



Melusyn Workshop

Synchrotron Radiation Based Radiobiology

Synchrotron SOLEIL - June 25 and 26, 2007

91190 SAINT-AUBIN (20 km from Paris)

Melusyn : Medicine and Synchrotron light

Topics

- *Molecular lesions and collective processes*
- *Primary steps of signaling*
- *Differentiation and radiosensitivity*

Scientific committee

Chairmen:

Y. Gauduel (LOA, Inserm, École Polytechnique-ENSTA, Palaiseau)
C. Miron (SOLEIL, St Aubin)

J. Cadet (CEA, Grenoble)
N. Foray (Inserm, ESRF Grenoble)
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S. Lacombe (LCAM, Paris Sud)
D. Markovitsi (LFP, CNRS-CEA, Saclay)
M. Martin (CEA, Evry)
L. Sabatier (CEA, Fontenay aux Roses)
M. Spothem Maurizot (CBM, CNRS, Orléans)

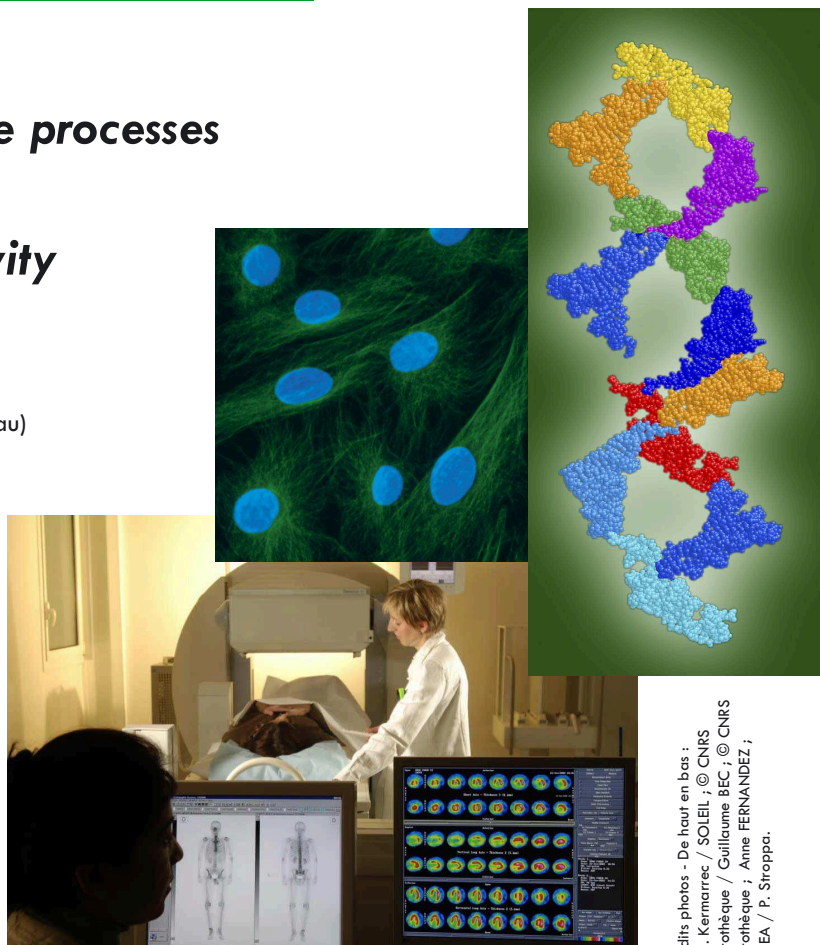
Local organizing committee

ECRIN : J.P. Gex, V. Sivan, D. Von Euw
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MELUSYN Workshop
Synchrotron radiation based radiobiology

June 25 and 26, 2007

Synchrotron SOLEIL, Saint-Aubin, FRANCE

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MELUSYN Workshop

Synchrotron radiation based radiobiology

June 25 and 26, 2007

Synchrotron SOLEIL, Saint-Aubin, FRANCE

SOLEIL, the new French synchrotron radiation facility, will provide the scientific community with one of the most advanced (brilliant, variably polarized and highly monochromatic) tunable light sources ranging from the infrared to the hard X-rays. The MELUSYN* workshops, created jointly through a collaborative work between SOLEIL and ECRIN are gathering together actors from the academic research, applied and medical research as well as private companies and clinicians, to take advantage of the high quality scientific and technological environment developed around SOLEIL.

One of the main transverse topics which are developed across MELUSYN is spatio-temporal Radiobiology involving radiation in the UV, VUV, soft and hard X-ray range. This represents an interdisciplinary field of study driven nowadays in strong synergy with the recent progress of complex medical imaging techniques or ionizing radiation therapies and namely cancer therapy. Using the unique properties of synchrotron radiation, as well as the wide panel of investigation techniques like spectroscopy and imaging, eventually operated in time resolved mode or with nanometric beams, SOLEIL is expected to provide new insight into yet unknown aspects of radiobiology.

