ABSTRACT BOOK

Third International MELODI Workshop

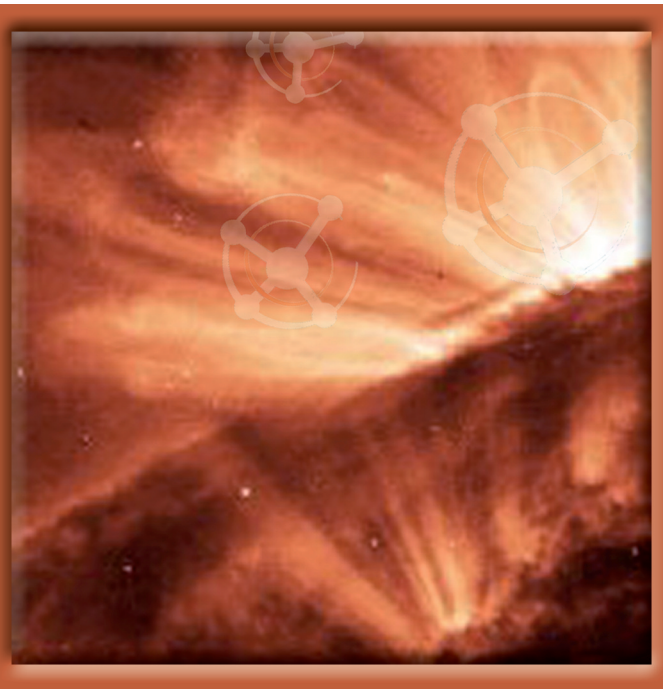
Rome, 2 - 4 November 2011

MELODI

ISTITVTO SVPERIORE DI SANITA

ENEA

ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES,
ENERGY AND SUSTAINABLE ECONOMIC DEVELOPMENT



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Rome (Italy) - November 2-4, 2011

Abstract Book

2011 ENEA

Italian national agency for new technologies, energy and sustainable economic development

Lungotevere Thaon di Revel, 76
00196 Rome

MELODI members

BfS Federal Office for Radiation Protection, Germany
CEA Atomic Energy Commission, France
HMGU Helmholtz Zentrum München German Research Centre for Environmental Health, Germany
HPA Health Protection Agency, United Kingdom
IRSN Institute for Radiological Protection and Nuclear Safety, France
ISS Istituto Superiore di Sanità, Italy
ITN Instituto Technologico e Nucleair, Portugal
KVSF Network of Competence in Radiation Research, Germany
Madrid University Universidad Autónoma de Madrid, Spain
NINCBP National Institute for Nuclear, Chemical and Biological Protection, Czech Republic
RIVM National Institute for Public Health and the Environment, The Netherlands
Rovira i Virgili University Laboratory of Toxicology and Environmental Health, Spain
SCK•CEN Belgian Nuclear Research Centre, Belgium
Stockholm University Centre for Radiation Protection Research, Sweden
STUK Radiation and Nuclear Safety Authority, Finland

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Foreword

Both natural and man-made sources of ionizing radiation contribute to human exposure and constitute a risk to human health. Exposure of the population to natural radiation is to some extent unavoidable and the medical use of radiation is now an indispensable part of modern healthcare. The exposure of workers and, to a smaller extent, of the public to low levels of radiation resulting from nuclear energy production and other industrial sources is a known consequence of our industrialized societies, and requires appropriate regulation. Radiation protection standards rely on the current knowledge of the risks from radiation exposure. Any over- or under-estimation of these risks could lead either to unnecessary restriction or to a lower level of protection than intended. Although much is known about the quantitative effects of exposure to ionizing radiation, considerable uncertainties and divergent views remain about health effects at low doses.

The purpose of MELODI, Multidisciplinary European Low Dose Initiative, is to promote and coordinate European research on the risks associated with low-dose exposure to ionizing radiation. MELODI is a research platform in accordance with relevant European Union policies, and will contribute to the definition of priority objectives and related programmes of low-dose risk research, identification of resources to devote to the achievement of these objectives, assessment of results, and dissemination of these issues among the various parties involved.

The ***Third International MELODI Workshop*** will offer attendants the opportunity to be updated on low-dose research issues and the possibility to actively participate in the discussion about the next steps for implementing the Strategic Research Agenda prepared and timely updated within MELODI.

Velio Macellari
Chair of the Third International MELODI Workshop

Programme

Wednesday, November 2

11:00 – 13:30 Registration
13:30 – 14:45 Welcome address

ISS	*Enrico Garaci*
ENEA	*Giovanni Lelli*
Italian Ministry of Health	*Fabrizio Oleari*
MELODI	*Jacques Repussard*
WHO	*Maria Del Rosario Pérez*
Japanese Low Dose Program	*Yutaka Yamada*
UNSCEAR	*Wolfgang Weiss*
EU	*Raffaele Liberali*

Opening session:

Chairpersons*: Velio Macellari and Carmela Marino*

14:45 – 15:45	MELODI Strategic Research Agenda	*Dietrich Averbeck*
15:45 – 16:15	*Coffee break*	

Epidemiology of ionizing radiation:

Chairpersons*: Elisabeth Cardis and Dominique Laurier*

16.15 – 17:15	DDREF: light and shadow	*Dale Preston*
17:15 – 18:15	Childhood leukemia and ionizing radiation	*Richard Wakeford*
18:15 – 18:30	General discussion	
18:30 – 20:00	*MELODI General Assembly*	

Thursday, November 3

The point of view of radioprotectionists:

Chairperson*: Peter Jacob*

08:30 – 9:15	What radiation protection can request from radiation research	*Patrick Smeesters*

Experimental models:

Chairpersons*: Sarah Baatout and Anna Saran*

09:15 – 10:15	Radiation effects on cancer- and non-cancer stem cells of human skin	*Michele Martin*
10:15 – 11:15	Radiation induced cardiovascular and cerebrovascular disease	*Fiona Stewart*
11:15 – 11:45	*Coffee break*	
11:45 – 12:45	Animal models and the analysis of the mechanisms determining individual sensitivity to radiation	*Michael Atkinson*

12:45 – 13:00	General discussion	
13:00 – 14:00	*Lunch*	

Biological mechanisms of radiation action:

Chairpersons*: Marco Durante and M. Antonella Tabocchini*

14:00 – 15:00	Age and gender relevance for the evaluation of radiation risk	*Wolfgang-Ulrich Müller*
15:00 – 16:00	The limitations of DNA double-strand break repair and checkpoint control after low radiation doses	*Markus Löbrich*
16.00 – 16:30	*Coffee break*	
16:30 – 17:30	Biomimetic models of radiation-induced radical stress and biomarker discovery	*Chryssostomos Chatgilialoglu*
17:30 – 17:45	General discussion	
17:45 – 18:30	Poster Session	
20:00 – 23:30	*Social Dinner*	

Friday, November 4

Targets of low dose radiation: physical and biological issues:

