signal downstream of NO, where TGF-beta1 could be released from irradiated cells and further caused DNA damage to bystander cells, but this RIBE was eliminated by anti-TGF-beta1.

On the other hand, we found that RIBE was regulated by the oxygen environment of cell growing. Under hypoxic condition, both HepG2 and T98G cells became radioresistance but still sensitive to bystander signals so that the contribution of the bystander response to total irradiation damage was greater than that under normoxic condition, which hints that RIBE may have important implication in radiotherapy of hypoxic solid tumour. Further investigation showed that, besides NO, HIF-1 and calcium ion channels were involved in the RIBE of hypoxic cells.

Finally, based on our findings, a crosslink among bystander signals and their regulating pathways was suggested.

Innovating approaches for radiotherapies – ST19

Precise millimeter beam positioning of a conformal radiation therapy system for the optimization and study of spatially fractionated radiotherapy

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We are developing an integrated radiation therapy system for small animal research to optimize and uncover mechanisms of action related to spatially fractionated (GRID) irradiation. Our system is capable of delivering image-guided 3D-conformal radiation therapy or precise patterns of submillimeter beams to address the typical mismatch between clinical, bench-top and animal radiation therapy research. The irradiation system provides precise, accurate, reproducible and quantifiable conformal delivery of radiation dose distributions to organs/tumors. The main components are a Seifert Isovolt Titan 225kV X-ray tube with beam collimating custom accessories and a robotic arm (Adept Viper s650) for precise animal positioning. The system is housed in a custom 6x6x6ft^3 shielded enclosure. The X-ray tube is mounted ~1 m from the floor, which is a primary barrier. The X-ray tube has focal spot sizes of 0.4 and 3 mm, it is mounted on a custom "gantry" and it has a special collimating assembly that allows field sizes with various geometries down to 0.5 mm diameter at ~34 cm SSD (designed at Johns Hopkins University). Current work is focused on characterization of the X-ray beam (at various energies, SSDs and field sizes) and programming of the six-degree-of-freedom robot. Specifically, the system has been found to be useful in experiments to test the cell and tumor biology associated with spatially fractionated radiation therapy (GRID). A 2 mm diameter square X-ray beam applied in a checkerboard GRID pattern was chosen for initial experiments using C57 mice implanted with B16 melanoma tumors on the hind leg. The tumors were irradiated at 225 kV, 13mA for a dose of 10 Gy at a 1 cm depth. Preliminary results demonstrate a high degree of beam conformality as measured by film and also by the detection of DNA damage response with H2Ax immunohistochemistry. The tumor cell survival after a single 10 Gy GRID exposure is reduced by 58%. This data has important mechanistic and practical implications in understanding how to optimize the already promising results we have observed with GRID treatment in combination with standard chemoradiation for head and neck cancer patients at our institution. Supported by Arkansas Biosciences Institute, Central Arkansas Radiation Therapy Institute and grant #CA107160 from the National Cancer Institute of the United States.

Innovating approaches for radiotherapies – ST20

New Opportunities of Near Infrared Radiation for Cancer Diagnosis and Therapy

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Near infrared radiation (NIR) is the most penetrating and least harmful to human tissues. The photon flux density at the Earth surface reaches its maximum also in this region of light (around 700 nm) and the same photons are potentially the most energy-effective for photocatalysis. On the other hand scientist are still blind to NIR opportunities in photomedicine, photodiagnosis etc. This spectroscopic region, in fact, suitable for such purposes as photodynamic methods since these wavelengths are able to penetrate deeply in the human body without interfering or damaging the healthy tissues. Photodynamic therapy (PDT) is a treatment modality for the selective destruction of cancerous and non neoplastic pathologies based on the use of electromagnetic radiation, photosensitizer and molecular oxygen to produce highly reactive oxygen species resulting in necrosis and/or apoptosis of the treated cells, shut down the tumor microvasculature and stimulation of the host immune system. The photosensitizer absorbs light and fluoresces, or reacts with substrate molecules in the tissues by electron or hydrogen transfer reactions, or transfers its energy to ground-state molecular oxygen generating singlet oxygen that attacks the tissues. The transparency of tissues is optimal above 700 nm and the irreversible formation of singlet oxygen requires sensitizers with singlet states below 800 nm. Thus, NIR photons are ideal for PDT and the absorption peak of halogenated bacteriochlorins at 750 nm is ideally suited to maximize both tissue penetration and efficient singlet oxygen generation. With bacteriochlorins it will become possible to treat efficiently larger tumours.

In this communication the usefulness of new class of halogenated bacteriochlorins in the photodynamic therapy of cancer is assessed and recommendations are given for the design of more effective PDT protocols employing such photosensitizers and near infrared radiation. Prior to biological tests (cytotoxicity, cellular uptake, dose-dependent phototoxicity), basic physicochemical characterization of the candidate systems is presented. This includes spectroscopic and photophysical investigations (singlet and triplet lifetimes, quantum yields of fluorescence, triplet state formation and singlet oxygen generation, including the mechanism of energy and/or electron transfer to oxygen or other acceptors excited state lifetimes and deactivation pathways).The crucial step is the efficacy of the treatment, using optimized light doses, concentrations and time of irradiation. Collected data is useful for redesigning the systems to fit to the phototherapeutic window, to increase phototoxic activity and tissue selectivity. Finally, the mechanistic details of excited state deactivation processes can be concluded from these data.

Ion-beam induced damage : Spectra of secondary electrons generated by carbon ions in tissue-like media

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Presently, encouraging results arising from ion beam cancer therapy have triggered a tremendous development of new medical facilities for cancer treatment. On the other side, radiation damage by
ion beams is becoming a major topic of research for shielding of human space missions by ESA and NASA. In fact these ions, not experienced on the earth, are present in the Galactic Cosmic Rays (GCR) and, even with small density, are able to produce tremendous damaging effects to human tissues as their Relative Biological Efficiency (RBE) is huge. Both curing and shielding purposes have the aim to understand and control as much as possible the clustered damage, i.e. the simultaneous occurrence of several damaging events on closeby regions of a DNA molecule, increasing drastically the probability of an irreparable break. More, the two phenomena involve similar initial ion energies (hundreds of MeV).

A key step in this understanding is in the initial abundance and energy distribution of secondary electrons formed in the process of ionization, as it is commonly accepted that they are mostly responsible for DNA damage, either by directly breaking the DNA strands, or by reacting with water molecules producing more secondary electrons and free radicals, which can also damage DNA. These quantities are then used as input for Monte Carlo track structure codes or other diffusion methods, to evaluate the propagation of secondaries and their damaging impact on DNA elements at a given distance from the track.
More, as the ionization of the medium is the major source of energy loss of the ion projectile, this microscopic quantity can be linked to a macroscopic one as the penetration depth, through the stopping of the ion.

We will overview the state of the art in research on ion-beam induced electron production that is mostly abundant in proton beams studies. Then we will focus on an ion that is much relevant both in therapy and space protection issues, C6+, reporting our results: we analysed liquid and vapour water as the tissue like media, with different approaches according to the validity ranges of the Born approximation, and thus the applicability of dielectric response approaches, and availability of experimental data . We produce Singly Differential Ionization Cross Sections (SDCS) that, after integration are consistent with a macroscopic calculation by Monte Carlo transport Code (GEANT4), returning a Bragg Peak position within 1% of deviation, and provide electron spectra for the whole energy range.

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Innovating approaches for radiotherapies – ST22

Time-dependent onset of cellular senescence in response to carbon ions: implications for hadrontherapy

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Stress-induced premature senescence (SIPS) is increasingly attracting interest as a cellular response to generalised sublethal stimuli, which include ionizing radiation (IR). Its timing can be modulated by the nature of the toxic agent while its underlying molecular mechanisms are not clear. Low doses of IR have been shown to induce SIPS in vitro in cell lines such as fibroblasts, this phenomenon being sometimes accompanied by premature differentiation. For the first time, here we demonstrate the occurrence of ectopic cellular senescence induced by varying LET radiation in human umbilical vein endothelial cells (HUVEC) and its correlation with shortening of mean telomere length as assessed by Interphase Quantitative (IQ)-FISH. Carbon ions from the plateau region and from the Spread-Out Bragg Peak (SOBP), of interest for normal tissue and tumour vasculature hadrontherapy effects respectively, were accelerated at GSI (Darmstadt). Clonogenically iso-effective doses of x-rays were used as reference. In our hands, the senescent phenotype was found to occur at early times post irradiation and was efficiently induced by doses as low as 0.1-0.5 Gy of carbon ions from the plateau region of the Bragg curve. A significantly higher fraction of senescing cells compared to sham-irradiated cells was also found to occur following SOBP irradiation despite the incidence of lethal damage being associated with the higher SOBP LET. Elevated frequencies of senescent cells were found to persist at later times in the irradiated progeny compared to unirradiated, physiologically senescing cells. Mean telomere length reduction, a known marker of senescence in HUVEC, was shown to accompany both physiological and radiation-induced senescence. However, telomere shortening was more pronounced after low-LET irradiation, whereas carbon-irradiated senescent cells showed a telomere shortening similar to that measured in controls. This suggests telomere length attrition as a driving molecular mechanism for SIPS after low LET-irradiation but of no apparent relevance for carbon ion-induced senescence. Telomere temporal stability is critical for maintenance of genomic stability and suppression of carcinogenesis. These results will be discussed in relation to their implications for normal tissue adverse effects as well as for desirable tumor vasculature impairment following hadrontherapy.

Posters

Each contribution benefits of 2 minutes Flash Poster Presentation (2 electronic slides).

Posters will be presented on May 17 (Session I) and May 18 (Session II).

***A poster prize** sponsored by EMBO will be awarded during the Conference Dinner*

Poster Session I

1	Abdullaev	Serazhutdin	RU	*Determination of mutations in tissue mitochondrial DNA of X-irradiated mice by means of CEL I nuclease*
2	Anderson	Rhona	UK	*Quantification and determination of size of radiation induced 53BP-1 foci over time in primary human lung cells*
3	Baragiola	Raul Antonio	US	*Radiation chemistry of ices and implications in astrobiology*
4	Benzina	Sami	FR	*Genome-wide screen for kinases involved in phosphorylation of histone H2AX, using siRNA microarrays*
5	Brun	Emilie	FR	*Parameters governing gold nanoparticle X-ray radiosensitization of DNA in solution*
6				*High sensitivituy of human centrin 2 toward radiolytical oxidation*
7	Bug	Marion	DE	*Nanodosimetric modelling of low energy electrons in a magnetic field*
8	Constans	Jean-Marc	FR	*4 year longitudinal MR1 and 1H single voxel MRS follow-up in 25 patients with oligodendoglial tumors or gliomatosis treated with temodal and radiotherapy*
9	De Wagter	Carlos	BE	*Spatio-temporal aspects of current advanced radiotherapy delivery methods and their dosimetric and potential biological implications*
10	Destraze	Maris-Eve	CA	*A time and place for everything mapping radiation-induced interstrand cross-links in DNA*
11	Doucet	Jean	FR	*X-ray synchrotron techniques for cell and tissue imaging: radiation induced skin changes revealed by microfluorescence*
12				*Time-resolved degradation of a hard alpha-keratin tissue irradiated with a high flux X-ray synchrotron microbeam*
13	Fournier	Isabelle	FR	*MALDI-MSI: from developments to clinical applications*
14	Francis	Ziad	FR	*A GEANT 4 based development for microdosimertic studies, adapted for applications on the molecular scale*
15	Granzotto	Adeline	FR	*X-rays-induced bystander effects and hypersensitivity to low doses: late DNA double strand breaks and novel observations with immunofluorescence*
16	Grau	Elena	ES	*Genetic polymorphisms on DNA damage influence the frequency of dicentrics and translocations in interventional radiologist*
17	Hervé du Penhoat	Marie-Anne	FR	*Ultrafast dissociation of a core-ionized biomolecules in liquid water: molecular dynamics simulations*
18	Ivanova	Katia	BG	*The proteasome inhibitor MG132 modifies the early radiation response in human lymphocytes*

19	Joubert	Aurélie	FR	*Radiation-induced DNA damage and signalling specific to neutron radiation*
20	Larivière	Damien	FR	*Realistic 3D modelling of cellular processes: a new approach for studying intracellular organization of integrated biological systems under radiative perturbations*
21	Lopez-Tarifa	Pablo	ES	*Theoretical investigation of the ultrafast dissociation of ionized biomolecules immersed in water: direct and indirect effects*
22	Markova	Eva	SK	*Possible early diagnostics of radiosensitivity and optimisation of radiotherapy using DNA repair foci in lymphocytes of breast cancer patients*