The scope of the two day workshop is bringing together physicists, chemists, biochemists, biologists, genetics experts as well as physicians with a common interest in using synchrotron radiation and related techniques to explore various aspects of modern radiobiology and radiation therapy. 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 signaling, genomic instability, apoptosis, radio sensitivity, Bystander effect etc. should have in the near future many practical consequences like the customization of radiation therapy or radioprotection protocols for instance.

The workshop will be organized at the SOLEIL site around four thematic sessions. The workshop time schedule and detailed scientific program are given here below.

*Medicine and synchrotron light

Scientific committee:

J. Cadet (CEA Grenoble)
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M. Martin (CEA Evry)
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L. Sabatier (CEA Fontenay aux Roses)
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D. Von Euw (ECRIN)

MELUSYN Workshop Synchrotron Radiation Based Radiobiology

June 25-26, 2007

Synchrotron SOLEIL – 91190 Saint-Aubin (France)

Time schedule

Monday – June 25 th		Tuesday – June 26 th	
		8 :30	Session 3 Primary steps of signaling
		<i>10:30-10:50 Coffee break</i>	
		12:10	
13:00	Welcome		Lunch
13:30	Introductory session	13:30	Session 4 Differentiation and radio sensitivity
14:30	Session 2		
<i>15:50-16:10 Coffee break</i>	Molecular lesions and collective processes	<i>15:30-15:50 Coffee break</i>	
17:30	Visit of SOLEIL	17:00	Round table Prospective synthesis
	Discussions around beamline posters		
19:30	Dinner at SOLEIL	18:00	<i>End of the workshop</i>

Scientific Program

Monday – June 25th, 2007

13:00 - 13:30 *Welcome and coffee*

Introductory session

13:30 - 13:45 Jean DOUCET (SOLEIL) - Jean-Pierre GEX (ECRIN)

13:45 - 14:00 Denis RAOUX - Directeur général de SOLEIL

14:00 - 14:30 Yann A. GAUDUEL (INSERM, LOA-CNRS UMR 7639, Ecole Polytechnique – ENSTA)
New challenges for spatio-temporal radiation biology

Session 2

Molecular lesions and collectives processes

Chair : D. Markovitsi – E. Sage

14:30 - 15:10 Katsumi KOBAYASHI (Tsukuba, Japan)
Spectroscopy of biological samples. From absorption to biological effects.

15:10 - 15:50 Alain TOUATI (IMPMC, Université Paris 6, Paris 7)
Radiobiological effects in cell. The role of DNA core ionisation, as probed by ultrasoft X-rays

15:50 - 16:10 *Coffee break*

16:10 - 16:50 Dimitra MARKOVITSI (Lab. Francis Perrin, CNRS URA 2453, CEA Saclay)
UV induced DNA damage studied by time-resolved spectroscopy

16:50 - 17:30 Christophe NICOLAS (SOLEIL)
Soft X-ray synchrotron radiation studies of isolated biomolecules: towards a realistic “bottom-up” description of radiation damage on DNA

17:30 - 19:00 **Visit of SOLEIL** – Discussion around beamline posters

19H30 *Dinner at SOLEIL*

Tuesday – June 26th, 2007

Session 3

Primary steps of signaling

Chair: C. Houée Lévin – L. Sabatier

8:30 - 9:10 Chantal HOUÉE LEVIN (Lab. Chimie Physique, CNRS UMR 8000, Université Paris Sud)
From free radicals to signal induction

9:10 - 9:50 Jean CADET (CEA Grenoble)
Molecular effects of ionizing radiation on cellular DNA

9:50 - 10:30 Georg BAUER (University of Freiburg, Germany)
Low dose radiation and intercellular induction of apoptosis: potential impact on the control of oncogenesis

- 10:30 - 10:50 *Coffee break*
- 10:50 - 11:30 François PARIS (INSERM U 601, Nantes)
Ionizing radiation induces p38-mediated endothelial cell death through ceramide generation and membrane remodeling
- 11:30 - **12:10** Chrysostomos CHATGILIALOGLU (ISOF-CNR, Bologna, Italy)
Trans fatty acids and radical stress

12:30 – 13:30 *Lunch at SOLEIL*

Session 4 **Differentiation and radio sensitivity**

Chair: M. Martin – F. Moati – J.M. Constans

- 13:30** - 14:10 Elizabeth SCHULTKE (College of Medicine, Saskatoon, Canada)
Microbeam radiation therapy - a therapeutic concept for the treatment of malignant brain tumors
- 14:10 - 14:40 Michèle MARTIN (Lab. de Génomique Fonctionnelle, CEA Evry)
Sensing radiosensitivity of human epidermal stem cells
- 14:40 - 15:10 Laure SABATIER (Lab. Radiobiologie et Oncologie, CEA Fontenay aux Roses)
Damage transmission in the progeny of irradiated human cells
- 15:10 - 15:40 Carine LAFFON (LCPMR, Université Pierre et Marie Curie)
Low energy NEXAFS spectroscopy: new tool for studying the radical chemistry induced by radiosensitizers
- 15:40 - 16:00 *Coffee break*
- 16:00 - 16:30 Nicolas FORAY (INSERM, ESRF, Grenoble)
Recent advances in anti-cancer strategies using synchrotron X-rays
- 16:30 - **17:00** Jean-Marc CONSTANS (CHU Caen)
About spectroscopic measures variability in the evaluation of the therapeutic answer of glial tumours