Chairpersons*: Mauro Belli and Mats Harms-Ringdahl*

09:00 – 10:00	Early events relevant for biological damage	*Andrea Ottolenghi*
10:00 – 11:00	Dose rate effects: spatial and temporal damage distribution	*Peter O'Neill*
11:10 – 11:45	*Coffee break*	
11:45 – 12:45	DNA damage response, senescence and cancer	*George Garinis*
12:45 – 13:00	General discussion	
13:00 – 14:00	*Lunch*	

DoReMi state of art:

Chairpersons*: Simon Bouffler and Jean-René Jourdain*

14:00 – 15.00	The EU NoE DoReMi: where we are	*Sisko Salomaa*

Workshop summary:

Chairpersons*: Dietrich Averbeck and Jacques Repussard*

15:00 - 15:45	Rapporteurs:	*Kevin Prise, Laure Sabatier*

Closing session:

15:45 – 16:00 *Carmela Marino, Velio Macellari*

Oral Presentations

MELODI Strategic Research Agenda

D. Averbeck[1,2] (chairman of SRA Working Group), D. Lloyd[3], P. O'Neill[4]

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[2]*Centre à l'Energie Atomique (CEA), Fontenay-aux-Roses, France;*
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The Multidisciplinary European Low Dose Initiative (MELODI) founded in 2009 has as its main goal, the promotion and consolidation of low dose radiation research on health risks and radioprotection in the European Community. To stimulate low dose risk research in Europe an open, integrative and holistic approach is proposed to attract new scientific competences from complementary disciplines, to ensure sustainability of infrastructures, education and training as well as communication with stakeholders, the public and international partners.
To achieve this, it is necessary to consolidate and promote European initiatives in research to better understand the health effects of low dose exposures by development of a Strategic Research Agenda (SRA) covering >20 years. A SRA is being developed by a working group taking into account the recommendations of the High Level Expert Group (www.melodi-online.eu), scientific outcomes from MELODI workshops, DoReMi, other international meetings and recent documents.

The second draft of the MELODI SRA is based on the key scientific research issues identified by HLEG to address the overarching questions: How robust is the current system of radiation protection and risk assessment? How can it be improved? What are the areas of uncertainties? What are the research needs, priorities and most suitable strategies?

Radiation protection at high radiation doses is established (ICRP) based to a large extent on epidemiological studies. Estimation of radiation risks at low doses <100 mGy, are expected to rely on mechanistic studies to fill the knowledge gaps as epidemiology does not have the statistical power at very low doses. The important question for low dose radiation-induced health risk is to determine the exposure levels at which existing internal regulatory and cellular defence systems in humans begin to be sufficiently perturbed by exposures to ionising radiation leading to cancer or non-cancer effects.

The SRA focuses on fundamental studies on regulatory mechanisms and pathways involving signalling/biodosimetry, metabolic perturbation and stress and pathological (disease specific) dysfunctions in normal cell, tissues and animal studies following low dose exposure. The SRA emphasizes the importance of developing susceptibility biomarkers associated with individual radiation sensitivity and related increased health risks. Appropriate epidemiological (pro- or retrospective) studies associated with molecular (mechanistic) studies (to increase validity in the low dose range) are included using bio- and data-banking facilities. Mathematical modelling is felt to be essential to complement mechanistic studies for estimating low dose radiation health risks. All the issues raised concern research on both cancer and non-cancer health effects to include studies incorporating the cross-cutting issues (radiation quality, tissue sensitivity and internal emitters) as well as gender, developmental stage and age effects. The SRA also addresses establishment of appropriate infrastructures education and training and interactions with stakeholders, the public and international bodies.
In the integration of low dose research activities, the MELODI SRA is subject to regular updating by the Scientific Advisory Committee of MELODI and the scientific community to also incorporate with future research developments.

DDREF: Light and Shadow

D. Preston

Hirosoft International, Eureka, CA, USA

Childhood Leukaemia and Ionising Radiation

R. Wakeford

Dalton Nuclear Institute, University of Manchester, UK

Leukaemia was the first cancer found to be in excess among the Japanese survivors of the atomic bombings of Hiroshima and Nagasaki. The increased risk of leukaemia among the survivors exposed as young children was particularly pronounced: the Excess Relative Risk (ERR, the proportional increase in risk above background) following an equivalent dose to the red bone marrow of 1 Sv rose to a marked peak approaching 100 a few years after exposure and then fell away quite rapidly, so that most of the excess risk was expressed as a wave within two decades of exposure – the minimum latent period for childhood leukaemia cannot be reliably determined from the survivors because systematic follow-up did not commence until 1950, and a notably increased risk was already apparent by then.

The particular sensitivity of children to radiation-induced leukaemia has been has been confirmed by studies of radiotherapeutic exposures, although not by all studies. Of some importance due to the low doses received during exposure are the case-control studies of childhood leukaemia and antenatal radiography, which show that fetal doses of ~10 mGy of X-rays are associated with an increased risk. The absence of childhood leukaemia among the Japanese atomic bomb survivors exposed *in utero* has been a matter of comment, but this may be related to the particular sensitivity of the fetal haematopoietic system to radiation-induced cell killing by moderate doses. Risk models for radiation-induced leukaemia have been developed principally from the experience of the Japanese atomic bomb survivors, and reflect the high ERR coefficient (ERR/Sv) for childhood leukaemia found among the survivors.

These risk models indicate that childhood leukaemia would be a good candidate disease for studies of low dose effects, provided that studies of sufficient size, and therefore of sufficient statistical power, can be conducted (which is a problem since leukaemia is a rare disease, affecting only 1 in 1800 children). The latest risk models suggest that ~15% of childhood leukaemia in Great Britain may be attributable to natural background radiation, mainly gamma radiation, although uncertainties are substantial; currently available childhood cancer incidence data may allow this prediction to be tested with reasonable power.

A further prediction is that a population prevalence of paediatric CT scanning of ~10% should be detectable by suitably designed epidemiological studies. The discovery of excesses of childhood leukaemia around certain nuclear installations has led to speculation that the risk of childhood leukaemia following exposure to radiation, especially from internally deposited radionuclides, has been grossly underestimated, but intense investigation has not confirmed this proposition. For example, a notable wave of childhood leukaemia did not occur after radioactive fallout during the period of intense atmospheric nuclear weapons testing in the late 1950s and early 1960s. The case-control studies of obstetric radiography and childhood leukaemia indicate that leukaemia risk models derived from data for moderate-to-high doses received at high-dose-rates are applicable to low dose or low dose-rate exposure circumstances, but confirmation of this from other studies is desirable, particularly given the predicted risk associated with paediatric CT scans, the prevalence of which is rising.

What Radiation Protection Can Request from Radiation Research

P. Smeesters

Belgian Federal Agency for Nuclear Control, Brussels, Belgium

There should be a fundamental and continuous loop linking research data about health and environmental effects of ionizing radiation, their evaluation for risk assessment, their potential implications for regulation and policy, the residual uncertainties and the need of further research. But there are in practice too few interactions between those coping with planning, financing or performing research and those interested in radiation protection (experts, practitioners, regulators, …). And there are even less interactions between the research community and the stakeholders in the society. This is particularly so with regard to radiobiology, whose language is often incomprehensible even for educated persons.