Poster Session II

23	Martin	Michèle	FR	*Analysis of radiation-induced damages on basal keratinocytes of human epidermis using a single cell model reveals heterogeneous functional consequences and acquisition of genomic abnormalities*
24	Moati	Frédérique	FR	*Towards a submicrometric dose determination in radioimmunotherapy*
25	Nicolas	Christophe	FR	*Molecular dynamics and elementary processes involved in damage induced by synchrotron radiation in the soft X-ray regime on biomolecules*
26	Paris	François	FR	*Radiation induced p38-mediated endothelial cell death through ceramide generation and membrane remodelling*
27	Pchelovska	Svitlana	UA	*Assessment of breast feeding risk on child growth and development for the radiation polluted regions of Ukraine*
28	Petrenyov	Danlil	BY	*Delayed macrophage activation as a system response to injure from ionizing and nonionizing radiation*
29	Porcel	Erika	FR	*Effect of platinum nanoparticles in ion induced damages in DNA*
30	Rebière	François	FR	*Radionuclides microdistribution by secondary ion mass spectrometry in a biological matrix after internal contamination*
31	Redon	Christophe Elian	US	*Gamma-H2AX as a biodosimeter for ionizing radiation exposure: an in vivo study with non-human primates*
32	Sabatier	Laure	FR	Telomere maintenance and chromosome instability in human fibroblast and keratinocyte cultures
33	Sedelnikova	Olga	US	*The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks*
34	Stankova	Katya	BG	*The natural product celasrol can modulate the radiation-induced changes in human lymphocytes*

35	Stashkevich	Dzmitry	BY	*Influence of iomozing radiation on hematologic and biochemical indicators of animal blood. Cardiorhythm regulation after radiation treatment*
36	Stypczynska	Agnieszka	UK	*Directly induced damage of biomolecules studied by means of X-ray photoelectron spectroscopy*
37	Vasilyev	Stanislav	RU	*Aneugenic effect of plutonium-239 in somatic cells of nuclear-chemical plant workers*
38	Vavrova	Jirina	CZ	*Response of peripheral blood lympocytes to DNA damage caused by fractionated irradiation in vitro and in vivo*
39	Villagrasa	Carmen	ES	*Low energy electron transport effect in proton track calculations*
40	Wien	Frank	FR	*VUV radiation impact on macromolecules observed with synchrotron radiation circular dichroism (SRCD)*
41	Wysokinski	Tomasz	CA	*05ID-2 beamline-radiation therapy facility at the Canadian light source*
42	Zaidi	Habib	CH	*Comparative methods for 18F-FET-PET guided delineation of target volumes in high grade glioma*
43	Zàrybnickà	Lenka	CZ	*Peripheral blood lymphocytes as biodosimetric marker?*

Poster session I - Poster 1

Determination of mutations in tissue mitochondrial DNA of X-irradiated mice by means of CEL I nuclease

Serazhutdin A. Abdullaev, N. A. Gulyaeva, V. G. Bezlepkin, and A. I. Gaziev

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Mitochondrial DNA (mtDNA) is critical target for genotoxic agents, and mutations of mitochondrial genome have been implicated in etiology of numerous diseases. In some investigations devoted to mtDNA as a more sensitive target for ionizing radiation compared to the nuclear DNA, the frequency of mtDNA deletions and point mutations was suggested as a marker of radiation damage in the cells of irradiated organism. In the present study, using CEL I nuclease which specifically recognizes base pair mismatches, we determined mutations in mtDNA from brain and spleen tissues of mice 8, 14 and 28 days after their exposure to X-radiation at a sublethal dose (5 Gy, dose rate was 1Gy per min). Non irradiated mice were used as a control. The presence of mutations was judged from cleavage by CEL I nuclease of heteroduplexes obtained by hybridization of mtDNA PCR products (fragments of ND3 gene and two D-loop regions of different sizes), from tissues of exposed and control mice. Simultaneously, the amount of mtDNA copies in mouse tissue was determined relative to the nuclear gene (beta-actin). The results show that heteroduplexes of PCR products of tissue mtDNA from one irradiated and one unirradiated mice were more prone to degradation by CEL I nuclease than those of mtDNA from two unirradiated (control) mice. A maximum degradation of heteroduplexes by CEL I nuclease was observed on day 8 after irradiation of mice. For heteroduplexes of mtDNA isolated on days 14 and 28 after irradiation, the cleavage by CEL I nuclease was significantly less. This was equally typical for heteroduplexes of both the ND3 gene products and the hypervariable sequences of the D-loop region (D-loop1 and D-loop2). The results point to formation of mutations in tissue mtDNA of irradiated mice during the first 8 days after exposure and to a decrease in mutant mtDNA copies in the same tissues in the subsequent days of the post-radiation period. Analysis also showed that the level of mutant mtDNA copies in brain tissue (14 and 28 days of post-radiation period) remained markedly higher than in the spleen. The total mtDNA copy number for both brain and spleen tissues of mice 8 to 28 days after their irradiation was reduced by 30-40% compared to the control. The results permit the suggestion that mutant mtDNA copies are eliminated from the tissues of irradiated animals in the post-radiation period. This process occurs more actively in the mitotically active tissue (spleen). The elimination observed can be considered as a result of both the selective degradation (“mitoptosis”) of mitochondria carrying mutant DNA copies and the radiation-induced death of cells.

Poster session I - Poster 2

Quantification and determination of size of radiation induced 53BP1 foci over time in primary human lung cells

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Ionising radiation (IR) is known to induce DNA double strand breaks (DSBs). Cellular mechanisms sense this damage and initiate a number of downstream responses including the recruitment of proteins associated with DSB repair. These can be visualised by immunofluorescence (IF) techniques and thereby provide a mechanism to directly visualise DSB within interphase nuclei. A number of studies have demonstrated the appearance and decline of radiation-induced foci (RIF) to be associated with the induction and repair of DSB, using 2-dimensional (2D) analysis. By increasing the resolution to 3D, information may also be gained on the relative volume of foci, proximity to each other or other nuclear structures and, whether this alters with time after IR. If observed, this could be suggestive of possible movement of DSBs for repair. To assess this, we are irradiating human bronchial epithelial (HBEp) cells with either ^{60}Co γ-rays ^{238}Pu high-linear energy transfer -particles. HBEp cells are fixed in 4% paraformaldehyde at various time points between 0 and 24 hrs after IR and DSB detected by immunofluorescence using anti-53BP1 monoclonal antibodies. We are analysing RIF by acquiring images in 2D and 3D using a Zeiss 200M widefield fluorescence microscope driven by AxioVision software. RIF are scored and categorised according to size (<0.5μm, ≥0.5μm to <1.0μm and ≥1.0μm). Preliminary 3D data shows the induction of ~20 RIF/nucleus within 6 minutes after exposure to 2 Gy ^{60}Co γ-rays. This level reduces to ~7 RIF/nucleus after 2 hrs, reaching sham levels of ~2 RIF/nucleus 16hrs post IR. Comparative 2D and 3D data for γ-rays will be presented.

Poster session I - Poster 3

Radiation chemistry of ices and implications in astrobiology

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Our laboratory studies the effects of ionizing radiation on condensed gas solids, including water, H_2O_2, NH_3, CO, and CO_2, using determined by Fourier Transform Infrared Spectroscopy, microgravimetry and mass spectrometry. The experiments are designed to measure the irradiation fluence dependence of the formation of radicals and subsequent chemical reactions. The use of low temperatures allow the slowing down of the reactions, and the use of amorphous solids simulate radiation processes in liquids, such as those of importance in biology. I will discus the applications to questions of astrobiology, particularly at the icy satellites Europa and Enceladus.

Poster session I - Poster 4

Genome-wide screen for kinases involved in phosphorylation of histone H2AX, using siRNA microarrays

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The introduction of Double-strand-breaks (DSBs) into DNA leads to a complex set of responses in eukaryotic cells. One of the earliest consequences in the cellular response to DSB is the phosphorylation of histone H2AX to form nuclear foci of phosphorylated form of H2AX (γH2AX) in the chromatin adjacent to DSBs sites. Following generation of DSBs, a rapid kinase based signalling pathway is activated that coordinates DNA repair with the induction of cell-cycle checkpoints. To date, two kinases, ATM and DNA-PK have been shown to phosphorylate histone H2AX in response to irradiation. However, molecular actors and mechanisms participating in the recruitment of γ-H2AX at foci after irradiation are still largely unknown.

To characterize all human kinases involved in H2AX phosphorylation, we have developed siRNA (small interfering RNA) microarrays to reverse transfect thousands of siRNA simultaneously into a human keratinocyte cell line (HaCaT). After transfection, microarrays were exposed to 2Gy ionizing radiations and foci formation was automatically analysed by immunofluorecence microscopy. We have screened 1296 siRNA targeting 648 human kinases. We have found 43 kinases involved in H2AX phosphorylation which unravel new signaling pathway in response to DNA damage in human.

Poster session I - Poster 5

Parameters governing gold nanoparticle X-ray radiosensitization of DNA in solution

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Radiotherapy is a prominent tool in oncology but it can be held responsible for important biological damages as ionizing radiations also induce degradation of healthy tissues. It is the reason why since more than five decades, great efforts have been devoted to increase its efficiency and tolerance. Different approaches have been developed to increase the dose to cancerous cells specifically. Herold and co-workers first showed that gold microspheres, suspended in cell cultures or distributed in tumour tissues, can produce an increased biologically effective dose when exposed to kilovoltage photon beams (1). Later, Hainfeld *et al.* demonstrated that gold nanoparticles (GNP) injected intravenously to mice bearing subcutaneous EMT-6 mammary carcinomas enhance 250 kVp X-rays radiotherapy (2), an experiment which was confirmed by theoretical studies (3). However, to optimize this treatment, a more complete understanding of the mechanisms responsible for such a radio-sensitization is necessary. For this reason, we studied the effect of X-rays on biologically relevant molecules (DNA, proteins) in the presence of GNPs.

Here, we present the results of an investigation of three key-parameters governing such radiosensitization in DNA, namely, DNA: GNP molar ratio, GNP diameter and incident X-ray energy. We performed irradiations with a clinical orthovoltage source and tested concentration ratios up to 1:1, five sizes of GNP from 8 to 92 nm and six effective X-ray energies from 14.8 to 70 keV. The most efficient parameters are found to be large-sized GNP, high molar concentration and 50-keV photons, which could potentially result in a dose enhancement factor of 6. The relevance of such parameters as regards the development of future therapeutic applications is discussed. To the best of our knowledge, this study constitutes the first report of systematic data on radiosensitization by GNP.

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Poster session I - Poster 6

High sensitivity of human centrin 2 toward radiolytical oxidation

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presented by **Emilie Brun**

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Centrins are calcium-binding proteins that play a significant role in the maintenance of the centrosomal organization, mainly in the continuity between centrosome and microtubular network. Recent data showed that centrosome duplication abnormalities, like overduplication for example, could be due to hydrogen peroxide, suggesting an important impact of oxidative stress. To challenge this hypothesis, we performed one-electron oxidation experiments with human centrin 2, with hydroxyl and azide radicals. Our results first revealed several intermolecular cross-links generating
dimers, trimers, tetramers and higher molecular mass species. Dimers result from the formation of a covalent bond linking the C-terminal tyrosines of each monomer. Second, the methionyl residue at position 19 was oxidized on the monomeric centrin.. Overall, these results show that centrin 2 is highly sensitive to ionizing radiation, which could have important consequences on its biological functions.

Poster session I - Poster 7

Nanodosimetric modelling of low energy electrons in a magnetic field

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The greatest amount of absorbed dose in tissue, deposited by any kind of ionising radiation, is due to low energy electrons with energies of a few eV. The presence of a magnetic field is assumed to modify the spatial distribution (track structure) of those electrons, without affecting the absorbed dose as a macroscopic quantity. This may result in a larger amount of events within nanometric segments of the DNA-molecule, leading to more complex DNA strand breaks and therefore to a higher relative biological effectiveness (RBE) of the radiation.

A Geant4 Monte Carlo simulation was performed to investigate the effect of a magnetic field on the track structure of low energy electrons. The model geometry of the simulation consisted of a liquid water cylinder of nanometric size as simplified, but accurate enough representation of a DNA-segment. An electron beam of monoenergetic electrons (50 eV - 10 keV) entered the water cylinder perpendicularly at half its height. The probability distribution of ionization events (cluster size distribution) with corresponding energy deposited within the volume was calculated.