Round table Prospective synthesis

Chair: C. Miron, Y. Gauduel

- 17:00 -18:00** Anne FLURY-HERARD (CEA) - Jean BOURHIS (IGR, Villejuif) – Géraldine Le Duc (ESRF, Grenoble) - Jacques BALOSSO (CHU Grenoble) - Hafid BELHADJ-TAHAR (Groupe Santé Recherche, Toulouse)
- 18 :00 End of the workshop

List of Abstracts

Introductory session

- IT-01** Yann A. GAUDUEL (INSERM, LOA-CNRS UMR 7639 , Ecole Polytechnique – ENSTA)
New challenges for spatio-temporal radiation biology

Molecular lesions and collectives processes

- IT-02** Katsumi KOBAYASHI (Tsukuba, Japan)
Spectroscopy of biological samples. From absorption to biological effects.
- IT-03** Alain TOUATI (IMPMC, Université Paris 6, Paris 7)
Radiobiological effects in cell. The role of DNA core ionisation, as probed by ultrasoft X-rays
- IT-04** Dimitra MARKOVITSI (Lab. Francis Perrin, CNRS URA 2453, CEA Saclay)
UV induced DNA damage studied by time-resolved spectroscopy
- IT-05** Christophe NICOLAS (SOLEIL)
Soft X-ray synchrotron radiation studies of isolated biomolecules: towards a realistic “bottom-up” description of radiation damage on DNA

Primary steps of signaling

- IT-06** Chantal HOUEE LEVIN (Lab. Chimie Physique, CNRS UMR 8000, Université Paris Sud)
From free radicals to signal induction
- IT-07** Jean CADET (CEA Grenoble)
Molecular effects of ionizing radiation on cellular DNA
- IT-08** Georg BAUER (University of Freiburg, Germany)
Low dose radiation and intercellular induction of apoptosis: potential impact on the control of oncogenesis
- IT-09** François PARIS (INSERM U 601, Nantes)
Ionizing radiation induces p38-mediated endothelial cell death through ceramide generation and membrane remodeling
- IT-10** Chrissyostomos CHATGILIALOGLU (ISOF-CNR, Bologna, Italy)
Trans fatty acids and radical stress

Differentiation and radio sensitivity

- IT-11** Elizabeth SCHULTKE (College of Medicine, Saskatoon, Canada)
Microbeam radiation therapy - a therapeutic concept for the treatment of malignant brain tumors
- IT-12** Michèle MARTIN (Lab. de Génomique Fonctionnelle, CEA Evry)
Sensing radiosensitivity of human epidermal stem cells
- IT-13** Laure SABATIER (Lab. Radiobiologie et Oncologie, CEA Fontenay aux Roses)
Damage transmission in the progeny of irradiated human cells
- IT-14** Sandrine LACOMBE (LCAM, Université Paris Sud)
Low energy NEXAFS spectroscopy: new tool for studying the radical chemistry induced by radiosensitizers
- IT-15** Nicolas FORAY (INSERM, ESRF, Grenoble)
Recent advances in anti-cancer strategies using synchrotron X-rays
- IT-16** Jean-Marc CONSTANS (CHU Caen)
About spectroscopic measures variability in the evaluation of the therapeutic answer of glial tumours

New challenges for spatio-temporal radiation biology

Yann A. Gauduel

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It is commonly admitted that the initial spatial distribution of energy deposition following the interaction of ionising radiations (UV and X rays, accelerated particles) with biomolecules and living targets is decisive for the behaviour and control of temporal radiation effects occurring on several orders of magnitude (early molecular damages, DNA repairs and cellular response mechanisms, signaling processes and gene expression, genomic instabilities, apoptosis, carcinogenesis and clinical effects).

The complex links between radiation physics and radiobiology concern the understanding of spatio-temporal events triggered by an energy deposition in confined sub- micrometric ionisation spaces (spurs). Microscopic radiation effects on integrated biological targets such as water molecules “the solvent of life” embedded in biomolecular architectures cannot be satisfactorily described from a dose delivery profile or a linear energy transfer (LET). Early biomolecular radiation damages being dependent on the survival probability of secondary electrons and radial distribution of short-lived radicals inside nascent ionisation clusters, a thorough knowledge of these processes require real-time probing of events on sub-micrometric scale, in the window $\sim 10^{-15} - 10^{-9}$ s.

In this context, a board panel of available radiation sources such as synchrotron radiation, pulsed laser systems and relativistic particle beams open opportunities for complementary investigations related to spatio-temporal radiobiology and low-high energy radiation medical physics, typically in the range $10^0 - 10^6$ eV. Some recent innovating advances will be illustrated by showing how a thorough knowledge of elementary phenomena that assist or impede early biomolecular radiation damages require a direct observation of transient electronic configurations in confined spaces. That implies a synergy between low and high energy radiation femtochemistry (LERF, HERF)*.