Yet there are essential *societal requirements* towards the research community (and by the way also towards the radiation protection community). Besides the obviously necessary competence, there is a societal implicit assumption of – and consequently a request for – neutrality, objectivity and priority concern about population's health and welfare. The compliance with these assumptions is in fact far from being self-evident.

Regarding neutrality, research depends on its financing and, in the case of radiation research, this financing – and the linked conditions- come often from national or institutional institutions with obvious conflicts of interest. This situation can obviously affect the expected degree of freedom and neutrality, self-censorship being then the easiest way for the researcher to avoid problems and to guarantee resources. Scientific independence is then a moral duty and a difficult challenge.

Secondly, science cannot avoid ethical issues, some of them being deeply imbricated (and often not seen by the researcher) *within* the area of the scientific work. Some examples are: active identification of the potential health issues, resistance to the pressure of the peers, temptation of selection of the sources, coherence and transparency of the rationale. Although frequently limited to the decision-making processes in situations of uncertainty, the precautionary approach is also relevant and appropriate in research. As underlined in the COMEST report from UNESCO, this approach includes a systematic search for surprises ("thinking the unthinkable"), particularly for possible long term effects, a responsiveness to the first signals ("early warnings") and, last but not least, a focus on risk plausibility rather than on hard evidence. Recent developments regarding the late recognized radiation effects of low to moderate doses on the lens of the eye and on the circulatory system are good illustrations of a lack of vigilance regarding early warnings that were described many years ago. Long term hereditary effects and unsuspected effects from *in utero* exposure are currently somewhat out of concern in the research community, but could cause bad surprises in the future. The same is true regarding the effects of chronic internal exposures, particularly during pregnancy and in infants and children.

Finally, science cannot escape from some intrinsic subjectivity. In an attempt to control this, one often appeals to *consensus* as a guarantee for objectivity. Doing so, one forgets that the scientific experts and researchers, coming from the same melting pot, spontaneously favour cognitive consonance and share the same interpretative language, the same paradigm (a whole of reference presuppositions, *which are often unconscious*).

On these grounds, interpretations of reality are not seen by them as subjective and have in their eyes an indisputable value. Stakeholder involvement is the appropriate remedy for this, allowing new views and perspectives to emerge and favouring creative thinking about mechanisms, scenarios or implications. Yet it is only possible if serious efforts are made to "translate" our scientific jargon into easily understood language. Unfortunately stakeholder involvement is currently often just a façade. The invited stakeholders and experts are very few and their opinion often considered as irrelevant and hardly taken into account.

Radiation protection has no lecture to give to radiation research but both need to undergo a cultural change to go "out of the box" and to be able to comply with the requirements of the society.

Sensing Radiation Effects on Stem Cells of Human Skin

M.T. Martin

CEA, Laboratory of Genomics and Radiobiology of Keratinopoiesis, Evry, France.

Skin is a well-known target of ionizing radiation as both complications in normal tissues and radiation-induced cancers have been documented. Epidermis is a multilayered tissue, each layer developing a specific differentiation and function that maintain skin homeostasis. We characterized in different layers the cells response to low and moderate doses of ionizing radiation.

Epidermis external layers are composed of differentiated keratinocytes. Although these cells are primed for elimination through the desquamation process, they are interesting because they participate to the maintenance of skin integrity after stress. Moreover, they can be removed by tape stripping to perform biological dosimetry. We found that differentiated keratinocytes responded to very low doses of gamma rays (10 cGy) by a very specific stress response involving a large number of genes [1]. Some of them were transcription factors that orchestrate the cell response, including GATA3 [2], which points to possible major regulators of the low dose response in epithelial cells.

Human epidermis is constantly renewed over a cycle of 28 days. This short-term constant renewal is due to a specific type of cells called the keratinocyte progenitors. They are located in the deepest layer of epidermis, or basal layer. These progenitors are highly sensitive to ionizing radiation [3], thus responsible for the short-term effects of radiation, such as radiodermatitis, dry and moist desquamation. Moreover, the progenitors that have resisted to radiation exposure exhibit a reduced capacity of DNA damage repair (4) and a long standing genomic instability. Keratinocytes progenitors appear thus to be major targets for cancer formation in skin.

Finally, long-term maintenance of skin homeostasis is due to rare (0.2%) and dormant stem cells, located in the basal layer of epidermis. We demonstrated that keratinocyte stem cells are radioresistant [3] and possess a high capacity of repair for all types of DNA damage [4]. Moreover, stem cells have developed specific mechanisms of protection, including modification of their niche through the FGF2 growth factor. Thus stem cell response to radiation appears to favour long-term tissue maintenance. However, as the error prone repair pathway NHEJ might be the main repair mechanisms in stem cells, tissue maintenance might be at the expense of genomic stability and overall stem cell response favour cancer formation [5].

The fact that both basal cell types appear to be direct targets of genotoxic stresses might explain why skin carcinoma is one of the most frequent tumour types in humans. However, as progenitors and stem cells respond to radiation through different mechanisms, a complete protection of skin should include several complementary strategies.

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Radiation-Induced Cardiovascular and Cerebrovascular Disease

F. A. Stewart

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Epidemiological studies have shown a clear association between therapeutic doses of irradiation and increased risks of both cardiovascular and cerebrovascular disease in long-term cancer survivors. Some studies also show increased risks after much lower total body radiation exposures, for example in the A-bomb survivors. The mechanisms whereby these effects occur and the relationship between radiation dose and other patient related risk factors are, however, poorly understood.

Experimental studies can help to unravel some of these mechanisms and may identify suitable strategies for managing these risks. Experimental studies show that doses of ≥ 2 Gy induce the expression of inflammatory and thrombotic molecules in endothelial cells of both microvasculature and large vessels. Circulating lymphocytes then invade the vessels and this causes thrombi formation and obstruction of microvessels. In the heart this leads to progressive loss of capillaries and eventually leads to reduced perfusion, myocardial cell death and fibrosis.

The animal data are supported by clinical studies demonstrating regional perfusion defects in non-symptomatic breast cancer patients 6 months after radiotherapy. In large arteries, radiation-induced inflammatory changes to the endothelium, in combination with elevated cholesterol, initiates atherosclerosis and predisposes to the formation of unstable lesions, which are prone to rupture and may cause a fatal heart attack or stroke. In the absence of elevated cholesterol, large arteries are rather resistant to the development of atherosclerosis, even after irradiation.