The Low-energy electron transport was modelled by the recently released Geant4-'DNA' extension down to 12.61 eV. Geant4 capability in nanodosimetry was assessed by comparing Geant4 to a well established Monte Carlo code, specific for nanodosimetry, developed at the Physikalisch-Technische Bundesanstalt.
A uniform magnetic field between 1 and 14 T was applied perpendicularly to the incident particle beam and also to the cylinder's main axis. The results obtained by the Geant4 simulation suggest that irradiation in a magnetic field does not change the track structure of low energy electrons significantly. However, the Monte Carlo simulation contains uncertainties, most importantly the cross sections, which cannot be determined experimentally for nanometrical volumes of liquid water.

Experimentally, a preliminary investigation on biological objects has supported the theory of an RBE enhancement in a magnetic field of 0.8 - 1.5 T. However, this effect could as well be due to other reasons, such as the extended lifetime of free radicals, increasing the probability of indirect biological effects. Furthermore, some studies show an influence of biological cell structure and chemical cell parameters by a magnetic field, altering also the probability of complex DNA strand breaks. The experimental and theoretical research on this topic will be continued.

Poster session I - Poster 8

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4 years longitudinal MRI and 1H single voxel MRS follow-up in 25 patients with oligodendroglial tumors of gliomatosis treated with temodial and radiotherapy

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Purpose: to better understand human glial tumor metabolism and post-chemotherapy and post radiotherapy in vivo spectroscopic biomarkers variations. To determine cerebral variation in MRS area, amplitude, and ratios of metabolites and spectral profiles during a 4 year longitudinal follow-up in 25 patients with oligodendroglial tumors (14) or gliomatosis (11) without initial hyperperfusion treated with Temodal and radiotherapy and to detect differences in infiltration or proliferation.

Methods: MRI: Sagittal T1, axial proton density, T2, FLAIR, diffusion, 3D T1 3 planes after gadolinium. MRS : 1H, single voxel (6 to 12 cm3), PRESS with multiple TEs on a 1.5 T MRI. Data processing : SA/GE software and home-written automated processing (SCI-MRS-LAB in Scilab cINRIA-ENPC code) yielding amplitudes, areas, ratios, and relative concentrations. Statistical analysis of longitudinal spectroscopic data (every 3 months over 48 months).

Results: quantitative studies in MRI with multi-spectral segmentation and tissular classification are ongoing. Without chemotherapy or radiotherapy spectroscopic profiles worsen with increases in Choline/N-Acetyl-Aspartate (Cho/NAA), Cho/Cr and Myoinositol/Creatine (mI/Cr) ratios, decreases in NAA/Cr and sometimes with increases in lactate. After chemotherapy, treated tumoral volumes, in MRI, change little between two exams while spectroscopic profiles and ratios do change. MRS could, in fact, be more sensitive than MRI and could, in some cases, be predictive of worsening. Cho concentration could be predictive and more sensitive than ratios. Cho concentration increased in 3 patients with aggravation later in 2 gliomatosis and one oligodendroglioma allowing early start of radiotherapy There was also a decreased Cho concentration before clinical improvement. Therefore, MRS could be more sensitive and detect changes earlier than MRI and sometimes is predictive.

Discussion and Conclusion : MRI remained stable for all patients, except for two late partial responses. MRS showed variable ratios of mI/Cr, Cho/Cr and NAA/Cr at baseline. We observed a decrease in Cho/Cr ratio and an increase in NAA/Cr ratio in patients whose clinical condition improved and opposite results for those whose conditions deteriorated. These spectroscopic changes occurred well before clinical deterioration and just before improvement. MRS allows non-invasive follow-up of treated cerebral tumors. There is a large variability, but repetition and modelization of spectroscopic measurements during follow-up could allow us to decrease this and

to improve prognostic evaluation.
Studying the relationship between MRS and PET measurements, segmentation and perfusion parameters could lead to a better understanding of therapeutic response, especially with regard to chemotherapy and radiotherapy, and in the future antiangiogenic molecules.

Poster session I - Poster 9

Spatio-temporal aspects of current advanced radiotherapy delivery methods and their dosimetric and potential biological implications

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From the point of view of the dosimeter sensor or the individual biological cell, current advanced radiotherapy delivers the dose in a temporally irregular way.

Examples will be given to demonstrate that a same dose distribution may be achieved in a multitude of ways that imply totally different dose histories for the dosimeter and the biological cell.

Poster session I - Poster 10

A time and a place for everything: mapping radiation-induced interstrand cross-links in DNA

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Interstrand cross-links (ICLs) impede critical cellular processes such as transcription and replication and are considered to be one of the most toxic types of DNA damage. Both natural products (psoralens) as well as several agents used in chemotherapy (such as carboplatin and Mitomycin C) induce ICLs. However, there is still very little information regarding the chemistry of ICL formation in cells. Recently, we reported the formation of ICLs by γ-rays that were specific for mismatched nucleotides within bromodeoxyuridine (BrdU) substituted DNA. Furthermore, when the irradiation products of various mismatch sequences were analyzed by gel electrophoresis, several distinct band patterns were observed. This suggests that multiple DNA cross-links could arise from a single radical, depending on the nature of the surrounding nucleotides. In order to probe the mechanism of formation of these ICLs, we used a modified version of the method developed by Hopkins and coworkers in 1991 to locate the cross-linking sites. We purified the ICLs by gel electrophoresis and then subjected them to mild acid hydrolysis to reveal the sites of modification, instead of using random cleavage by hydroxyl radicals as in the method of Hopkins. This modification of the mapping technique allows the direct localization and relative quantification of the damaged sites, using very small amounts of starting material (100 - 300 ng of purified ICLs). Using this method, we compared the pattern of cross-linked structures obtained from ionizing radiation with those obtained from UV light (313 nm) as a function of DNA sequence. In the case of ionizing radiation, γ-induced ICLs extend as far as four DNA bases from the site of BrdU substitution. This damage is more pronounced on the 5′-side of BrdU and occurs on both the brominated and the opposite strands. In contrast, UV-induced ICLs arise almost exclusively at the site of the BrdU, and the major target is the base on the opposite strand immediately facing BrdU. Thus, using this assay, we were able to resolve multiple cross-link structures, as well as to gain a better understanding of the chemistry of interstrand cross-link formation in BrdU-substituted DNA.

Poster session I - Poster 11

X-ray synchrotron techniques for cell and tissue imaging: radiation induced skin changes revealed by microfluorescence

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presented by Jean Doucet
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New micro-imaging techniques take advantage of the unique characteristics of synchrotron radiation beams: submicrometer resolution and high intensity. Among these techniques infrared microspectroscopy, X-ray microdiffraction and X-ray microfluorescence (XRµF) can be performed on biological samples under conditions close to the natural physiological environment without any sample preparation, providing highly selective chemical or molecular structure information. The capabilities of XRµF will be illustrated by the analysis of the distribution of chemical elements across a full thickness human epidermis and its modifications after submission to ionizing radiations.

Changes in the skin ionic concentrations can induce perturbations in its barrier function. A deeper understanding of the processes involving metals in epidermis requires a precise knowledge of their state and distribution inside each epidermis layer. Numerous experimental techniques allow chemical elements detection but they present various limitations. XRµF offers a particularly powerful combination of capabilities in terms of resolution, sensitivity, penetration and multi-elemental analysis. Surprisingly, probably because XRµF technique is only available at a few synchrotron centres, no high resolution 2-d mapping of elements in epidermis cuts has been yet obtained.

Using XRµF at ESRF beamline ID21, we got insight into chemical elements distributions, including Fe, Ca, K, P and S, in normal human epidermis. These distributions were significantly different in the various layers. Importantly, in the living part of epidermis, the analysis revealed a preferential localisation of iron and sulphur within the basal layer (the deepest layer which contains the keratinocyte stem cells). Thanks to the high spatial resolution, it was possible to evidence high extra-cellular sulphur and iron amounts in the basal layer. Then we addressed whether the acute effects of ionizing radiation could be detected and quantified. Both a high dose (10 Gy, ^{60}Co) and a clinically relevant dose (2 Gy) of γ-Rays induced within 24 hours alterations that were detected from the elemental contents and their spatial distribution. The most significant difference was a decrease in iron content in the basal layer, without any increase in the upper layers of epidermis, which could be detected as soon as 6 hours after exposure. In conlusion, XRµF is a suitable method to detect acute effects of radiation exposure in the most critical layer of human epidermis.

Poster session I - Poster 12

Time-resolved degradation of a hard alpha-keratin tissue irradiated with a high flux X-ray synchrotron microbeam

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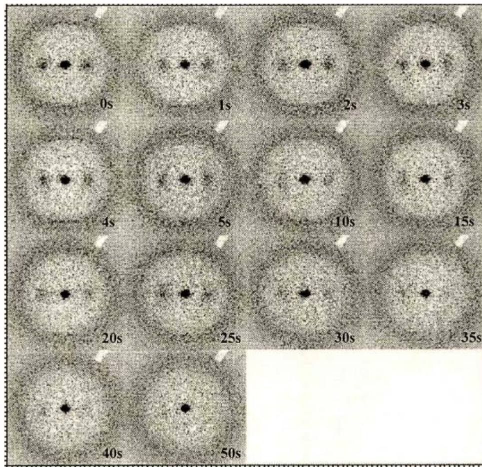
X-rays interact strongly with biological organisms. For radiotherapy applications most studies of the radiation damages have been focused up to now on DNA alterations. New synchrotron radiation sources deliver very intense photon fluxes in the X-ray range within micro- or submicro cross-section beams, resulting in doses million times higher than the medical ones. The relevance of synchrotron radiation analyses of biological materials is therefore questionable since such doses can cause huge damages, not only on DNA but also on proteins and lipids. Very little data on this topic have been published in litterature.

We have analysed the structural phenomena which occur when a model tissue (human hair) is irradiated by a synchrotron X-ray micro-beam. The choice of hair is supported by its hierarchical and partially ordered keratin structure which gives rise to various X-ray diffraction features. The three structural hierarchical levels that characterize hard alpha-keratin architecture in hair and give it its exceptional mechanical properties can be summed up as follows. At high resolution, two 450 Å long chains are assembled into dimeric molecule with a rod-like central part, composed of alpha helical coiled-coils and two globular C- and N-terminal domains. This regular coiled-coil packing gives rise to the wide-angle X-ray scattering (WAXS) meridian arc located in the 5 Å region. The coiled coil packing is at the origin of a diffuse equatorial peak in the 9.5 Å region. At medium resolution the molecules are assembled both longitudinally and laterally, forming long cylinder-shaped filaments called intermediate filaments (IF). The strong 67 Å meridional reflection arises from an axial stagger between molecules along the IF which is stabilized by interactions between the terminal ends located at the surface of the IFs and extrafibrillar proteins; in particular through disulfide bond linkage. At low resolution, bundles of IFs are embedded into a sulphur-protein rich matrix of extrafibrillar proteins, called keratin associated proteins (KAPs). The IFs are located at the nodes of a distorted hexagonal two-dimensional array, giving rise to a strong equatorial reflection observed around 90 Å.

To assess the damages caused by hard X-ray micro-beams (1 μm^2 cross-section), 1 s long exposure time scattering SAXS/WAXS patterns have been recorded at beamline ID13 (ESRF) after various irradiation time (ranging from 0 to 50 s) corresponding to doses of the order of 10^6 Gy/s. Various modifications of the scattering patterns are observed, they provide fine insight of the radiation damages at the various hierarchical levels. It appears that the high resolution structural level, i.e. the alpha helices which are stabilised by hydrogen bonds and the alpha-helical coiled coils which are stabilised by hydrophobic interactions, is more sensitive to radiation than

the low resolution level, i.e. the global architecture of the keratin filament and the filament packing within the KAPs matrix which is stabilized by disulphide bonds.



Series of WAXS patterns of human hair collected after irradiation times (from 0s to 50s) using a $1 \mu m^2$ *diameter beam.*
The X-rays beam is perpendicular to the hair axis which is vertical

Poster session I - Poster 13

MALDI-MSI: from developments to clinical applications

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Elucidating changes at the proteome level for better understanding of cell signalisation pathways modifications in abnormal cells is a complex task that requires the development and use of dedicated and new tools. In this respect, MALDI Imaging has shown growing interest for proteomics applications in biology and clinical fields by allowing pathological biomarkers to be discovered and their distribution to be followed directly from tumour tissues. On tissue proteomics is a highly interesting approach for biomarkers hunting and has shown to avoid several of the problems classically encountered with fluids proteomic. However, even if the continuously growing number of publications of MALDI imaging applications to pathologies, methodology strategies still require to be improved.