New challenges offer exciting perspectives for the sensitisation of confined environments (aqueous groove of DNA, protein pockets, sub-cellular entities) to ionising radiation, for which target volumes of mass per area in size of about 1×10^{-6} g cm⁻² correspond to 100 Å at a density of 1.0 g cm⁻³. This domain would foreshadow the development of radiobiology in the prethermal regime (real-time nanodosimetry, selective pro-drogue activation using quantum states of short-lived radicals). Regarding the specificities of SOLEIL synchrotron, we attempt to address open questions on radiation biology in the framework of a tenuous borderline between direct and indirect damages of living matter.

* Gauduel et al., J. Am. Chem. Soc., vol. 122 (2000) ; Rad. Phys. Chem., vol. 72 (2005); SPIE “Genetically Engineered and Optical Probes for Biomedical Applications”, vol. 6449 (2007)

Spectroscopy of biological samples *From absorption to biological effects*

Katsumi Kobayashi

Photon Factory, IMSS, KEK, Japan

All atoms and molecules can absorb photons of certain energy. Absorbed photon energy excites the atom or molecule into an excited state. Excitation energy is sometimes dissipated in the surrounding system without leaving any changes. In some cases, the energy causes changes not only in photon-absorbing molecule itself, but also in many surrounding molecules. These processes have been intensively studied by atomic physicists, solid state physicists and photochemists, depending on the system they are interested in. Measurement of photon-energy dependence is a commonly used method in these researches, and due to this reason, this type of research work could be classified as “spectroscopy” in a wide sense. When a photoabsorption event occurs in biological systems such as living cells, this event may cause some significant effects on the system (photobiological effects). Initiating processes are clearly the same as those studied in atomic physics or photochemistry. Induced molecular changes are recognized and modified by various enzymes in intracellular environment, and some of them are repaired and unrepaired changes, or damages, may cause macroscopic changes, such as cell kill or mutation, in the biological system.

Before 1980, photobiological studies were restricted to the wavelength region above 190 nm, due to the lack of practical light source. Soon after synchrotron radiation became available, Japanese research group started to study photobiology of vacuum ultraviolet radiation at INS-SOR ring in 1977, and extended their study to soft X-ray region up to 20 keV at the Photon Factory in 1983. Research works have been focused on the effect of transition from photoexcitation to photoionization, and on the effect of inner-shell ionization followed by Auger effect. Photo-electron energy dependence of some biological effects has also been studied from radiobiological point of view. Summary of these studies for over 30 years will be presented.

Radiobiological effects in cell. The role of DNA core ionisation, as probed by ultrasoft Xrays

Alain Touati

IMPMC, Université Paris 6

Looking to the paradoxical behaviour of inactivation cross sections versus LET of heavy incident charged particles, we had demonstrated (1) (2) , for a large range of energetic ions, a striking correlation between the inactivation of various cells and inner-shell ionization cross section in carbon, nitrogen and oxygen atoms of the DNA molecule.

This so called **core event** not only includes the inner- shell ionized DNA atom, but also the damages which are created in its vicinity by the Auger and ejected electron *via* interactions directly within DNA or indirectly through reactions with radicals produced in the surrounding water molecules.

To study the role of such a critical primary physical process, ultrasoft X-rays are a very powerful and essential tool. To do this study, we realised a great number of experiments at the LURE facility (Orsay) and the results for various end points for V7 cells are presented here:

The relative biological efficiency (RBE) of ultrasoft X rays to induce cellular inactivation (2) (3), exchange type chromosomal aberrations (4) and double strand break induction, were found to strongly correlate the number of core events. More over, the DSB repair data indicate a significant decrease in the reparability of DSB when triggered by core ionization.

Finally, calculation based on Monte Carlo simulation (5), here presented, is giving an estimate of 75 ± 25 % contribution of core events to cell inactivation efficiency for gamma rays .

(1) Chetioui A. et al. *Int. J. Radiat. Biol.* 1994 ; 65 : 511-522.

(2) Hervé du Penhoat M.A. et al. *Radiation Research* 151: 649-58.

(3) Fayard B. et al. *Radiation Research* 157: 128-40.

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(5) Boissière A. et al. *Radiation Research* – 2007- 167: 493-500.

Soft X-ray synchrotron radiation studies of isolated biomolecules: towards a realistic “bottom-up” description of radiation damage on DNA

Christophe Nicolas, Catalin Miron and Paul Morin

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

A novel research program, concerning the interaction between gas phase biomolecules and soft X-rays delivered by the new French synchrotron light source SOLEIL will be carried out on the PLEIADES beamline¹.

Since photoabsorption is the first step of the sequence leading to biological consequences, photoabsorption spectroscopy will contribute to a better understanding of the radiobiological processes. This is why, synchrotron radiation, with its continuous spectrum over a wide photon energy range (from VUV to soft X-rays in the case of PLEIADES) is the most appropriated light source to perform such studies. Moreover atomic inner-shell relaxation processes in biomolecules create localized ballistic electron sources *via* the production of photoelectrons and Auger electrons. Several 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.² Being able to determine the energy distribution of the ejected electrons for a specific dissociation channel of the biomolecule or the different electronic states involved in its breakdown could be very helpful as new inputs in radiation damage models at the molecular level. In addition, even at constant low dose, the energy of the incident photons would certainly impact on the physical properties of the created particles, for example *via* resonant channels or by opening new dissociation pathways.