In contrast to the inflammatory processes initiated by localized irradiation with doses > 2 Gy, whole body doses of 0.1-0.6 Gy actually inhibit inflammatory cell adhesion to endothelial cells, therefore other mechanisms are probably responsible for cardiovascular effects. Persistent increases in circulating pro-inflammatory cytokines and long-term impairment of T-cell-mediated immunity may, however, be involved. Monocyte killing and increased levels of chemoattractant proteins may also play a role in initiation and progression of atherosclerosis. Key features of radiation-induced cardiac and large artery damage, as identified from animal models, will be described. The results will be discussed in the light of possible methods for intervention to reduce the risks of cardio and cerebrovacular damage after irradiation.

Animal Models and the Analysis of the Mechanisms Determining Individual Sensitivity to Radiation

M. Atkinson

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(On behalf of the EURATOM consortia: CARDIORISK, DoReMi, GENRISK-T, ProCardio, RISCRAD)

Epidemiological studies have not allowed us to quantify the risk of adverse health effects from low doses of radiation. Extrapolation of available epidemiological data into the lower ranges of the dose response curve may be possible using mathematical models describing the underlying cellular processes.

There are many gaps in our knowledge of the process behind the development of radiation-induced effects. Missing information ranges from an understanding of how, or even if, initial DNA damage is involved, through to deciphering the mechanisms by which individual sensitivity is modulated by genetic (or even epigenetic) factors.

Our strategy has been to develop and deploy animal models of both carcinogenesis and cardiovascular disease, and to use these in conjunction with in vitro models to examine mechanistic and genetic processes. Using examples we will show how an integrative biological approach can start to unravel the complexities underlying tissue level radiation damage following exposure to low doses.

Age and Gender Relevance for the Evaluation of Radiation Risk

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Radiation risk

Historically, there are four fields of radiation risk: death, malformations, malignancies, hereditary diseases. It seems that hereditary diseases are not very relevant for human beings, but that cardiovascular problems may play a significant role. Malformations and death are typical medium (more than 100 mSv) to high dose (more than 1 Sv) problems. It is not yet clear, whether cardiovascular diseases can be induced below about 500 mSv. Because of the open questions in the low dose range (up to about 100 mSv), the focus of the presentation will be on malignancies.

Age relevance

In general, radiation risk is very high at younger ages and declines with increasing age. Partly, this has to do with the higher cell proliferation rate and with the longer life expectancy of young individuals. There are indications, however, that in older individuals (starting at around 50 years) radiation-induced cancer risk may rise again, at least for some tumour types. Explanations could be impaired repair capacities and reduced immune functions.

Gender relevance

Some epidemiological studies suggest that, based on the excess relative risk, women are two times more sensitive to acquire a radiation-induced tumour compared to men and, based upon excess absolute risk, one point five times more sensitive. When one looks at all available data, the picture is not as clear as it may seem at a first glance. The major problem is that, up to now, no studies have been carried out explicitly looking for gender differences in radiosensitivity. The data available are in a sense byproducts of studies aimed at completely different aspects. In addition, not all epidemiological studies show an increased risk for women. The clinical studies after radiotherapy are not suitable to answer the question, and the biological studies show conflicting results. Thus, the German Commission on Radiological Protection (Strahlenschutzkommission, SSK) recommended not to take into consideration possible gender differences in radiosensitivity in radiation protection regulations right now. This may change in the future, when more reliable data are available.

The Limitations of DNA Double-Strand Break Repair and Checkpoint Control after Low Radiation Doses

M. Löbrich

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The DNA damage response pathways involve processes of double-strand break (DSB) repair and cell cycle checkpoint control to prevent or limit entry into S phase or mitosis in the presence of unrepaired damage. Checkpoints can function to permanently remove damaged cells from the actively proliferating population but can also halt the cell cycle temporarily to provide time for the repair of DSBs. Although efficient in their ability to limit genomic instability, checkpoints are not foolproof but carry inherent limitations.

Recent work has demonstrated that the G1/S checkpoint is slowly activated and allows cells to enter S phase in the presence of unrepaired DSBs for about 4-6 h post irradiation. During this time only a slowing but not abolition of S-phase entry is observed. The G2/M checkpoint, in contrast, is quickly activated but only responds to a level of 10-20 DSBs such that cells with a low number of DSBs do not initiate the checkpoint or terminate arrest before repair is complete. At the time of release from the G2 checkpoint, cells have started but not completed DSB repair by homologous recombination (HR). Such cells are able to progress through mitosis but exhibit elevated damage levels in G1 due to de novo DNA breakage generated during mitosis.

We suggest that HR-intermediates between sister chromatids combined with a negligent G2 checkpoint response leads to formation of additional DSBs in mitosis, an issue that may have hitherto unappreciated bearings for the impact of IR and other clastogens on genome integrity and cell survival. Here, I discuss the cellular consequences of the limitations in checkpoint control and DSB repair.

Biomimetic Models of Radiation-Induced Radical Stress and Biomarker Discovery

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The biological consequences of the free radical production are the central subject of a very lively scientific debate, focusing on the estimation of the type and extent of damage, as well as the efficiency of the protective and repair systems. When studying free radical based chemical mechanisms, it is very important to establish biomimetic models, which allow the experiments to be performed in a simplified environment, but suitably designed to be in strict connection with cellular conditions.

The biomimetic modeling approach has been coupled with physical organic chemistry methodologies and the basic knowledge of free radical reactivity, thus allowing the molecular basis of important processes, as well as molecular libraries of products concerning unsaturated lipids, sulfur-containing proteins and nucleic acids, to be identified.

In this context, radiation-induced transformations have been considered in depth together with the systematic study of all possible factors that drive reactivity in aqueous medium and the characterization of reaction or degradation products.

This research leads to the discovery of new biomarkers and molecular libraries to be used for the evaluation of radiation effects. Ongoing projects in our group deal with lipidomics, genomics and proteomics of free radical stress and some examples will be described: (i) radical generation in the different positions of the sugar residue or purine base, and clarification of important reaction intermediates by pulse radiolysis;[1] (ii) cis-trans isomerisation of unsaturated fatty acids with the formation of trans lipids in liposome systems;[2] (iii) tandem damage of lipids and sulfur-containing proteins, with the event of post-translational modifications of amino acid sequences containing cysteine and methionine.[3]

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Early Events Relevant for Biological Damage

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Track structure studies have disclosed that a good knowledge of the radiation track characteristics is necessary to understand the evolution of biological damage, together with sound based hypotheses about the initial targets and initial damage giving rise to the various biological endpoints at sub-cellular, cellular, tissue, organ and organism levels.

In the past few years, with the evolution of experimental detection techniques, also the vision of the biological damage evolved, passing from a focus on the damage in terms of the DNA molecule, to a new one, wherein the final results of the radiation exposure is seen as a broader response of the system (e.g. DNA and non-DNA targets, collective multicellular response, etc.) to the perturbation induced by the radiation exposure. To cope with that a systemic view is necessary, however, a reductionist approach – wherein the overall response to the radiation insult is separated into individual steps – is the best strategy to get insights on the initial/localized mechanisms (pattern of energy deposition, fixation of damage) characterizing the different radiation exposures.