A first part of developments concern MALDI imaging of Formalin-Fixed and Paraffin-Embedded Tissues. Such samples are highly advantageous for pathologists since they do present a great stability in time. Although, such samples are not well adapted for MALDI experiments. We have studied new methodological strategies for retrieving information and imaging old FFPE samples. *In situ* controlled enzymatic digestion of tissues has proved to be the easiest solution to image and get structural information on peptides and proteins from FFPE samples.
In situ chemical derivatization of peptides obtained after enzymatic digestions were also studied. Derivatizations have shown to be very helpful for peptides/proteins identification by either allowing de novo sequencing or leading to high increase in identification score after databanks interrogation.

On the other hand, we have also developed a new concept for both using MALDI imaging as a validation tool in clinical researches as well as broadening the range of analysable molecules. This so-called specific imaging is a targeted methodology for specifically tracking a probe of interest. We have developed new types of reporters adapted to MS detection that can be combined with different types of probes such as antibodies, oligonucleotides or lectin probes by constructing reporters including photocleavable moieties. By combining our specifically tagged probes with different hybridization techniques we can now image antigens, mRNA and glycoprotein's by imaging of the reporter peptide released during MALDI experiments under laser irradiation. This makes MALDI Imaging becoming an interesting tool for biomarkers validation with possible correlation at the transcriptome/proteome level.

These developments were used in several applications including ovarian cancer and animal models for Parkinson for biomarkers hunting and validation. This proves that MALDI imaging has now found is way to be considered as an efficient tool for pathological proteomics.

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Poster session I – Poster 14

A GEANT4 based development for microdosimetric studies adapted for applications on the molecular scale

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Noticeable progress has been made lately in particle track simulation domain, through the development of many Monte-Carlo codes. However on the micrometric or even the nanometric scale these “classic pattern” codes (MCNP, GEANT4…) become unreliable and one needs specific codes dedicated for microdosimetric studies. This work was done in the frame of the GEANT4LowEnergy collaboration group. Based on the First Born Approximation and papers published by D. Emfietzoglou and M. Dingfelder, we developed several cross section table sets concerning low energy electrons (starting from 7 eV till 10 keV), fast protons (energies from 100 eV till 100 MeV) and alpha particles (100 eV till 10 MeV). We used the GEANT4 Monte-Carlo open source code as a base; the cross sections were integrated in the code as new physics processes. The resulting toolkit allows us to simulate track structures for ionising particles in water, taking into account the inelastic interactions with the water molecules (5 excitation states and 5 ionisation states). Simulations were carried out calculating the proximity functions for electrons and protons in liquid water, results were compared with data from Kellerer et al.

Proximity functions tend to give a fairly good description of the specific energies distribution inside the cell nucleus, thus revealing the concentration of the considered lethal energy depositions inside the sensitive biological volume. Several sublethal events, created at a certain distance limit, can combine together to form a lethal event. It becomes possible, taking into account the proximity functions of two or more radiation types, to compare these radiations and deduce their respective relative biological effect (RBE). An example is presented in this study for melanoma radio-resistant cells irradiated with 14 MeV neutrons (at 5 cGy / hour) at the LPC Clermont-Ferrand. The simulation of the experiment was done using GEANT4 and the especially dedicated processes, concentrating mainly on recoil protons (~80 % of recoils are protons) and secondary electrons.

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Poster session I - Poster 15

X-rays-induced bystander effects and hypersensitivity to low doses : late DNA double-strand breaks and novel observations with immunofluorescence

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Radiation-induced bystander effects (RIBEs) describe biological events occurring at the vicinity of irradiated cells. Various experimental procedures were used to investigate RIBEs. Interestingly, most of microirradiation experiments have been performed with alpha-particles whereas media transfers have been done with X-rays. To propose a unified model for RIBEs, we used X-rays synchrotron radiation making possible both micro-tracks and irradiated media transfers. X-rays micro-tracks and media transfers were found to produce late DNA double-strand breaks (DSBs) recognized by both pH2AX and MRE11 immunofluorescence with an intriguing temporal coincidence (most DSBs appear 4 h after irradiation). From these experiments, three major observations have been made: 1) the use of media rich in phosphates like PBS inhibited the formation of such damage while the use of media poor in calcium increases the production of RIBE-induced damage. A mechanistic model involving radiation-induced free Ca2+ ions release is therefore proposed to explain the intensity of RIBEs. 2) Cells concerned by RIBEs show ``tiny`` pH2AX foci whose pattern changes drastically with the ``standard`` pH2AX foci observed in cells that are irradiated directly. Such foci pattern was also encountered in cells treated with UV, heavy metals or from certain genetic diseases. After further investigations about the impact of chromatin decondensation on the foci pattern, a mechanistic model is proposed to explain the consequences of RIBEs on chromatin and the potential artefacts when using immunofluorescence. 3) Cells concerned by RIBEs show a low but significant number of DSBs as observed in cells irradiated at low doses of radiation (1mGy - 50 cGy). Interestingly, the DSB repair rate of cells irradiated at such doses depends clearly on genetic status and notably on their radiosensitivity at high doses. A model is proposed to predict the biological consequences of RIBEs on cells from different radiosensitivity.

Poster session I - Poster 16

Genetic polymorphisms on DNA damage influence the frequency of dicentrics and translocations in interventional radiologist workers

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Cytogenetic dose estimation based on analysis of dicentric chromosomes and translocations is used to assess acute doses and evaluate protracted doses like those received occupationally. Recently the study of polymorphisms in DNA repair genes or genes involved in folate metabolism has gained importance due to their association with high levels chromosomal aberrations. The aim was perform a cytogenetic and molecular genetic study in workers exposed to ionizing radiation to identify the putative received dose.

Blood samples belonged to 9 exposed workers to X radiation. Blood samples from voluntary donors with no history of radiation exposure were used as negative controls.In the cytogenetic study, chromosome aberrations were detected by fluorescence plus Giemsa staining and by FISH. Dose estimates were obtained by extrapolating the yield of dicentrics and translocations to their respective dose-effect curves. Genotypes analysis of polymorphisms *hOGG*^{326}, *XRCC1*^{194}, *XRCC1*^{399}, *XRCC1*^{280}, *XRCC3*^{241}, *MTHFR*^{677}, *MS*^{2756}, *XPD*^{312} and *XPD*^{751} was performed as described in original articles.

Our sample series displayed higher frequency of chromosomal alterations than background level. In five individuals the estimated doses by translocations were higher than those estimated by dicentrics. In 4 individuals no difference between doses estimated by dicentrics and translocations was observed. In genotype analysis we obtained genotypic frequencies for each gene according to literature except for *hOGG*^{326}, *XRCC3*^{241} and *MTHFR*^{677}, where there was an increment in the heterozygous and mutant homozygous variant. Samples group showed higher frequency of dicentrics and translocations than background level, but we found discrepancies in the physically recorded doses respect to the cytogenetic findings. In five cases the dose estimated by translocations was higher than by dicentrics, indicating that the overexposure is not recent. The high dicentrics rate in three samples suggests a non-uniform exposure.

The discrepancies observed between the physical recorded dose and the biologically estimated doses are probably due to these cases did not always wear their dosimeters or that in most cases the dosimeter was not in the radiation field.Regarding to genotype frequencies observed and focusing in the 3 cases (4, 7 and 8) with the highest physical and estimated dose, all of them showed Val/Val *MTHFR*^{677} as well as Thr/Met *XRCC3*^{241}, both of them associated high levels of chromosome breaks. The presence of those polymorphisms would influence in the high rate of chromosomal aberrations found in these cases with increased exposures.

Poster session I - Poster 17

Ultrafast dissociation of a core-ionized biomolecules in liquid water: molecular dynamics simulations

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Chetioui et al. proposed that inner shells ionizations in DNA constituent atoms could be partly responsible for cellular inactivation upon heavy ion irradiation [1]. Core-hole states have a strongly dissociative character. In particular, they may induce ultra-fast dissociation reactions in which the nuclear dynamics takes place on the same time scale of the lifetime of the core vacancies. Moreover, in the relaxation process following the inner-shell ionization events, Auger transition probabilities are nearly 100% in DNA constituent atoms, leading to the multi-ionization of the atom and to the emission of two low-energy electrons, the Auger and secondary electrons. These electrons can, in turn, locally damage DNA *via* interactions directly within DNA (direct effect) or indirectly through reactions with radicals produced in the surrounding free water molecules (indirect effect). This whole set of events could induce complex DNA damage.

Experiments have aimed at investigating specific fragmentation resulting from core hole vacancies in thin films of DNA components [2]. In the condensed phase, it proved difficult to distinguish experimentally damage to DNA resulting from the core ionized molecule from that due to the secondary electrons. We have thus recently started to investigate theoretically the fragmentation dynamics of single core ionized biomolecules (such as water or DNA bases) in liquid water within the framework of ab initio Density Functional Theory-based Molecular Dynamics simulations [3].

Our results predict that the dissociation of core-ionized water molecules may be reached during the lifetime of inner-shell hole (about 5fs). Indeed, our calculations lead to neutral OH as primary outcomes. We also observe a second fragmentation channel in which total Coulomb explosion of the ionized molecule occurs. Preliminary results on the uracil molecule are also presented.

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Poster session I - Poster 18

The proteasome inhibitor MG132 modifies the early radiation response in human lymphocytes

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Regulated proteolysis plays important role in cellular physiology. The inhibition of the ubiqutin proteasome system concerns several essential cellular functions such as gene transcription, cell cycle regulation, stress response, cellular differentiation and DNA repair. It creates diverse biological effects in normal and tumor cells. Proteasome inhibitors are novel efficient antitumor agents which induce apoptosis in the transformed cells. They are also valuable new tools for evaluation of the cellular stress response caused by different stimuli, including ionizing radiation.

The aim of the present study is to characterize the effects of the proteasome inhibitor MG132 at two different concentrations (100nM and 10μM) on the early radiation response in human lymphocytes by analyzing their capacity to repair radiation-induced double strand breaks in DNA and changes in their enzymatic antioxidant defense system.

We found lower levels of DNA double strand breaks in pretreated with MG132 and subsequently irradiated cells when compared to the irradiated cells. These effects were exerted in the dose range of 1 to 8Gy by MG132 at 100nM and at doses higher than 2Gy by MG132 at 10μM, suggesting necessity of threshold for activation of the cellular repair systems.

The antioxidant status of lymphocytes after their treatment with MG132 followed by irradiation was analyzed by changes in the levels of three antioxidant enzymes: catalase, manganese super oxide dismutase (Mn-SOD) and glutathione-S-transferase (GST). We found increased expression of MnSOD at both concentrations of MG132 after irradiation in the dose range from 1 to 8Gy. The expression profiles of GST and catalase were more complex and were dependent on both, MG132 concentration and the irradiation dose.

Summarized, the results of the present study showed that the proteasome inhibitor MG132 can modify early radiation response increasing both, the repair capacity of lymphocytes and gene expressions of the antioxidant enzymes Mn-SOD, GST and catalase which might be of interest for radioprotection and radiotherapy.

Poster session I - Poster 19

Radiation-induced DNA damage ans signaling specific to neutron radiation

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Neutrons irradiation results in the production of various secondary particles such as protons, alpha-particles, electrons and gamma-rays, and should generate therefore a complex spectrum of DNA damage. Particularly, DNA double-strand breaks (DSBs) are considered to date as the most significant lesions that, if unrepaired, can result in cell lethality, or if misrepaired, can cause potential cancer onset. In G1 cell cycle phase, DSBs activate two major repair pathways, namely non-homologous end-joining (NHEJ) and MRE11-dependent recombination whose impairments lead to different clinical consequences such as immunodeficiency and radiosensitivity or genome instability and cancer proneness, respectively.

Here, we have investigated the molecular and cellular response of human cells exposed to different neutrons fields, by focusing notably on the temporal formation, spatial distribution and repair rate of DSB. The untransformed human 1BR3 fibroblasts have been irradiated with a ^{252}Cf source or with mono-energetic neutrons (from 300 keV to 16 MeV) produced by AMANDE accelerator (IRSN, France). The immunofluorescence technique that permits the detection of the relocalisation of specific DNA repair actors such like H2AX, DNA-PK, ATM and MRE11.