The sophisticated spectroscopic tools and methodologies available on the beamline using tunable soft X-rays delivered by SOLEIL or lasers are the key points in obtaining these data. The time structure of the synchrotron radiation could also be used for time resolved experiments. For example, the stability of elementary bricks of DNA or polypeptides will be probed either by photoelectron spectroscopy or combined with mass spectrometry in Auger electron/ion coincidence schemes. Thanks to the possible control of the solvation degree of biomolecules, gas phase studies should also provide the relevant answers concerning the role of the solvent (secondary electrons, free radicals etc.). An important issue will also be understanding the physical chemistry changes related to hydrogen bonding between the biomolecules and one or several water molecules.

¹ PLEIADES: <http://www.synchrotron-soleil.fr/portal/page/portal/Recherche/LignesLumiere/PLEIADES>

² B. Boudaïffa *et al.* Science 287 (2000).

From free radicals to signal induction

C. Houée-Levin

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The interaction of ionizing radiation with matter leads to electron ejection. As a consequence, free radicals are formed, which is the first step of radio-induced modifications. It is well known that DNA is a major target for free radicals since DNA modifications can induce cell death, genomic instabilities, cancer.... However it has been recently shown that free radical reactions with other cellular targets could also lead to dramatic events. The production of molecules able to cross membranes and to induce signals either intracellular or intercellular is proposed. Unfortunately the nature of these molecules and thus the identification of key cellular deletions are not unambiguously elucidated.

Among the possible important targets are cytosolic proteins, membrane proteins and lipids. The main chemical modifications induced on biopolymers are known. For instance the interconversion in the disulfide/dithiol system is known to trigger numerous events leading to the liberation of nuclear factors (NF- κ B) that control oxidative stress. Some of the reactions that lead to the production of small molecules like lipid peroxidation can also be invoked. The radiation chemistry of biomolecules should thus be revisited with the aim of identification of the entities that could trigger or control reactions leading to signal induction.

Molecular Effects of Ionizing Radiation on Cellular DNA

Jean Cadet, Thierry Douki, Noureddine Belmadoui, Peggy Regulus
and Jean-Luc Ravanat

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The main oxidative decomposition pathways of purine and pyrimidine nucleobases arising from the direct (one-electron oxidation) and indirect effects (mostly $\cdot\text{OH}$ radical) of ionizing radiation have been elucidated in isolated DNA and model compounds. This was inferred from the characterization of the transient radicals involved in these reactions and the final decomposition products. Among the 70 single base lesions identified, only 10 of them have been detected so far in cellular DNA upon exposure to gamma radiation. This has led to the validation of several of the main $\cdot\text{OH}$ radical-mediated radical oxidative pathways of thymine, guanine and adenine that were previously assessed in model studies. For this purpose a sensitive and accurate high-performance liquid chromatography - electrospray ionization tandem mass spectrometry assay has been designed. As other striking data we may quote the overwhelming formation of 8-oxo-7,8-dihydroguanine in the DNA of cells exposed to high intensity 266 nm laser pulses. This was rationalized in term of initial bi-photon ionization of the pyrimidine and purine bases of DNA, followed by hole migration to guanine bases acting as sinks. Evidence was recently provided for a new class of radiation-induced lesions both in isolated and cellular DNA as the result of initial OH radical-mediated oxidation of the sugar moiety at C4'. The highly reactive aldehyde thus generated while being still attached to the DNA backbone is able to add to proximate cytosine residue giving rise to 4 diastereomeric lesions. The cytosine adducts together with the DNA nick produced at the site of the oxidized sugar moiety constitute the first class of clustered damage involving a modified base identified so far. It would be of interest to apply the above strategy in order to investigate the damaging effects of monochromatic X-rays provided by synchrotron sources on cellular DNA including those associated with Auger processes.

Low dose radiation and intercellular induction of apoptosis: potential impact on the control of oncogenesis

Georg Bauer

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Transformed target cells are subject to efficient apoptosis induction exerted by nontransformed effector cells. This reactive oxygen (ROS)-mediated process has been termed „intercellular induction of apoptosis“ and has been shown to be relevant for a variety of effector and target cell systems. In addition to intercellular induction of apoptosis, transformed cells can also be eliminated by ROS-mediated autocrine selfdestruction. In this process, the effector molecules are generated by the transformed target cells themselves and establish ROS-mediated signaling between transformed cells. Sensitivity to intercellular induction of apoptosis and autocrine selfdestruction are strictly correlated to the expression of the transformed state.