Moreover, for equal absorbed doses densely ionizing (high-LET) radiations are known to be considerably more effective than sparsely ionizing radiations (low-LET, such as γ-rays) in leading to biological effects, including the induction of cancer. Qualitative and quantitative differences between the biological responses (e.g. possible linearity or non-linearity of the biological effects at low doses) are thought to arise from the different spatial and temporal energy deposition properties of the different radiation types, at the nanometer, micrometer and higher levels. Definitely, much is still to be understood on the mechanisms by which different initial events during the physical and chemical stages, drive the induction of different low dose radiation responses.

This presentation will particularly discuss DNA damage, DNA repair and examples of non-DNA targets.

The results of investigations on the role of radiation quality and track structures in inducing DNA damage, will be reported. Theoretical investigations on the induction of DNA damage in terms of DNA fragmentation spectra will be presented, together with an evaluation of RBE for different radiation qualities. To allow the study of low dose effects, examples on DNA damage evaluated through γH2AX, will also be discussed.

Possible non-DNA initial targets will be considered, of importance for potential alterations of cell signaling and of the cell homeostasis (and consequently for cancer and non-cancer risks). Examples of targets will be proposed, such as cellular membrane and organelles (for instance mitochondria, lysosomes or ribosomes) that could be damaged by radiation, resulting in possible cytoplasmic degradation, interruptions of respiration and even induction of apoptosis.

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Dose Rate Effects: Spatial and Temporal Damage Distribution

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Predictions from biophysical models of interactions of radiation tracks with cellular DNA indicate that clustered DNA damage sites including double strand breaks (DSB), defined as two or more lesions formed within one or two helical turns of the DNA by passage of a single radiation track, are formed in mammalian cells. These complex DNA damage sites are regarded as a signature of ionizing radiation exposure particularly as the likelihood of clustered damage sites arising endogenously is thought to be low. The induction of radiation-induced non-DSB clustered DNA damage sites in mammalian cells has been confirmed experimentally, with both high and low LET radiations. The spatial distribution of damage depends upon the ionization density of the radiation track. For low LET radiation it is thought that clustered DNA damage sites arise mainly from low energy electron 'track-ends' and are less complex, few lesions per cluster, than those formed by densely ionising radiation. The time required to repair clustered DNA damage sites increases as the ionization density of the radiation increases. The increased biological effects such as mutagenesis, carcinogenesis and lethality with increasing complex DNA damage are consistent with these predictions.

The spatial and temporal aspects of damage distribution will be discussed on the ability of ionizing radiation to produce clustered DNA damage sites, including DSB, against a plethora of endogenous damage induced and that the complexity of the clusters increases with ionization density of the radiation. I will concentrate on developing the theme that damage complexity is important and is consistent with the hypothesis that radiation-induced clustered DNA damage sites and complex DSB are less repairable. For non-DSB clustered damage the reparability is less than that for isolated single lesions, e.g. those caused by aerobic metabolism, and as a consequences may be highly mutagenic and harmful. The question arises whether the increased biological effects such as mutagenesis, carcinogenesis and lethality is in part related to DNA damage complexity and/or spatial distribution. With particle radiation it is also important to consider not only delta-rays which may cause clustered damaged sites and may be highly mutagenic but the non-random spatial distribution of DSB which may lead to deletions.

In summary the aim is to emphasis the link between the spatial distribution of energy deposition events related to the track structure, the temporal responses of damage repair to identify sub-sets of damage that may contribute to biological effects and to stimulate discussion on outstanding challenges to low dose effects.

DNA Damage Response, Senescence and Cancer

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Ageing is a complex biological process that is influenced by genetic, environmental and stochastic factors. Human efforts over the last centuries have succeeded in substantially lengthening lifespan, allowing ageing to become a common feature of western societies. However, despite intense research, the molecular basis of the processes that cause loss of bodily functions and degeneration of cells and tissues is still unresolved. It is now widely accepted that ageing is not caused by active gene programming but by evolved limitations in somatic maintenance, resulting in a build-up of stochastic damage accumulation (Kirkwood, 2005). Within the complex chemical machinery of each cell, all biomolecules (proteins, lipids and nucleic acids) are subject to indiscriminate damage caused by spontaneous chemical reactions and by numerous endogenous and exogenous reactive agents. It is, therefore, plausible that damage to multiple cellular constituents accounts for ageing. However, evidence suggests that ageing is subject to regulation by evolutionarily highly conserved molecular pathways (Guarente and Kenyon, 2000; Kenyon, 2005; Partridge and Gems, 2002). In mice, several aspects of ageing can be accelerated or delayed by single gene mutations. For instance, inborn defects in DNA metabolism are almost exclusively linked with an extending class of syndromes and associated mouse models with phenotypes resembling accelerated ageing pointing to genomic damage as a major culprit in the ageing process (Garinis et al., 2008; Hasty et al., 2003). On the other side, long-lived mouse models all show suppression of the lifespan regulator growth hormone/insulin growth factor 1 (GH/IGF1) axis, dwarfism and a significantly increased lifespan compared to wild type control mice (Bartke and Brown-Borg, 2004; Liang et al., 2003).

We have previously established a direct link between unrepaired cytotoxic DNA damage and the suppression of the GH/IGF1 axis, the oxidative metabolism and energy pathways in mice (Niedernhofer et al., 2006; van der Pluijm et al., 2006). Recently, we also showed that essentially these pathways are shared among premature ageing (DNA repair-deficient animals), long-lived (mutant dwarfs, calorie-restricted animals) and naturally aged mice (Schumacher et al., 2008a; Schumacher et al., 2008b). Thus, both intrinsic and environmental stressors (e.g., ageing, intrinsic genome instability, or food shortage) induce common stress responses aimed at overcoming crisis and extending lifespan. This notion led us and others to propose that, whereas random damage drives functional decline with advancing age, the existence of universal mechanisms that are able to promote longevity may set the pace on how rapidly damage builds up and function is lost (Garinis et al., 2008; Kirkwood, 2005).

We will discuss how such universal mechanisms are dynamically coordinated at the genome-wide level over the entire organismal lifespan and propose a testable hypothesis to explain how distinct, yet highly conserved stress responses are coordinated under diverse environmental and intrinsic cues during ageing.

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The EU NoE DoReMi – Where Are We Now?

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The aim of the DoReMi consortium is to promote the sustainable integration of low dose risk research in Europe. This will facilitate efforts to resolve the key policy questions identified by the 'High Level Expert Group (HLEG) on Low Dose Risk Research' (www.hleg.de). These are the shape/s of cancer dose-risk relationship/s, variation in risk between individuals, differences in tissue sensitivities for cancer, effects of radiation quality, risks from internal exposures and the risks of non-cancer effects. DoReMi provides an operational tool to continue the development of the MELODI platform (Multidisciplinary European Low Dose Risk Research Initiative) that represents the major national bodies and research programmes with a long term commitment to low dose risk research in Europe. Strategic planning of DoReMi activities is carried out in close collaboration with MELODI.