Exposure to neutrons leads to the formation of nuclear pH2AX foci as clusters and/or tracks and whose spatial distribution depends on the neutrons energy. Interestingly, our findings suggest that such anti-pH2AX immunofluorescence may serve as a spatio-temporal indicator of the energy microdeposition. Our study revealed also that neutrons irradiation inhibits the DNA-PK dependent NHEJ repair process, providing a molecular explanation of the already known severity of DSB induced by neutrons. Furthermore, it appears that such severity may over-activate the MRE11-dependent pathways, which increase the mutagenesis of the DNA damage specific to neutrons, as well. Altogether, our findings support that neutrons generate late unrepairable DSBs that impact upon the ATM-dependent stress signalling pathway by favouring propagation of errors. Such findings should help to consider more carefully the biological action of neutron.

Poster session I - Poster 20

Realistic 3D modelling of cellular processes: a new approach for studying intracellular organization of integrated biological systems under radiative perturbations

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Thanks to NMR, X-ray crystallography and electron microscopy, it is possible to resolve the 3D structure of many cell components. While many software exist for visualizing the 3D shape of individual biological molecules, a visual discovery tool is needed to link the individual component view with a system view.
Setting proteins back into their cellular context should help biologists and teachers to better understand and describe which conformation (scale of protein cluster) and organization (scale of the cell) support a cellular process in a crowded medium. The benefit of spatial modelling is likewise to measure architectural details in order to constrain and refine computational models. This approach is particularly relevant to get insight into preferential DNA compaction providing cells with protection against radiations.

The prototype that has been developed enables to map the cell components (reconstructed from their PDB structures) organized into large supramolecular structures and to navigate in real-time within the cell at various scales from a complete cell down to a single macromolecule. The tool has 3 main features:

- A user interface to navigate inside a cell at various scales,
- A graphical tool to build 3D scenes and manipulate objects,
- An annotation tool to organize and share information about a 3D scene or its components.

Cellular process under current investigation and modelling :

Bacterial cytokinesis is initiated by the formation of a circumferential ring of FtsZ proteins (the Z ring) around the inner surface of the cytoplasmic membrane. The tubulin-like FtsZ is a GTPase that provides at least part of the contractile force for septal invagination through dynamic remodeling of the Z ring. In addition, the cell division machinery promotes the switch from lateral to preseptal peptidoglycan synthesis, and also provides a scaffold for the recruitment of downstream divisomal proteins essential for cell division. In Escherichia coli more than 10 divisomal proteins are recruited to the division site in a hierarchical order.

We created a 3D model of the E. coli septosome in order to address and characterize the spatio-temporal coordination of cell division proteins, including the septal protein FtsK that links cell division and chromosome segregation.

The LifeExplorer software is an open source project. The project page is http://www.lifeexplorer.eu.

Poster session I - Poster 21

Theoretical investigation of the ultrafast dissociation of ionized biomolecules immersed In water: direct and indirect effects

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Theoretical simulations are particularly well suited to investigate, at a molecular level, direct and indirect effects of ionizing radiations in DNA, as in the particular case of irradiation by heavy ions, such as those used in hadron therapy. In the present work, we have studied the dissociation of ionized biomolecules in the gas phase and in the liquid phase.

We modelled the ionization of liquid water and water clusters generated by swift ions with first principle Car Parrinello Molecular Dynamics simulations [1, 2]. In order to complete this study, the early stages of the Coulomb explosion of a doubly ionized uracil molecule in both, liquid and gas phase, have been investigated with Time-Dependent Density Functional Theory molecular dynamics simulations (TD-DFT MD) as described in [3]. Our aim was to verify that the double ionization of one target uracil molecule immersed in liquid water leads to the formation of an atomic oxygen as a direct consequence of the dissociation of the molecule. To that end, we used TD-DFT MD in which effective molecular orbitals are propagated in time. We have investigated the dissociation process of one target uracil molecule immersed in bulk water following the removal of two electrons from one of its three deapest molecular orbitals. We show that the doubly charged molecule explodes into four atomic fragments in less than 30 femtoseconds in gas phase in a very good agreement the experimental measurement [4], while leading to the formation of one isolated oxygen atom when uracil is embedded in water.

We are able to simulate the evolution of chemical primary event, in a few tens of femtoseconds, either when the holes are created in water (indirect effect) or in the biomolecule (direct effect).

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Poster session I - Poster 22

Possible early diagnostic of radiosensitivity and optimisation of radiotherapy using DNA repair foci in lymphocytes of breast cancer patients

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In this study, 53BP1 DNA repair foci were analyzed in lymphocytes from breast cancer patients with the final goal to develop new sensitive assay for prediction of radiosensitivity of patients undergoing radiotherapy.

Twelve breast cancer patients were subjected to radiotherapy: 25 fractions, 2 Gy/day each fraction. No cytostatic treatment was applied. The acute reactions of patients were classified according to the RTOG criteria. Blood samples were taken 2 times before treatment, 24h, 48h, 72h, 1 week and 2 weeks from the beginning of irradiation. The blood samples were also taken from some patients following one and three month after the end of radiotherapy. The samples were prepared for immunostaining of the 53BP1 DNA repair foci. Our results have shown that all patients belonged to the Grades 0-1 as classified by the RTOG acute reactions. The patients will be followed up to analyze the postponed radiation complications. The acute and postponed reactions of patients will be correlated with dose responses and kinetics of DNA repair foci.

The data have shown statistically significant increase in the background level of 53BP1 foci in breast cancer patients as compared to healthy persons. The radiation-induced DSB repair foci were observed in the lymphocytes of breast cancer patients already at 24h following first local fraction. At all time points during radiotherapy, significant increase in the number of DNA repair foci was detected in cells from all patients, although some variation between patients was observed. Interestingly, the levels of radiation-induced DNA repair foci were rather stable during the whole course of radiotherapy. This data correlate with the data obtained by us in experiments with lymphocytes irradiated *in vitro*. Similar levels of the radiation-induced 53BP1 DSB repair foci were observed during the radiotherapy and even 1-3 month after radiotherapy. This stability in the levels of radiation-induced DNA repair foci may be related to radiation-induced increase in constitutive DNA breakage rather than to unrepaired DSB in the residual DNA repair foci. Such stability allows limiting blood sampling at the beginning of the radiotherapy in order to accomplish analysis of DNA repair foci and possibly to provide recommendations for optimization of radiotherapy based on the predicted individual radiosensitivity.

Poster session II - Poster 23

Analysis of radiation-induced damages on basal keratinocytes of human epidermis using a single-cell model reveals heterogeneous functional consequences and acquisition of genomic abnormalities

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Epidermis is the first target of external irradiation received for therapeutic or diagnosis purposes. The basal layer is the deeper part of epidermis. It is particularly important for skin carcinogenesis, as it contains both stem cells and keratinocyte progenitors, which are the cells at risk for the development of skin carcinomas. Progresses in the field of carcinogenesis will be closely linked to the development of efficient experimental systems suitable for investigations at a clonal level. We developed a new method that allows multi-parallel clonal cultures of basal keratinocytes. Immediately after extraction from tissue samples, cells are sorted by flow cytometry based on their high integrin- 6 expression and plated individually in microculture wells. At the end of the primary culture (14 days), the distribution of clone size, which ranged from abortive clones to highly proliferative clones containing 1.7×10^5 keratinocytes, was well representative of the hierarchical organisation of the basal layer. In long-term cultures, some highly proliferative clones could sustain extensive expansion (180 population doublings [PD] over 28 weeks) and exhibited epidermis reconstruction potency, thus fulfilling stem cell functional criteria, whereas other clones exhibited the more limited growth potential of progenitors (~50-70 PD). We have used the clonal microculture system to analyze the consequences of an exposure of integrin-α6 high keratinocytes to a single 2 Gy dose of ionizing radiations (γ rays, 0.6 Gy/min). These cells correspond in majority to progenitors. Examination of the primary clonal growth profile obtained with these irradiated keratinocytes revealed a marked increase of the frequency of abortive clones. However, we observed a significant proportion of resistant clones, which were capable of preserving their long-term growth capacity after radiation exposure. Cytogenetic studies were performed on these resistant clones at 20-25 PD. They revealed long-term genomic instability, including Non Reciprocical Translocations and deletions. Transmission of genomic abnormalities to the long-term progeny of resistant clones (> 100 PD) was then investigated using comparative genome hybridization (CGH) arrays. This analysis revealed different specific aberrations in the progeny of individual keratinocyte clones, including large duplications of genomic loci. In conclusion, the single-cell method described here appeared efficient to explore the long-term effects of a clinically relevant dose of irradiation in cells at risk for carcinogenesis. This method will be next used to investigate the effects of very short (femto second scale) high energy monoenergetic laser-plasma-based electron pulses, in collaboration with LOA (Polytechnique, France).

Poster session II - Poster 24

Towards a submicrometric dose determination in radioimmunotherapy

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The complex links existing between radiation physics and radiobiology concern the understanding of spatio-temporal events triggered by energy depositions in confined spaces.

Tumour targeting antibodies (mAb) labelled with β^- emitters gives hope for treatment. 3D biodistribution of absorbed dose, assumed to be homogeneous, is defined with gamma-emitting despite differences in chemical linkage or metabolization. Simulation with an anthropomorphic phantom results in disappointing dose effect relationship. Software packages investigated at the (multi)cellular scale confirm multiple heterogeneities. Autoradiography is not adapted to follow tracks deposit of high energies (2 MeV).

Deleterious β^- radiation damages triggered on biological targets are dependent on the survival probability of secondary electrons and radicals distribution inside micrometric and sub-micrometric clusters of ionization (stochastic processes), a thorough understanding of these radiobiological processes at the local order requires the precise pure β^- emitter localization.

The aim of this research is to determine the dose absorbed effects by the identification and the mapping of biochemical alterations of cells/tissues submitted to ionizing radiations taking as ionizing source β^- mAb ^{90}Y-Zevalin, an antiCD20 used for the treatment of lymphoma in association with Rituximab another antiCD20 and by the observation of the spatial distribution and local concentration of Zevalin through 2D imaging. Imaging (digital autoradiography) in mouse femur of grafted mice, with human mantle lymphoma cell line treated with ^{90}Y-Zevalin, were correlated with an heterogeneously bone marrow infiltration.

Synchrotrons constitute an opportunity to approach the absorbed dose in radioimmunotherapy using β- mAb in Human B normal and lymphoma cell line (without treatment, incubated with Rituximab, ^{90}Y-Zevalin, or with these two mAb) and in grafted mice. Detecting subtle structural changes within normal and tumoral cells/tissue requires a precise spectral quality for a high lateral resolution. Vibrational infrared absorption spectroscopy and chemicals mappings of lipids, proteins and nucleic acids are investigated by FTIR (Soleil, SMIS). With Sensitive synchrotron SXRF microanalysis 2D imaging and scanning of ^{90}Y/^{90}Zr spatial distribution would be obtained.

First IR study shows, using principal composent analysis, contributions of ^{90}Y-Zevalin vs Rituximab on the DNA/RNA (1245, 1080 cm^{-1}) or on the amide (1650) bands sensitive to protein secondary structure.

In synergy with complementary micro-analytical techniques such as Atomic Force Microscopy to image morphology changes of cells or Secondary ion mass spectroscopy to quantify distribution of ^{90}Zr, the synchrotrons imaging and mapping (sub)cellular radiation damages would provide guidance for significant advances in sub-micrometric dosimetry of biomedical interest, more particularly for selective immuno-radio-therapies of cancers.

Poster session II - Poster 25

Molecular dynamics and elementary processes involved in damage induced by synchrotron radiation in the soft X-ray regime on biomolecules

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Biological consequences of ionizing radiation on living organisms result from physical processes taking place in the early stages following exposure. The identification and the understanding of these primary physical events are important for the building up of knowledge in radiobiology.

A novel research program, concerning the interaction between gas phase biomolecules and soft X-rays delivered by the synchrotron light source SOLEIL, will be carried out on the PLEIADES beamline.[1]

Although not best suited to radiation therapy, soft X-rays have been shown to be a unique tool to understand the early stages of DNA damage by ionizing radiation. Tuning the photon energy enables either to target specific DNA atoms and/or to select the energy of the secondary (Auger associated to various thresholds and photo-) electrons emitted. Synchrotron radiation, with its continuous spectrum over a wide photon energy range is thus the appropriate light source for such studies. Due to the increase by a factor two of the yield of inner-shell ionizations in DNA, Fayard et al. have shown for example that soft X-rays above the C-K threshold are twice more efficient at cell killing than below it.[2] Moreover, atomic inner-shell relaxation in biomolecules creates localized ballistic electron sources via the production of Auger electrons. Studies have shown that such electrons, even with kinetic energies as low as 3 eV, can efficiently induce single and double strand breaks in DNA.[3] In addition, specific fragmentation of the core ionized molecule may occur.