Selectivity as well as efficiency of intercellular induction of apoptosis are based on reactive oxygen and nitrogen species. Extracellular superoxide anions generated by transformed target cells represent the central element in this signaling system. They allow and control four different signaling pathways, based on HOCl / hydroxyl radicals, NO / peroxynitrite, nitrylchloride and on the metal-catalyzed Haber-Weiss reaction. Dependent on the spatial situation of the cells and on the concentrations of the various players in this system, these four signaling pathways may act synergistically or may show negative interference. The central elements of this extracellular signaling chemistry have been elucidated through the use of specific scavengers and through small interfering RNA (siRNA)-mediated genetic knock-down.

Tumor formation and progression *in vivo* seem to depend on the selection of transformed cells that are resistant against intercellular induction of apoptosis and autocrine selfdestruction. Intercellular induction of apoptosis and autocrine apoptotic selfdestruction are discussed as a hitherto unrecognized control system during oncogenesis.

Low dose irradiation of nontransformed or transformed cells enhances intercellular induction of apoptosis. This interaction is controlled by TGF-beta. It seems to depend on the induction of peroxidase release in nontransformed as well as in transformed cells. In addition, low dose radiation enhances superoxide anion generation of transformed target cells. The stimulating effects of low dose radiation on intercellular induction of apoptosis might explain the well-known inhibitory effect of low dose radiation on detectable transformation frequency. However, modifications of the complex intercellular ROS - based signaling system may also lead to configurations in which low dose radiation protects transformed cells from elimination. These findings may be relevant for the understanding of the complex picture of low dose radiation effects *in vivo*.

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

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Department de Recherche en Cancérologie, INSERM U601, Nantes, France

Radiotherapy protocols were defined to target and kill cancer cells. New developments showed that tumor endothelium is a putative target to enhance radiotherapeutical efficiency. First observations have been made in normal tissue model. Gastrointestinal syndrome (GI) is a common radiotoxicity side effect which induces destruction of the small intestines. Single dose of 15 Gy induced destruction of the intestinal mucosa and subsequent death of the animals. Apoptotic TUNEL assays revealed that the first cells to die are endothelial cells located in the *lamina propria* and around the crypts (Paris et al Science 2001). Protection of the endothelium compartment pharmacologically, by injection of basic fibroblastic growth factor or Sphingosine 1 Phosphate, or genetically, by disruption of the acidic sphingomyelinase gene (*asmase*), enzyme involved in the generation of the pro-apoptotic factor ceramide, prevented occurrence of the GI syndrome and subsequent death of mice. If aSMase/ceramide pathway is clearly involved in endothelial radiosensitivity, the p38 MAPK transduction pathway have been recently described to be also activated during radiation-induced microvascular endothelial cell death (Kumar JBC 2004). However, connection between the two pathways has never been reported. In this present abstract, we demonstrated that radiation-induced ceramide generation activates the p38 pathway through the membrane remodeling and the coalescence of rafts in large lipidic platforms.

First, we confirm that high dose of radiation activates the p38 death pathway using several primary endothelial cells, such as HUVEC and HMVEC, but also in SV40-transformed endothelial cell line HMEC-1. Besides, this activation was mimicked by pharmacological application of aSMase and ceramide. The p38 blockade by MAPK inhibitor III decreased radiation induced death of HMEC-1 by 30%(±11.3). Concomitantly, ceramide generation in the membrane appears in HMEC-1 within minutes after exposure to 15 Gy, followed by the deep relocalisation of the raft-marker ganglioside GM1, from a scattered, discret pattern, to large areas on the cell membrane. Finally, disorganization of rafts by drugs as nystatin, hindered the activation of p38 and the subsequent death-induction of microvascular cells (death-decrease by 35.03±1.8%). Proof of concept is supported by the aSMase inhibition, preventing both rafts-aggregation and radiation-induced death.

These results clearly demonstrate a connection between ceramide-induced membrane remodeling and radiation-induced death signaling leading to microvascular apoptosis, and suggest a specific membrane-controlled death mechanism, independent of the DNA damage generation, in endothelial cells submitted to radiotherapy.

Trans Fatty Acids and Radical Stress

Chryssostomos Chatgililoglu

ISOF, Consiglio Nazionale delle Ricerche, Bologna, Italy

Trans fatty acids are well known to have an exogenous origin, since those present in the diet can be incorporated in tissues. Their biological role and adverse health effects have been largely studied.

However, some trans fatty acids found in humans can only arise via an endogenous transformation of the naturally occurring cis structures and are correlated with radical stress produced during physiological and pathological processes.¹⁻³ Based on biomimetic chemistry studies, diffusible sulfur-centered radicals are quoted to be the candidate isomerizing species. An example of these species is methanethiyl radicals derived from the reductive modification of methionine residues in peptides and proteins.⁴⁻⁶ The use of trans fatty acid library can be advantageous in lipidomic research to distinguish between endogenous (radical stress) and exogenous (diet) source, and in particular for investigation of health conditions associated with radical stress. This knowledge has been used by the spin-off company LIPINUTRAGEN to develop a tool for lipidomics of radical stress and trans fatty acid detection such as the FAT-PROFILE®.⁷ By this approach a global evaluation of the individual lipid metabolism can be obtained for the personalization of therapies and implementation of the quality of life.