Since the beginning of the DoReMi Network of Excellence in January 2010, there has been rapid progress in the establishment of a European research platform to focus on questions of low dose risk. DoReMi continues the initial work of HLEG by contributing to the development of the long-term SRA of MELODI, and by establishing the more detailed shorter-term DoReMi TRA. Engaging the broader scientific community via exploratory workshops has proven to be an efficient way in developing the research agendas. The research agendas provided by MELODI and DoReMi have helped to identify priorities for low dose risk research not only by the organisations involved but also in national, European and global contexts. The planned enhancement of the DoReMi network through the inclusion of partners with new expertise was initiated through the first competitive call. This was launched in September 2010 and resulted in 10 new organisations joining the network from the beginning of July 2011. This very successful activity has enhanced the competence of the consortium in several key areas, by integrating research experts in biomarker identification, immunological/inflammatory pathways, and the effects of chronic low dose exposures.

DoReMi has implemented research programs addressing the three key research areas: shape of dose-response curve for cancer, individual radiation sensitivity for cancer and non-cancer effects. The research activities are all performed at appropriately low doses. DoReMi defines these low doses as those of 100 mGy or less. Low dose rates are defined as 0.1 Gy/h or less for low LET radiations. For high-LET radiations, dose and dose-rates of interest are lower, e.g. for alpha radiation by an order of magnitude. Low dose studies are complemented by higher dose/dose-rate studies to inform judgments on extrapolation from moderate and high doses/dose-rates to low doses/dose-rates. All RTD activities address the cross-cutting issues of radiation quality, tissue sensitivity and internal emitters. During the first 18 months, several workshops were convened to develop strategies that focus on the most promising lines of research for the three areas. Experimental programs have been launched in all three areas, including a number of feasibility studies preparing the field for large international collaborative efforts. The RTD approaches have been closely coordinated through discussions on needs for research infrastructures and analytical platforms, as well as targeted stimulation of training and education of next-generation researchers at the European level.

The planned duration of DoReMi is 2010-2015. There are currently 22 partners in the DoReMi Consortium: Radiation and Nuclear Safety Authority, Institut de Radioprotection et de Sûreté Nucléaire, Helmholtz Zentrum München, Commissariat à l'Energie Atomique, Health Protection Agency, University of Pavia, Istituto Superiore di Sanità, Belgian Nuclear Research Centre, Bundesamt für Strahlenschutz, University of Stockholm, Centre for Research in Environmental Epidemiology, Institut Curie, Universitaetsklinikum Erlangen, Johann Wolfgang Goethe-Universitaet, Frankfurt am Main, Universitaet Rostock, Norwegian University of Life Sciences, Norwegian Radiation Protection Authority, Nasjonalt Folkehelseinstitutt, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico Sostenibile, Institute for Environmental Sciences, Dublin Institute of Technology, and Erasmus Universitair Medisch Centrum Rotterdam.

Poster Presentations

Low Dose Research in Thyroid Cancer Biology at SCK•CEN

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REarranged during Transformation/Papillary Thyroid Carcinoma (RET/PTC) is an oncogenic translocation that was found to be enough to be an initiating step for the development of PTC and is found predominantly in radiation-induced cases of thyroid carcinoma. After the Chernobyl accident many cases of childhood PTC with the RET/PTC translocation were found in areas surrounding the accident site where the population received anywhere from low to high doses of radiation. We have attempted in this work to uncover differences in the response of normal vs. RET/PTC-positive thyroids to low vs. moderate to high doses of X-rays using both *in vitro* and *in vivo* models.

Using immunostaining, multiplex bead assay, Western blots, and fluorometric and flow cytometric assays, we have found that TPC-1 cells, a papillary thyroid carcinoma cell line with RET/PTC1 translocation, undergo cell cycle checkpoint activation at a low dose of radiation (62.5 mGy) but display signs of senescence at 0.5 Gy-4 Gy as opposed to another cell line with BRAF V600E point mutation.

Using Affymetrix microarray chips, we analyzed the transcriptomic response of TPC-1 cells, RET/PTC3-positive murine thyroids, and thyroids from normal C57BL/6 mice to a low (62.5 mGy), moderate (0.5 Gy) and high (4 Gy) doses of X-rays. The response at moderate to high doses of X-rays was up-regulation of the p53 pathway in RET/PTC-positive cells while TGF-β pathway was up-regulated at all doses in wild-type thyroids. A common set of response genes between TPC-1 and RET/PTC3-positive thyroids was confirmed using Western blotting and compared to wild-type thyroids. Analysis of the epigenetic response was done using MBD2Seq with which highly methylated regions of the genome were isolated and sequenced using the Illumina Solexa Genome Analyzer II. Results indicate alterations in the methylation status in some genes involved in the p53 and senescence response.

In conclusion, our results indicate that the response of RET/PTC-positive thyrocytes differ from that of normal thyroids and that both differ in response to low and high doses of external X-irradiation. Furthermore, results could indicate that the risk from low doses of radiation could be higher than expected in normal thyroids with several growth promoting pathways activated on the transcriptional level.

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NF-kB Pathway and Its Perturbation: a Systems Radiation Biology Approach

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The nuclear factor-kappa B (NF-kB) signaling pathway is a multi-component pathway that regulates the expression of hundreds of genes that are involved in diverse and key cellular processes, such as cell proliferation, cell survival, cellular stress response, innate immunity and inflammation. Not surprisingly, mis-regulation of the NF-kB pathway, either by mutation or epigenetic mechanisms, is involved in many human and animal diseases, especially ones associated with chronic inflammation, immunodeficiency or cancer [1]. NF-kB is activated by IkB Kinase (IKK) in response to extracellular stimuli, being therefore modulated by a wide range of spatio-temporal concentration gradients of signaling molecules [2]. Once the transcription factor is activated, it moves into the nucleus to induce the expression of specific target genes, including its own inhibitors, before being rapidly stabilized due to negative feedbacks.

Due to the highly coordinated response of such a complex system, this implies the investigation of molecular and cellular response, which is always present in order to guarantee and re-establish the cell homeostasis, following ionizing radiation exposure, through an integrated theoretical and experimental approach.

Adopting the same experimental protocols as for the studies of bystander signaling proteins, NF-kB modulation was measured for different conditions such as change of the medium, irradiation with different γ-rays doses, or the presence of signaling proteins activating this pathway, showing the typical negative feedback response. These studies were performed using different experimental techniques (i.e. Western Blot, Immunocytochemistry and ELISA Assay) in order to obtain more data about its localization and its quantification in human skin fibroblasts (AG01522). Data were afterwards analyzed on the basis of the models, reported in literature ([3],[4],[5],[6]), in order to understand how the interaction network leads to these damped oscillations of nuclear NF-kB concentration experimentally observed. These studies suggested a key role of IKK activation, and the subsequent NF-kB nuclear import and gene transcription, due to radiation exposure.