The sophisticated spectroscopic tools and methodologies available on the beamline, like photoelectron spectroscopy or Auger electron/ion coincidences, combined with tunable soft X-rays delivered by SOLEL will make possible the determination of the energy distribution of the ejected electrons for a specific dissociation channel of the biomolecule, or the different electronic states involved in this break down. Those measurements should be very helpful as new inputs in radiation damage models. Stability of elementary bricks of DNA or polypeptides will be investigated together with the resonant processes or the opening of new dissociation channel that will be quantified.

Thanks to the possible control of the solvation degree of biomolecules, gas phase studies should also give key answers about the dissipative effects due to solvent. An important issue will also be understanding the physical chemistry changes related to hydrogen bonding between the biomolecules and one or several water molecules.

Let's also emphasize that experimental data on such complex systems will need molecular dynamics simulations for an accurate interpretation.

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Poster session II - Poster 26

Radiation induces p38-mediated endothelial cell death through ceramide generation and membrane remodeling

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Microvascular endothelial cell apoptosis has been reported to be a critical event in severe tissue toxicity and tumor response after irradiation (Paris et al., Science 2007). Despite the involvement of sphingolipid ceramide generation and its metabolic enzyme activation, the acid sphingomyelinase (ASM), apoptotic molecular mechanisms are still poorly understood. Recently, high-dose irradiation has been shown to induce the p38 stress pathway in endothelial cell apoptosis. In tumor cells, p38 activation through its phosphorylation has been shown to be dependant of DNA damage and reactive oxygen species (ROS) activation. In this present study, we demonstrated a straight connection between the radiation-induced ROS, ASM/ceramide generation and p38 activation, which explained endothelial apoptosis.

Using the HMEC1 endothelial cell line, we confirmed that ASM activation and ceramide generation in the membrane appears within 1 to 5 minutes following 15 Gy. Radiation-induced apoptosis within 24 hours was inhibited by treatment with pharmacological ASM inhibitors (Bonnaud, Cancer Research, 2007) proving the use of those cell line as a good *in vitro* model to study the ceramide mediated apoptosis after exposure to ionising radiation. Furthermore, 15 Gy-induced HMEC-1 death was dependent of p38 activation as seen by the rapid p38 phosphorylation visualised by phosphoblot and immunofluorescence and by the inhibition of apoptosis by p38 MAPK inhibitor III or shRNA.

Link between ceramide generation and p38 activation has been elucidated by studies on cell membrane reorganisation. Ceramide induces coalescence of raft microdomains after a large spectrum of stresses, such as H_2O_2 or cytokines. Raft-marker, ganglioside GM1, was relocalised from a discrete pattern in the cell surface, to large and polarised areas following irradiation. Finally, the two concomitant phenomena, i.e. ceramide-induced raft coalescence and p38 death-pathway activation, has been connected by use of drugs, such as nystatin or bacterial ASM, which respectively inhibited and activated radiation-induced raft coalescence, p38 activation and the subsequent death. bASM do not induce H2Ax foci demonstrated that p38 dependant HMEC-1 death was not related to DNA damage induction. Use of gluthatione before irradiation was inhibiting membrane reorganisation, p38 activation and death. Furthermore, H2O2 treatment resumed the molecular cascade observed in irradiated HMEC1, confirming of ROS dependence of the ceramide/p38 apoptosis.

By immunohistology on small intestines slides, we showed that p38 was rapidly phosphorylated in endothelial cells from 15 Gy-irradiated wild type, but not ASM invalidated mice, proving a physiological relevance of the connection between ceramide and p38 signalling.

Poster session II - Poster 27

Assessment of breast feeding risk on child growth and development for the radiation polluted regions of Ukraine

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In recent years, the benefits of breast-feeding have increasingly been recognized. Human breast milk offers the optimal nutrition for all infants and provides immunological, developmental, psychological, economic, and practical advantages when compared to artificial feeding. However, ecological situation in the most regions of Ukraine is not so favourable for the childbearing and maternity because of widespread pollution from heavy industry and agriculture. Moreover in 1986 the Chernobyl accident caused the additional pollution of territories by radionuclides and heavy metals, in particular lead. Consequently, pursuing of research dealing with the assessment of breast-milk quality in feeding mothers to forecast the consequences of mother and breast milk contamination by different pollutants on child's growth and development as well as children's morbidity are of urgent need regarding demographic problems in Ukraine.

The proposed research is intended to identify the chemical and radiation contamination of breast milk in feeding mothers who are inhabiting radiation polluted territories of Ukraine, specifically Rivno Region.
For the experiments 80 primiparous women-volunteers who gave birth or are going to give birth to the first child have been found and used in randomized cohort studies. Collection of breast-milk has been performed either using electric breast pump or manually. In breast milk pesticides, dioxins and dioxin like substance, heavy metals and radionuclides were determined. Evaluation of contaminant release kinetic in the course of breast milk feeding and assessment of possible accumulated dose in mothers for every contaminant of breast milk was done using computer simulation and mathematic approaches.

On the basis of analytical analysis the contaminants present in breast milk of feeding mothers have been elucidated and used for the quantification of total burden of pollutants accumulated in each mother's organism during their life-time on the radiation polluted territories. Finally, the prognosis concerning the risk of breast milk feeding and fetus bearing on children's development and morbidity has been drawn up for particular highly radiation polluted region of Ukraine.

The research is supported by NATO Reintegration Grant.

Poster session II - Poster 28

Delayed macrophage activation as a system response to injure from ionizing and nonionizing radiation

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Many studies have investigated delayed health effects of ionizing radiation (IR). A growing body of evidence supports the possibility of adverse effects of nonionizing radiation (NIR) from different sources as well. It seems to be some delayed effects of IR and NIR are very similar by nature and could be classified as stochastic effects. Their pathogenesis include system reaction to primary injure. The mechanisms of these delayed radiation effects are unclear. We consider the resident macrophages could play significant role in tissue damage and increase cancer risks via generating of clastogenic factors, including inflammatory mediators and free radicals.

To compare radiation effects of IR and NIR, 3 month aged male Wistar rats were exposed to acute whole body irradiation with gamma-rays at dose 1 Gy (0.92 Gy/min, ^{137}Cs) and during 14 days (4+4 hr per day) fractionated pulsed electromagnetic field of GSM-900 band (0.2-0.3 mW/cm^2, Ch 35, 897.2 MHz) respectively. Animal were sacrificed on 3, 10, 30, 90 days after irradiation with IR and on 1, 7, 15, 21, 28, 34 days after last fraction of NIR. Resident peritoneal macrophages were isolated by lavage. The basal and stimulated reactive oxygen (ROS) and nitrogen (RNS) spices production were estimated by luminol-enchanced luminescence and nitrite accumulation in media respectively. All experiments were conducted under local ethics committee approval.

The increasing in basal production of ROS and RNS were found at month after action of gamma rays as well as radiowaves. While animal irradiated with IR demonstrate almost uniform increasing in basal RNS production, this effect were identified only in 30 % of animal exposed to NIR. Obtained data indicate inflammatory-type response of organism in late term after irradiation and demonstrate what macrophage activation could be involved in pathogenesis of delayed untargeted radiation effects via increased level of free radical production. Hence, monitoring and management of reactive oxygen and nitrogen spices production could be used for delayed radiotoxicity amelioration.

Poster session II - Poster 29

Effect of platinum nanoparticles in ion induced damages in DNA

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The hadrontherapy is a rapidly developping tool for the treatment of cancer. The specific physical property of fast atomic ions interacting with matter stems from the strong dose deposition at the end of the particle tracks (the Bragg Peak). This property results in the irradiation of the well-defined volume of the tumour, whilst preserving the healthy tissue around. It differs strongly from the electrons and photons, which deliver the dose in an exponentially-like decreasing function versus the depth penetration in tissue. In order to further enhance the targeting and the efficiency of the treatments, an amplification of the cell death rate specifically in the tumour is of strong interest.

As shown recently, nanotechnology provides essential breakthroughs in the fight against cancer. The combination of 100 keV range X-rays with the addition of gold nanoparticles is proposed as a new alternative to improve radiotherapy protocols. However, these low energy radiations are not relevant for conventional treatments.

We show for the first time in this work that the combination of coated platinum nanoparticles with fast ion irradiation enhances strongly DNA damage, in particular double strand breaks. These effects can be explained by the multiple ionization and by the self amplified Auger cascades taking place in the platinum nanoparticles.

This opens the perspective of combining nanocompounds and hadrontherapy in order to enhance the efficiency and the spatial resolution of the dose deposition in cancer treatments.

Poster session II - Poster 30

Radionuclides microdistribution by secondary ion mass spectrometry in a biological matrix after internal contamination

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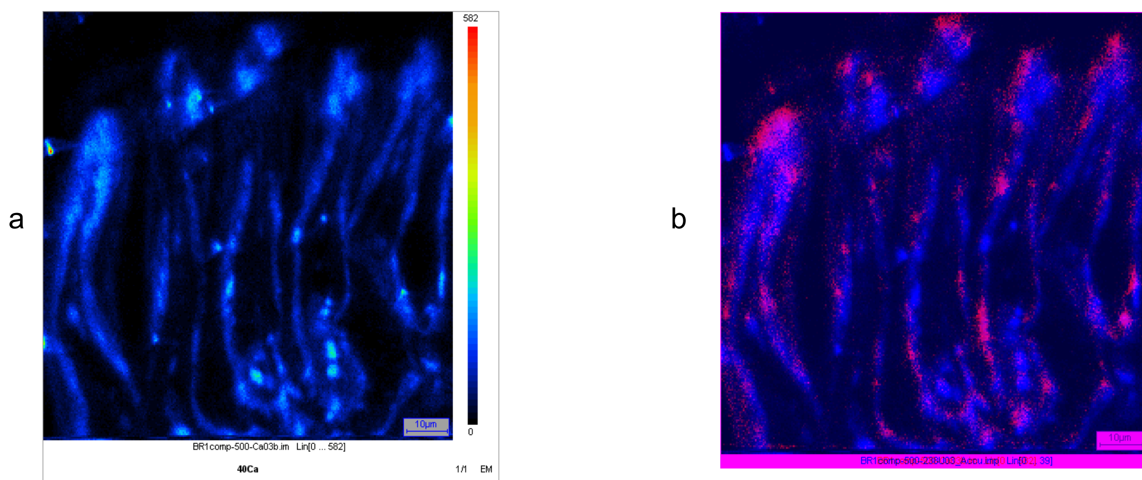
The ENVIRHOM research program supported by the Institute for Radiation protection and Nuclear Safety (IRSN) is intended to improve risk assessment to the general public and ecosystems induced by chronic exposure to low levels of radioactive contaminants. The main objectives are to study speciation, transfer, biokinetic and accumulation processes of radionuclides and also biological effects to man and environmental species correlated with this exposure using the human model (rats, mice) and environmental organisms (algae, molluscs, crustaceans, fishes, plants, etc.). Uranium has been the first element studied in this program. Chronic exposure of uranium may occur in naturally contaminated areas (underground water) or as a result of human activity (nuclear fuel cycle, agricultural use, military use of depleted uranium).

The understanding of transport and transfer mechanisms of incorporated radionuclides, according to the chronic mode in ecosystems and in the public, requires the contaminants distribution mapping of the biological structures targeted as bioaccumulation sites. Among the micro-imaging analysis techniques, the ion microscopy based on Secondary Ion Mass Spectrometry (SIMS), was used as an efficient tool for characterizing the preferential sites of radionuclides accumulation in tissues and cells.



Ionic images of *Corbicula* gills: 500 µg/L, 10 days uranium exposure, (a) $40Ca^+$; (b) $238U^+$ (red) overlaying $40Ca^+$ (blue) (image field 100 µm X 100 µm)

In this work, the microdistribution of uranium in gills of freshwater bivalve *Corbicula fluminea* following chronic and direct exposure to this radioelement has been investigated. Different exposure levels and exposure durations have been studied. For each bivalve analysed area, mass spectra around the 238 uranium isotope mass and ion images have been obtained with a SIMS CAMECA 4F-E7. Thanks to $^{40}Ca^{+}$ images the histological structure of the bivalve gills could be given and ^{238}U fixation sites in these structures were showed using $^{238}U^{+}$ images (Figure). Uranium bioaccumulation has been displayed also in some other structures: rat kidney, neuron, hepatic cells.