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- (7) See, <http://www.lipinutragen.it>

**Microbeam radiation therapy:
a therapeutic concept for the treatment of malignant brain tumours**

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The concept of microbeam radiation therapy (MRT) has been developed during the 1990s at the NSLS in Brookhaven, USA and at the ESRF in Grenoble, France. Instead of using a broad beam in the MeV range for tumour irradiation, which is typical for the hospital environment, highly collimated synchrotron beam in the keV range is split with the help of a special collimator into an array of small near-parallel beams in the micrometer range. This creates a characteristic profile of dose peaks and dose valleys in the irradiated tissue. MRT allows us to deposit X-ray doses into the tumour that are higher by two orders of magnitude compared to the doses used in hospital-based radiotherapy programs. This could prove a therapeutic advantage especially where the target tumours are extremely radioresistant. Animal studies have shown that therapeutic success can be achieved where the concept of MRT is combined with grid therapy, a radiotherapy concept that had been explored in clinical trials earlier in the 20th century. Our research group has studied the effects of MRT in small animal models of malignant brain tumour and data from those studies will be discussed.

Functional genomics and radiosensitivity of human keratinocytes: from differentiated to stem cells

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Skin epidermis is a physiological barrier that protects the organism against pathogens, and chemical or physical damage. Functional role of keratinocytes differ according to their differentiation level. Undifferentiated keratinocytes proliferate in the basal layer, which contains both the proliferating progenitors and the quiescent stem cells. Then keratinocytes migrate to the upper layers and progressively differentiate up to the terminal differentiation in the horny layer. Despite improvements in radiation techniques, patients still experience radiation toxicity of the skin during and after radiotherapy. Although basal keratinocytes have long been proposed as the main targets of radiation, the exact roles of these cells are still largely unknown. Thus we started a research program to define the radiosensitivity of keratinocytes according to their differentiation status. In a first study, we characterized the response of differentiated keratinocytes to low and high doses of γ -rays. We found that keratinocytes from the upper layers of epidermis are radioresistant. Using large scale gene expression analysis (microarrays), we found that the molecular response of differentiated keratinocytes to a 2 Gy dose was characterized by a global reprogramming of genes involved in energy production. The response to a low dose (10 mGy) was completely different. We identified low-dose specific genes, including transcription factors of the GATA family. To characterize the radiosensitivity of undifferentiated, basal keratinocytes, we used flow cytometry to isolate the stem cells and the progenitors directly from normal human skin. We demonstrated that stem cells were more radioresistant than the progenitors. Moreover, we found that stem cells exhibited a more rapid and efficient repair of DNA double strand breaks. In conclusion, human epidermis is a fascinating model to analyse a complex tissue response to radiation, with both sensitive and resistant cell populations. We will discuss how synchrotron-based technology may help in the future to get further knowledge on the response of the skin tissue to irradiation.

Damage transmission in the progeny of irradiated human cells

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Studies on the transmission of radiation-induced damages through several cell generations have highlighted the complexity of the mechanisms involved. Over the last two decades there is increasing evidence that accumulation of DNA damage continues in the progeny of irradiated cells. Most of these chromosome changes are similar to those seen in cancer cells. When explanted from tissue into in vitro culture, normal cells undergo a finite number of divisions and thereafter enter a non-replicative state termed replicative or cellular senescence. Senescence is accepted as being the result of cumulative oxidative damage and telomere shortening, probably acting at different degrees according to cell or environment. The **role of telomere** was also demonstrated in the **chromosomal instability** detected in the long term progeny of irradiated human fibroblasts. These end-to-end fusions could be linked to telomere shortening associated with cell divisions or to telomere loss due to a DNA double-strand break occurring near the end of a chromosome. In both cases this leads to formation of ter-ter dicentrics that could initiate a more extensive chromosomal instability leading to **large LOH** (Mb) due to the loss of whole chromosome arms.

Our results let us propose the following hypothetical scheme of **radiation oncogenesis** : a) induction of recessive gene mutations (direct effect of radiations) b) accumulation of genomic alteration in the irradiated tissues with aging and proliferation of irradiated and non irradiated cells c) unmasking, amplification... of radiation induced or pre-existing mutations d) loss of tumour suppressor functions (aneuploidy), e) gain of proliferative advantage f) on going instability: cycles of b-e steps g) initiation and progression of multistage carcinogenesis. One of our running hypothesis is that the heterogeneity of telomere length (for each telomere of each chromosome) would permit or not the unmasking of radiation-induced mutations (via chromosome imbalances, LOH). This unmasking events might be crucial in the long term effect of IR because the large majority of the **radiation-induced mutations** are **recessive** i.e will remain silent until the occurrence of a mutation on the second allele (highly improbable at low dose) or **LOH**.