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Radioresistant Stem-Like Breast Cancer $CD24^{-/low}$ Cells are Involved in Memorization and Transmission of Radiation-Induced Genomic Instability

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The aim of our work is focused on the importance of non-targeted effects of ionizing radiations in human breast cancer cells. Experimental evidence indicates that cancer stem cells (CSC) could be responsible for breast cancer radiation resistance. Moreover, progeny of surviving irradiated cells display long-term radiation-induced genomic instability, transmitted through many generations.

Effectively, we identified $CD24^{-/low}$ labelling as a marker of radioresistance among seven breast CSC markers. We demonstrated that $CD24^{+}$ progeny of irradiated breast cancer cells exclusively descends from $CD24^{-/low}$ cells. We also confirmed that irradiated cell lines display chromosomal instability more than 35 population doublings after a 10 Gy irradiation. Finally, we showed that delayed chromosomal instability is only expressed by $CD24^{+}$ cells, but is transmitted by stable surviving $CD24^{-/low}$ cells. So, for the first time a CSC marker, CD24, was associated with the transmission of genomic instability.

These results indicate that our model is relevant to study persistent chromosomal instability. Because the majority of cells survive radiation exposure at lower doses, delayed effects, occurring in the progeny of irradiated cells, may have significant implications for evaluating risks associated with radiation exposure and provide mechanistic insights into radiation carcinogenesis. The next step of our work is to understand how irradiation directly and indirectly modulates epigenetic factors implicated in chromosomal instability.

A Preliminary Mechanistic Model of X-Ray Promoted Atherosclerosis in ApoE-/- Mice

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Mechanistic models may help to determine how ionising radiation promotes the development of atherosclerosis. For this purpose a preliminary model has been formulated that allows for radiation to contribute to the process of atherosclerosis in several ways. The model is based on what is known from the scientific literature on plaque formation and the possible influences of radiation on this process.

The current formulation has been tailored to experiments carried out at the Dutch National Cancer Institute (NKI) with ApoE-/- mice. Plaque formation in this genetically modified mouse model is considered to partly mimic the process of atherosclerosis as it occurs in humans. At NKI the carotid arteries of groups of ApoE-/- mice were exposed to 0, 2, 8 or 14 Gy X-ray doses. After approximately half a year the arterial tree was removed from these mice and examined for plaques. It was observed that the number and surface area of plaques grew with increasing dose. Moreover, exposed mice demonstrated plaques of a more vulnerable phenotype.

The mechanistic model developed at the Dutch National Institute for Public Health and the Environment (RIVM) therefore aims to adequately predict number, size and phenotype of the observed plaques. The fact that possible radiation effects are included in several steps of the model will help to indicate where radiation action is required to explain the data and where it is not. This is important for the more accurate determination of radiation risk for vascular disease, but it also constitutes significant input for new, tailored experiments aimed at advancing our knowledge on the influence of radiation on atherosclerosis.

Head and Neck Lesions Prevalence in Individuals Submitted to Childhood X-ray Epilation for *Tinea Capitis* Treatment

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Background
X-ray scalp epilation was largely used, more than fifty years ago, to treat *tinea capitis* infection. In Portugal, similarly to other countries, the Adamson-Kienbock method was used, applying 325-400 R per field, in 5 fields of the scalp. Sometimes the treatment had to be repeated. Several studies have shown that these irradiated individuals were more prone to head and neck neoplasias, namely basal cell carcinoma (BCC), thyroid cancer and meningioma.

Methods
We had access to a registry of 5356 individuals that were submitted to this treatment in a Health Institution in Porto, Portugal, between 1951 and 1963. It included age at irradiation, gender, *tinea capitis* diagnosis and irradiation dose. We have traced the individuals at the present moment, contacted them, and proposed a clinical observation. Upon the clinical examination all head and neck suspicious skin lesions were proposed for surgery; a cervical ultrasound was requested. Blood and oral mucosa cells were collected for biological studies.

Results
We supposedly traced 3686 individuals; 240 refused to come, 91 were living abroad and 326 have deceased. So far we have clinically observed 1350 individuals from the original cohort, 811 women and 539 men. We found a BCC prevalence of 8.7%, with 32 out of the 117 patients (27%) diagnosed by us. Higher radiation dose (2 or 3 treatments) increased the risk to develop BCC in these *tinea capitis* irradiated individuals. We found a prevalence of thyroid carcinoma of 2.6%, with 16 out of the 35 patients (46%) diagnosed by us. Thyroid nodules were found in 54% of the scans. Female gender and younger age at irradiation increased the risk for its development. Women and younger irradiated individuals were significantly more prone to have benign lesions surgically removed.

Conclusions
The high prevalence of thyroid carcinoma and BCC found in this cohort is in accordance to the higher risk referred by others in irradiated patients and justify a close follow-up of the cohort in order to be able to identify the undiagnosed benign and malignant lesions. As we have been collecting biological samples from these individuals, our ultimate goal would be to uncover possible radiosensitivity markers.

Cytokines Cascades after Low Dose Gamma Irradiation

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For many years one of the key step in radiation biology was the direct interaction of radiation with specific unique cellular targets (such as DNA molecules). This process was assumed as a necessary prerequisite for manifestation of the biological effects of ionizing radiation (IR) exposures [1].

Experimental evidence started to accumulate since 1990, showing that IR could lead to effects in non-irradiated cells [2]. These secondary effects, named "*radiation-induced bystander effects"*, play a not negligible role in affecting cell functions after exposure to low doses of radiation and they are usually the results of redundant signaling systems between exposed cells and not irradiated ones (bystander cells) [3].

Different reports demonstrated that, in bystander effects, two ways of communication are mainly involved, *i.e.* gap junction and secreted soluble signaling factors [4], which are based on an ever-growing number of molecules that have been found to be responsible for conditioned-medium mediated bystander effects. Aim of the present work was to investigate (experimentally and theoretically) the perturbation induced by radiation on the proteins involved in the signaling mediated by soluble factors (e.g. cytokines). Experimentally, an evaluation of the cytokine levels in the medium of irradiated cells at different times was performed, observing an effective role of radiation in modulating the release of these signals.

In particular we evaluated the expression of different cytokines before and after cells irradiation using different techniques such as ELISA Kit, immunofluorescence and Western blot. In our experimental investigations, AG01522 human fibroblasts were irradiated with doses ranging from 0.25 Gy to 1 Gy of gamma-rays, studying the induced activation of signaling pathways and their subsequent return to its homeostatic equilibrium. Amongst the several signaling proteins, we experimentally investigated the Transforming Growth Factor-beta (TGF-b), Interleukin-6 (IL-6), Interleukin-8 (IL-8), reactive oxygen species (ROS) and reactive nitrogen species (NO). Subsequently, in order to look at the actual precursor mechanisms involved in the different release of the cytokine, also the transcription factors related to the transcription of the cytokines were measured at different times, such as p53, ciclo-oxigenase II (COX-II) and nuclear NF-kB expression.