Poster session II - Poster 31

Gamma-H2AX as a biodosimeter for ionizing radiation exposure: an *in vivo* study with non-human primates

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Exposure to Ionizing Radiation (IR) leads to DNA double-strand breaks (DSBs). Upon DSB induction, hundreds of H2AX molecules at the DNA break site are rapidly phosphorylated on a serine residue near the C-terminus (gamma-H2AX). Therefore the detection of gamma-H2AX is a good candidate for monitoring DSB *in vivo*. We have developed diagnostic tests for *in vivo* IR exposure based on detection of gamma-H2AX using Western blotting and immunocytochemistry. By sampling peripheral blood lymphocytes and plucked hairs, we assessed the usability of gamma-H2AX as a possible biodosimeter using a nonhuman primate (NHP) radiation model (60Co gamma-rays at 0.6 Gy/min). A total of 22 male and female adult (3 to 6 years old) Rhesus macaques (*Macaca mulatta*) were used for this study: 3 groups of 6 NHPs each received a single whole-body irradiation dose of 1, 3.5 and 6.5 Gy respectively while a fourth group of 4 NHPs was sham-irradiated. Plucked whisker and eyebrow hairs were examined 1 and 2 days post-IR. Blood samples were analyzed prior to irradiation and from 6.5 hours for up to 23 days post-IR. Gamma-H2AX was still detected in hair samples 2 days after treatment with 3.5 and 6.5 Gy. In lymphocytes significant signals for both individuals and the pooled radiation dose cohorts were still detected after 1, 2, and 4 days following treatment with 3.5 and 6.5 Gy; p values for the pooled dose cohorts were 0.022, 0.005, and <0.001 respectively. Moreover, data in blood samples for up to 2 and 4 days after 3.5 and 6.5 Gy treatments were fitted to a linear dose response. Finally, low residual DNA damage was still detectable for 2 weeks in lymphocytes after 3.5 and 6.5 Gy exposures suggesting that some DSBs remained unrepaired. As a rapid response to nuclear or radiological exposure is crucial, this study shows that gamma-H2AX formation to monitor DNA damage *in vivo* using minimally invasive blood and plucked hair samples could be a robust biodosimetric measure of IR exposure in humans. Such a methodology would allow clinicians to screen for humans exposed to IR and treat its immediate and long-term medical effects. Additional studies are required to evaluate the influence of potential confounders (i.e., inter-individuals variations, dose fractionation, response of special populations (i.e., elderly, etc.) for use of this assay for radiation injury and dose assessment.

Research supported by DARPA's Radiation Biodosimetry Program - MIPR entitled: Nonhuman Primate Testing for Biodosimetry.

Poster session II - Poster 32

Telomere maintenance and chromosome instability in human bibroblast and ketarinocytes cultures

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In spite of the significant progress in cancer biology and modern radiation biology in the last decades, it is still an open question how exactly different human cell types respond to ionizing radiation. Most studies have been done on two types of human cells, skin fibroblasts and peripheral blood lymphocytes, due to their relatively easy access in individuals. Other cell types such as normal epithelial cells have been used on a much smaller scale and consequently there is relatively little data on how normal epithelial cells respond to ionizing radiation. The study of epithelial cells is highly relevant for the elucidation of radiation-induced carcinogenesis as the majority of human solid cancers will develop from epithelial cells. The work presented here is the characterization of karyotypes and telomere loss in non-irradiated fibroblast and keratinocyte populations from the same donor during the different stages of proliferation in vitro and results for long term transmission of radiation-induced damage in keratinocytes. An increase in the incidence of telomere loss with doublings in vitro was noted; however, it followed different kinetics in the two cell types. An increase in clonal and de novo chromosomal aberrations was observed in both fibroblasts and keratinocytes as proliferation progressed into senescent phases. Clonal emergence from the second senescent plateau, characterized by progressive instability of the genome and different kinetics of telomere maintenance, was observed in keratinocytes. After irradiation, fibroblasts and keratinocytes showed different kinetics of γH2AX foci, with apparent higher initial induction in fibroblasts, but faster disappearance of the induced foci.

Using a keratinocyte cell culture set-up developed by M. Martin and N. Fortunel, we studied the follow-up of the post-irradiation progeny of single cells. We observed that chromosomal instability can occur during the proliferation of irradiated human keratinocytes, i.e. 20-25 population doubling after 2 Gy γ-rays. This de novo chromosomal instability can be characterized by the accumulation of non-reciprocal translocations (NRTs) induced after one telomere loss and cycles of breakage-fusions-breakage. Such instability is rarely observed in human fibroblasts and has been described only after high-LET exposure.

The radiosensitivity of each cell type and the transmission of radiation-induced damage could largely differ according to the cell type status at irradiation (fibroblasts/keratinocytes, young/senescent) and their role in the long-term occurrence of radiation-induced tumours needs to be characterized.

Poster session II - Poster 33

The complexity of phosphorylated H2AX foci formation and DNA repair assembly at DNA double-strand breaks

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Presented by **O. Sedelnikova**

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Histone H2AX, a key protein to efficient DNA double-stranded break (DSB) repair, becomes phosphorylated at the sites of DNA DSBs. A nascent DSB is rapidly, over a period of 10 minutes, flanked by hundreds to thousands of phosphorylated H2AX molecules (here after referred as γ-H2AX), to form a structure which appears as a focus upon staining by a variety of means. These foci are sites of rapid accumulation of many species of DNA repair and/or cell cycle checkpoint proteins into assemblies that are critical for efficient DNA DSB repair. The kinetics of these foci formation is also a critical factor for the maintenance of genome stability. In fact, defects in H2AX and/or other DNA repair proteins result in cells and organisms with increased radiation sensitivity, cancer incidence, and genomic instability, and in some cases with well characterized diseases, such as Nijmegen Breakage syndrome (NBS1) or Ataxia Telangiectasia (ATM). Therefore, understanding the nature of DNA damage foci formation will provide insights into the efficient repair of DNA DSBs and into the maintenance of genome stability.

Using a variety of techniques to visualize, expand, and partially disrupt chromatin, we are showing that DNA damage-induced γ-H2AX foci are complex, differentiated, and dynamic structures. Certain accumulated protein species bind homogeneously to γ-H2AX foci while others bind only to certain regions after ionizing radiation. In addition, while γ-H2AX foci exist throughout the cell cycle, including mitosis, some protein species are stably present in these foci while others dissociate in G2 and re-associate in the following G1. These data suggest that components of DNA damage-induced foci are reorganized through cell-cycle. Our findings all demonstrate that γ-H2AX foci are complex dynamic assemblies.

Poster session II - Poster 34

The natural product celasrol can modulate the radiation-induced changes in human lymphocytes

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Celastrol, a quinone methide triterpene, is a natural product derived from the *Celastraceae* family of plants. It is characterized by anti-inflammatory, anti-tumor, anti-oxidant and cytoprotective properties. The present study was designed to analyze the impact of celastrol on radiation induced changes in gene expression of the anti-oxidant enzymes catalase, manganese super oxide dismutase (MnSOD) and glutathione S-transferase (GST) in human lymphocytes isolated from peripheral blood of healthy donors. The effects of two concentrations of celastrol, 100 nM and 1 μM, were studied and lymphocytes pre-incubated for 2 hours with celastrol were irradiated with different doses ranging from 0.5 to 8 Gy. Changes in the expression levels of the enzymes in the pretreated with celastrol and subsequently irradiated lymphocytes were compared to those in irradiated lymphocytes.

Pre-treatment of lymphocytes with 1 μM celastrol followed by irradiation increased the expression of MnSOD in the whole dose range (0.5 to 8 Gy). 100 nM celastrol exerted quite different effect. It suppressed the expression of MnSOD at 0,5 Gy and did not affect the protein levels in the range of 1 to 8 Gy.

Pre-incubation of lymphocytes with 100 nM celastrol did not affect the catalase expression in lymphocytes after irradiation with doses from 0,5 to 4Gy but 1 μM celastrol exerted suppression on the enzyme level in the same dose range. A clear increase in catalase expression was registered at both celastrol concentrations after irradiation with 8Gy.

Similar to catalase, GST expression was also suppressed in the dose range of 0,5 to 4 Gy by 1 μM celastrol and increased at 8 Gy. The expression profile of the same enzyme was more complex at 100 nM celastrol showing decrease at 0,5 and 1Gy, and increase above 2 Gy.

The concentration dependence of the celastrol effects was confirmed by the Comet test. It registered very low levels of DNA double strand breaks in pretreated with 100 nM celastrol and irradiated cells in contrast to the effect of 1 μM celastrol which showed increase in radiation induced DNA damages with increasing the radiation dose. Thus, celastrol can modify the early radiation response in dose and concentration dependent manner and exhibits promise as a new active regulator of the radiation-induced stress response in lymphocytes.

Poster session II - Poster 35

Influence of ionizing radiation on hematologic and biochemical indicators of animal blood. Cardiorythm regulation after radiation treatment

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The problem of the radiation exposure to organism is obviously actual after 23 years since Chernobyl accident. Also it does not raise the doubts position about possibility of an adverse influence on a clinical course of radiating and emotional stress. Research objective: to study the features of hematologic and biochemical indicators of rat's blood at action of an ionizing radiation against sharp stress influences; to consider irradiation influence on extracardial regulation of a cardiorhythm.

Experiments were spent at institute of Radiobiology NAS of Belarus on outbred rats-females 6 month age; weight was 170-230 g. All rules of work with experimental animals were observed. Animals were irradiated on IGUR equipment by ^{137}Cs in a dose 1.0 Gr at capacity of a dose of 0.9 Gr/min. The ionizing radiation in this dose does not cause of development of clinical signs of radiation sickness in rat organism. For 1 day after an irradiation the rats were subjected to influence of stress by rigid fixing in back position during 6 hours. Not irradiated rats were subjected to stress simultaneously with the irradiated ones. Thus in experiment used 4 groups of animals: 1) control rats; 2) rats + irradiation in dose 1.0 Gr; 3) rats + stress; 4) rats + irradiation in dose 1.0 Gr + stress. The results of analysis of hematologic and biochemical indicators of the rats blood developing under the influence of stress and radiation factors are represented. It is ascertained that on the 4th twenty-four-hour period after the influence of sharp ionizing radiation against a background of sharp stress impact some changes become evident. They appear in reducing of total number of leucocytes, haemoglobin, hematocrit and general albumen, and also in increase of such ferments activity as lactate dehydrogenase, creatine phosphokinase, aspartate aminotransferase, alanine aminotransferase.

For studying the opportunities of adaptation of cardiovascular system the researches of mechanisms of extracardial regulation of a cardiorhythm on 3 and 10 days after influence of ionizing radiations in a dose 1.0 Gr (sharp and chronic) have been carried out. In all cases shift of vegetative parameters aside the sympathetic activity which is responsible for emergency mobilization of energetic and metabolic resources at any kinds of stress has been marked.

Poster session II - Poster 36

Directly induced damage of biomolecules studied by means of X-ray photoelectron spectroscopy

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Of all the contemporary surface characterization methods, X-ray Photoelectron Spectroscopy (XPS) is one of the most widely used. The popularity of XPS as a surface analysis technique is related to its ability to provide quantitative information on almost all chemical elements present, its flexibility in addressing a wide variety of samples and its well understood theoretical basis [1]. In the current work chemical transformations of lyophilized double stranded deoxyribonucleic acid from calf thymus and poly-L-arginine have been studied by means of X-ray Photoelectron Spectroscopy.

To study the effect of soft X-ray irradiation of the aforementioned compounds measurements were performed using a Kratos XSAM 800 spectrometer equipped with an anode source, a hemispherical electrostatic electron energy analyzer and a channeltron detector. The spectra were recorded using Mg Kα radiation in the medium pass energy of 20eV and fixed analyzer transmission (FAT) mode. In order to monitor detectable changes samples were irradiated continuously for five and a half hours.

The XPS spectra have complicated shapes due to contribution of several functional groups and shake-up satellites. Analysis of high resolution photoelectron spectra recorded for O, N, C and P regions within lyophilized double stranded deoxyribonucleic acid from calf thymus before and after irradiation identified several decomposition pathways. Both the oxygen and nitrogen peaks decrease during X-ray exposure, mostly due to decomposition and desorption of small fragments. Significant changes in the shape of the photoelectron peaks were also observed for the carbon peak, which can be attributed to formation of new products during irradiation [2].