Association of NEXAFS spectroscopy and cryogeny for studying the effect of radiosensitizers on water radiolysis

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Oxygen derived radicals are known to play a fundamental role in the biochemistry of living cells exposed to ionising radiations. In particular the damages induced in irradiated DNA and its components are attributed to the effect of OH° radicals. In presence of radio-sensitizers, the amplification of the DNA damages irradiated by X-Rays or fast ions is commonly attributed to an increase of the production of OH° radicals (1 and references therein). Such conclusions stems from studies based on chemical methods such as the use of OH° quenchers (ex: DMSO). However it exists no direct evidence of the production of OH° by radi osensitizing agents.

In recent studies, the association of the X Ray Absorption Spectroscopy (NEXAFS) coupled to cryogeny has been improved for studying the production of transient species in molecular films. In particular, the group has demonstrated with this method, the production of radicals such as O°, OH° and HO₂° in the irradiated water ice, in addition to the m olecular products such as O₂ and H₂O₂ (2). The induced reactivity is strongly perturbed by the environment, in particular in presence of molecular oxygen.

This tool could be used for investigating the production of all the transient species produced in condensed water in presence of molecular radiosensitizers such as Cisplatine, chloro-terpyridine platine but also platinum nanoparticles. These experiments could take place in SOLEIL synchrotron.

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Recent advances in anti-cancer strategies using synchrotron X-rays

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Synchrotrons are opening a new gate in innovating anti-cancer radiotherapy strategies. Indeed, fluence of X-rays induced by synchrotrons is so high (10^6 higher than standard medical irradiators) that it makes possible the production of powerful X-ray beams tunable in energy (*monochromatic* beams) and in size (*micrometric* beams). *Monochromatic* synchrotron X-rays beams theoretically permit to *photoactivate* high-Z elements introduced in or close to tumours in order to increase the yield of damage by enhanced energy photoabsorption. This is notably the case of attempts with iodinated contrast agents used in tumour imaging (the CT therapy approach) and with platinated agents used in chemotherapy (the PAT-Plat approach). *Micrometric* synchrotron X-rays beams theoretically permit to accumulate very high radiations dose into tumours by using arrays of parallel microplanar beams that would spare surrounding tissues (the MRT approach). All these anti-cancer applications of synchrotron radiation are mainly developed at the European Synchrotron Radiation Facility and deal with glioma, one of the most refractory tumour tissues to standard treatments. Here are reviewed the molecular and cellular mechanisms involved in these three approaches, in the frame of new advances of radiobiology. Furthermore, by considering the unavoidable biases, we attempted to propose a comparison of the different results obtained in preclinical trials dealing with rats bearing tumours.

About spectroscopic measures variability in the evaluation of the therapeutic answer of glial tumours

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Proton MRS of the brain enables us to identify, by using a standard MRI machine and non-invasive means, and quantify resonances of neurochemical compounds to be used in the study of neuronal and glial metabolism, and especially that of glial tumor metabolism. Taking into account normal variability and reproducibility and also anatomical region reduces variability. However, it is still variability due to anatomical location (brainstem or sustentorial), instrumental factors, tumoral tissue types and grades, tumoral evolution, heterogeneity and biology, type and combination of treatments (chemotherapy and radiotherapy), delay between the end of a treatment and the MRS exam, MRS measures and relaxation times, tumoral environment. Furthermore, type of therapeutic responses: oxidative stress, mitochondrial, pH and metabolic responses to treatment and, finally, tumoral individual variability and other unknown factors are also sources of variability.

One potential solution is to better understand this variability by longitudinal follow up and improved, automated segmentation to determine cerebral variations in MRS (with multiple TEs) and MRI of areas, amplitudes, and ratios of metabolites or spectral profiles during a longitudinal follow-up of patients with glial tumor treated with chemotherapy or radiotherapy. Data processing yields amplitudes, areas, ratios, and relative concentrations. Statistical analysis of longitudinal spectroscopic data of magnetic resonance spectroscopy (MRS) signals and longitudinal statistical study allows us to improve quality of ratios or/and concentrations variations estimations. Quantitative studies in MRI with multi-spectral segmentation with spatial matching fusion and tissular classifications are ongoing as well as spectral differential analysis between 2 groups of glial tumors. Without chemotherapy spectroscopic profiles worsen with variable increases of Choline/N-Acetyl-Aspartate (Cho/NAA), Myoinositol/Creatine (Myo/Cr) ratios, NAA/Cr decreases and sometimes lactate increases. Treated tumoral area and volumes, in MRI, changes little between two exams while spectroscopic profiles and ratios change more and sometimes revert back towards more normal values. In some other cases ratios remain stable or worsen. MRS could in fact, be more sensitive than MRI.

Conclusion : MRS allows sensitive non-invasive follow-up of treated cerebral tumors. There is a large variability but the repetition and modelisation and quantitation of spectroscopic measures during longitudinal follow-up could allow us to diminish it and to improve prognosis evaluation and variability assessment. To study the relationship with perfusion parameters and angiogenesis studying vasculature on histological tissues and animal models is also an important approach. Lastly, the goal is to try to better understand therapeutic response variability parameters, especially to radiation, chemotherapy and antiangiogenic molecules. This could be done with synchrotron techniques on cell cultures, histological tissues, animal models and finally, in the distant future in patients and be complementary to other techniques.