In the case of poly-L-arginine, changes in the XPS spectra after exposure were also detected. The intensities of photoelectrons ejected from all elements present, i.e. C, O and N decrease during irradiation, also mostly due to fragmentation of the sample. Observable changes in the shape of photoelectron lines were also observed for C1s, which can be attributed to radiation induced rupture of a peptide bond in the studied molecule [3].

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Poster session II - Poster 37

Aneugenic effect of plutonium-239 in somatic cells of nuclear-chemical plant workers

Stanislav A. Vasilyev, V.A. Timoshevsky, I.N. Lebedev

Aneugenic potential of ionizing radiation especially of high-LET α-emitting radionuclides such as plutonium-239 is poorly understood. Therefore the aim of our study was to analyze the aneugenic effect of incorporated plutonium-239 in comparison with clastogenic activity and to assess the origin of emerging aneuploidy as a result of different mechanisms, namely chromosomal non-disjunction and loss. The exposed group comprised by 27 male workers of Siberian chemical plant (Russian Federation, Seversk) with activity of incorporated plutonium-239 from 370 to 6956 Bq was investigated by FISH in cytokinesis-blocked binucleated lymphocytes. A group of 33 health male individuals of the same age living in Seversk and having no connection with ionizing radiation in their professional occupation was studied as control. FISH with pancentromeric human DNA probe was used to distinguish aneugenic (centromere-positive micronuclei – MnC+) and clastogenic (centromere-negative micronuclei - MnC-) components of the incorporated plutonium-239 influence. In addition double-color FISH with centromere-specific DNA probes for chromosomes 2 and 8, 7 and 12, X and Y were used for the analysis of impact of chromosomal non-disjunction and loss mechanisms to overall aneuploidy frequency. A significant increase of the total micronuclei frequency in the exposed group (8.6‰) as compared with the control (5.1‰, $p<0.001$) were found. This increase was mostly due to higher frequency of centromere-negative micronuclei (MnC-) in the group of workers (4.7‰) than in the control (2.4‰, $p<0.001$). But significant increase in the centromere-positive micronuclei (MnC+) frequency was observed also in the exposed group (3.9‰) in compare with the control (2.7‰, $p<0.05$). There was a significant increase of the non-disjunction frequency of chromosomes 2, 7, 8, 12 and Y in the exposed group ($p<0.05$). A significantly higher frequencies of chromosome 7 and 12 loss in the exposed group ($p<0.05$) were observed also. As regards to analysis of the mechanism of aneuploidy origin it was found that almost all of autosome segregation failures was due to chromosome non-disjunction (94%). Incidence of sex-chromosomes non-disjunction events among all segregation failures was 75% and 82% in the exposed group and 63% and 68% in the control, respectively.

For the first time it was confirmed that the influence of complex of occupational factors including incorporated plutonium-239 in addition to well known clastogenic effect (MnC-) results in significant increasing of hypoploidy frequency (MnC+). Furthermore, our results indicate that chromosome non-disjunction is predominant mechanism of aneuploidy origin under high-LET influence with some variation between the chromosomes.

Poster session II - Poster 38

Response of peripheral blood lymphocytes to DNA damage caused by fractionated irradiation in vitro and in vivo

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Very quickly upon induction of double strand breaks (DSB) by ionizing radiation also changes in DSB flanking chromatin occur. Within minutes after irradiation nucleosomal protein histone H2AX is phosphorylated on serine 139 (γ-H2AX) by ATM kinase. Other proteins are then bound to modified chromatin, e.g. adaptor protein 53BP1 and protein foci are formed around each DSB. These foci are also known as IRIF – ionizing radiation-induced foci. In our work we studied effect of single dose irradiation and fractionated irradiation by the dose of 4 Gy on formation of these foci in lymphocytes irradiated in vitro and in vivo.

We observed dose dependence of integral optical density (IOD) of γ-H2AX in IRIF 1 h after the in vitro irradiation of isolated human lymphocytes in the dose range 0.5-5 Gy. The dose of 2 Gy delivered by both, in vitro and in vivo (whole-body irradiated patient) irradiation also caused increase of H2AX IOD 1 h after irradiation. 24 h after this first irradiation DSB were repaired and almost all IRIFs disappeared (significant decrease in IOD, the values were not significantly different then IOD of nonirradiated cells). Then the cells were irradiated by second fraction of 2 Gy, formation of IRIFs 1 h after the second irradiation was comparable with response to the first fraction (as well as IOD), but the cells were unable to repair all DSB within next 24 h, and in 52% of PBMC significant amount of γ-H2AX persisted. In the detected foci γ-H2AX colocalized with adaptor protein 53BP1, which indicates presence of unrepaired DNA lesion. Furthermore we demonstrate that similar pattern can be observed in peripheral lymphocytes collected from the blood of patients undergoing fractionated radiotherapy. When apoptosis induction in lymphocytes irradiated by single dose of 4 Gy and by fractionated irradiation (2+2 Gy, 24 h interval) was compared, no significant changes were observed 72 h after the last irradiation.

These results indicate that although 24 h after the irradiation of lymphocytes by the dose of 2 Gy no changes in the cells are apparent, but the repair capacity of these cells is decreased and 24 h after the second irradiation foci around unrepaired DSB persist.

Poster session II - Poster 39

Low energy electron transport effect in proton track calculations

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Understanding the behavior of biological matter after ionizing radiation is one of the main issues for the scientific community nowadays. For therapy or preventive reasons, it is necessary to characterize and quantify the radiation damages created in irradiated cells in order to understand the induced repair mechanisms and their consequences on the cell structure.

Three main stages can be distinguished when describing the interaction of ionizing radiations within a cell: the physical track of the ionizing radiation in the cell matter (leading to energy deposition and radical creation), the diffusion and reactions of the created chemical species and finally the bio-chemical and biological stage that accounts for the chemical changes produced in the cell and the induced biological repairing mechanisms.

The work presented here is focused on the first stage. Two different Monte Carlo codes (GEANT4 (1) and a water radiolysis code LQD (2)) have been used in order to calculate the track structure of 5 MeV protons in liquid water. The GEANT4 code used in this work has a complete set of low energy electron cross-sections from 7 eV until 10 keV (see Z. Francis presentation in this conference) whereas the LQD code transports the secondary electrons until thermalization (0.025 eV). Taking into account the transport of very low energy electrons (< 7 eV) during the track can be important in order to accurately model the chemical stage. The proximity functions are calculated from the distances between energy transfer points in the track and characterize the interaction topology of the radiation with the medium. Results of the proximity functions calculated with the two Monte Carlo codes will be presented here showing the effect of the very low energy electron transport.

Poster session II - Poster 40

VUV radiation impact on macromolecules observed with synchrotron radiation circular dichroism (SRCD)

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Synchrotron Radiation Circular Dichroism (SRCD) is an absorption spectroscopy method, which measures the differential absorption of circular left and right polarized light by chiral biological macromolecules. The spectral analysis of characteristic electronic transitions of the peptide bonds permits the secondary structure prediction of macromolecules in solution. It has been shown that synchrotron radiation, which produces high photon fluxes such as available on new 3rd generation synchrotron light sources, causes rapid deterioration of signal amplitudes due to denaturation of the protein sample. The origin of the denaturation is in direct correlation with the Vacuum Ultraviolet (VUV) radiation, in terms of the impact on the macromolecular structure and observed changes to tertiary spatial arrangement or folding pattern with exposure to the radiation. A series of experiments were done to distinguish between two possible mechanisms (local heating vs free radical formation) for the deterioration in signal. Using different proteins shown to be more or less susceptible to this radiation effect and inducing a conformational change of a test protein (albumin), secondary structural changes were observed in consecutive scans (increasing radiation exposure) on various international high flux SRCD beamlines. The data is consistent with the principal cause being denaturation due to local heating, rather than degradation due to free radical formation.

Whilst there was no detectable overall heating of the sample during the course of the experiment (as measured by a probe placed directly inside the sample cell), the heating could be specifically localised to microscopic regions within the sample. It is proposed that the process of radiation-induced heat denaturation involves heating of water molecules which are either bound to or buried inside the proteins, and thus form an integral part of the three dimensional structure of the protein.

The significance of these findings will be high-lighted and shall stimulate the discussion about low energy radiation damage of macromolecules in vitro with high flux light sources.

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A. J. Miles, Robert W. Janes, A. Brown, D. T. Clarke, J. C. Sutherland, Y. Tao, B. A. Wallace & S. V. Hoffmann (2008). *Light flux density threshold at which protein denaturation is induced by synchrotron radiation circular dichroism Beamlines.* J.SynchrotronRadiation **15**, 420–422

Poster session II - Poster 41

05ID-2 Beamline – Radiation Therapy Facility at the Canadian Light Source

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The BioMedical Imaging and Therapy (BMIT) facility will provide synchrotron specific imaging and therapy capabilities that will be used to rectify conditions in human medicine, veterinary medicine, agriculture, and other biomedical areas. The facility is comprised of 05ID-2 and 05B1-1 Beamlines and supporting laboratories.
The ID beamline will have a filtered white beam capability for microbeam radiation therapy (MRT). Filtered white beam for MRT will deliver up to 3000 Gy/s. Therapy is also foreseen with monochromatic beams, including Synchrotron Stereotactic Radiation Therapy (SSRT). Monochromatic X-ray flux of up to 10^{13} ph/s/cm^2 will be available.

Beamlines of the biomedical imaging and therapy facility at the Canadian light source--Part 2 T. W. Wysokinski, D. Chapman, G. Adams, M. Renier, P. Suortti, and W. Thomlinson, Nuclear Instruments and Methods in Physics Research Section A In Press, (2009).

Beamlines of the biomedical imaging and therapy facility at the Canadian light source--Part 1 T. W. Wysokinski, D. Chapman, G. Adams, M. Renier, P. Suortti, and W. Thomlinson, Nuclear Instruments and Methods in Physics Research A 582 (2007) 73-76.

Poster session II - Poster 42

Comparative methods for 18F-FET-PET-guided delineation of target volumes in high grade glioma

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Poster session II - Poster 43

Peripheral blood lymphocytes as biodosimetric marker

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Irradiation of human organism activates in each person biological processes which could be used for estimating the recieved dose of irradiation. Physical dosimetres dispose with many restrictions, which bring along relative high divergence in measurement absorbed dose of irradiation and information obtained by this way do not predicate enough about the condition of irradiated pacient. Beside that, in the case of nuclear accidents, there would be important deficiency of these data. The biological effect of irradiation is individual, depending on many factors. Biodosimetry is potencial excellent way how to establish the level of influence of each person in relative short time and due to this, set them the best treatment.

Decline of hematopoiesis belongs to one of symptoms of acute disease of irradiation. Peripheral blood lymphocytes are known as radiosensitive cells which respond to irradiation by inducing apoptosis. Nevertheless individual subpopulations of lymhocytes embody different sensitivity against irradiation which could be potential important for biodosimetry. In our work we focused on radiosensitivity of B lymphocytes, cytotoxic T lymphocytes (T_C), helpers – T lymphocytes (T_H) and natural killers (NK) cells. On the experimental animal model of large white pig we described changes of representation of individual peripheral blood lymphocyte populations depending the absorbed dose of irradiation and the time of analyse. At the same time we set forward integrated results comparing *in vivo*, *in vitro* and *ex vivo* experimental design.

Porcine peripheral lymphocytes were immunophenotypized by indirect double color surface immunostaining and analysed by flow cytometry to determine radiosensitivity of small lymphocytes which were sort out by gating within forward scatter versus side scatter. Populations and subpopulations were classified by CD markers (IgM, CD2, CD3 and CD8) and were immuphenotypizated by mouse anti-porcine monoclonal antibodies visualized by secondary antibodies conjugated with fluoresceins FITC and PE. Results of changes were expressed by ratio of percentages of irradiated versus non-irradiated results (IVNIR).

Obtained data embodied B cells (IgM^+) as the most radiosensitive lymphocyte population. A weak decline of T lymphocytes ($CD3^+$), proved T cells as a biodosimetric marker in later analyses. Very important was the fact, that within *in vitro* analyse of non-irradiated negative control sample T_C cells ($CD3^+CD8^+$) embodied higher tendency to spontaneous apoptosis then T_H cells ($CD3^+CD8^-$). In irradiated samples this trend changed and T_H lymphocytes were found out more radiosenzitive then relative radioresistent T_C cells. The most radioresistant lymphocytes in porcine peripheral blood were NK cells (CD3-CD8+).

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