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Non-Linear Effects Induced by Low Doses of Ionizing Radiations of Different Quality

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In the last 20 years a body of experimental evidences *in vitro* has shown the presence of a plethora of phenomena occurring after low-dose irradiation (including hypersensitivity and induced radioresistance, adaptive response, and non-targeted phenomena like bystander effect and genomic instability [1]), which might imply a non-linear behaviour of cancer risk curves in the low-dose region and question the validity of the Linear No-Threshold (LNT) model for cancer risk assessment through extrapolation from existing high-dose data.

In this framework a systematic investigation on non-linear effects at low doses has been undertaken in terms of different radiation quality and cellular radiosensitivity, focusing on bystander effect (BE) and hyper-radiosensitivity and induced radioresistance (HRS/IRR), in terms of various biological end-points including cell survival, micronuclei induction and chromosomal aberrations.

The gathered results on HRS/IRR and on bystander effect in V79 Chinese Hamster cells irradiated with protons of various energies seem to show the absence of HRS/IRR [2], in contrast with the previous findings by using gamma-rays and helium-4 ions [3], as well as the absence of any bystander effect (BE) [2].

The investigation has been recently extended to T98G human glioblastoma cells and to carbon ions. Results related to HRS/IRR and BE after gamma-rays, protons, helium-4 and carbon ion irradiations in rodent and human cells are presented and discussed here.

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The Italian Military Biodosimetry Laboratory: Multiparametric Approach for Radiation Dose Assessment

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The risk of accidental human exposure is linked to the use of ionizing radiation sources in medical, research and industrial areas. Furthermore, the possibility of terrorist attack using radiological or nuclear devices must be considered. Dose estimation is the first important step for medical treatment of subjects exposed to ionizing radiation. For this purpose, clinical signs and symptoms and biological dosimetry methods are the two main approaches for assessing radiation exposure.

Biological dosimetry is a method that uses biological markers to measure the amount of ionizing radiation dose received by an individual. This type of dosimeter is useful when an individual is accidentally exposed and physical dosimetry is not available or uncertain.

The most validated assays for biodosimetry and radiation injury assessment are Dicentric Chromosome Assay (DCA), the gold standard, and Cytokinesis Block Micronucleus assay (CBMN assay). Otherwise these methods require days to weeks until dose estimations are available.

Currently automated scoring is the best strategy to speed up the cytogenetic analyses achieving faster availability of the data and reducing the variability due to subjective evaluation. Besides for better and rapid dose assessment other new assays are under development such as the gamma-H2AX focus assay or gene expression analysis of radiosensitive genes. For dose assessment it is necessary a reference curve which correlate dose with biological effects, the so called calibration curve. On the principle of the comparability of *in vitro* and *in vivo* irradiation effects, it is possible to generate calibration curves (dose-response) by *in vitro* exposure of peripheral lymphocytes at sequential increasing doses. In order to establish a biodosimetry laboratory, the manual and automated calibration curves are generated for DCA and CBMN. Furthermore, a calibration curves for gamma-H2AX and gene expression for two candidate genes are also elaborated. In this study are compared manual and automated cytogenetic markers scoring and the results of the different biodosimetric approaches are discussed. Finally, an inter-assay comparison is also evaluated. This multiparametric approach is very promising for a more accurate dose assessment but needs to be validated and standardized.

Leukemia in Atomic Bomb Survivors: Towards a Biophysical Model

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We apply a biologically motivated mechanistic model to describe radiation-induced leukemia incidence in survivors of the atomic bombs on Hiroshima and Nagasaki. The model yields risk estimates that can be compared with results from epidemiological studies, but more importantly, it provides insight into the impact of radiation action on leukemogenesis and forms a biologically motivated basis for the transfer of risks from acute exposures of the Japanese population to chronic, low dose exposures of any population.

Inputs for the model are individual data on exposure to both gamma radiation and neutrons and on the incidence as well as mortality of leukemia in the Life Span Study cohort. Data were available for 112,932 individuals, constituting approximately 3.5 million person-years. These data are combined with biologically based information from the literature on the form of the mathematical equations that describe DNA damage by ionizing radiation and on the cells that are the target for radiation induced leukemogenesis. Parameters related to both radiation action and baseline leukemia incidence are determined with a maximum likelihood method using a simulated annealing routine.

Baseline cancer rates vary across populations, but since the spontaneous incidence of cancer is described separately from radiation action in the model, risk transfer to populations where leukemia incidence is different is feasible: values for the parameters that describe baseline cancer incidence can be determined by fitting the model to mortality or incidence data of national cancer registries. We will present preliminary fitting results, with corresponding derived risk estimates and show how these estimates translate to low-dose leukemia risk in a general population. These results provide important input for radiation protection measures.

Are Changes in the Cell Redox Status and Oxidative Metabolism a Sensitive Tool to Monitor Exposure to Low Doses of IR?

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A key issue when analysing the effects of low doses of radiation is the selection of an end-point sensitive enough to allow measurement of what are often subtle changes. In this study we have exploited the use of intracellular ROS levels and metabolic profiles as end-points to characterize the hypersensitivity to ionizing radiation (IR) of primary fibroblasts derived from Cockayne syndrome (CS) and Xeroderma pigmentosum (XP)-A patients.

We have previously shown that CS-A cells are hypersensitive to IR and oxidizing agents (D'Errico et al., Oncogene, 2007). Here, we show that the hypersensitivity of CS cells to IR is detectable at doses as low as 0.5 Gy. We present the first evidence that also human XP-A primary fibroblasts are hypersensitive to IR (including low doses) and accumulate oxidative damage in their DNA upon exposure to oxidizing agents.

To gain insights into the molecular mechanisms underlying the sensitivity of these cells to oxidative stress, the redox status of CS primary fibroblasts was analysed. The intracellular concentration of ROS was determined using the oxidation-sensitive fluorescent probe, 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCF-DA) and detected by flow cytometry. The steady-state ROS levels were 2-fold higher in primary fibroblasts from both CS-A and CS-B donors as compared to normal. These results were confirmed by electron spin resonance (ESR) analysis.

An increased intracellular ROS concentration is expected to affect the cell metabolic profile. A comprehensive analysis of the metabolic profile of CS-A and CS-B fibroblasts was carried out by ^{1}H-NMR and compared with that of normal fibroblasts. To this end, aqueous extracts of primary fibroblasts from CS-A, CS-B and normal donors were prepared and analyzed. The levels of a number of metabolites belonging to several pathways, namely glycolysis, oxidative metabolism, glutaminolysis, choline phospholipid metabolism and osmoregulation were consistently higher in CS compared with control cells. These metabolites allow clear discrimination of CS from normal fibroblasts.

We propose that the perturbation of oxidative metabolism as detected in CS cells might account for their hypersensitivity to IR. The potential of these techniques to detect the effects of low doses of radiation in normal cells will be discussed.

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Direct Assessment of Low Dose Effects in Children Undergoing a Computed Tomography Examination

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Computed Tomography (CT) is a diagnostic imaging technique that uses X-rays for assessing a variety of disorders including cancer detection and surveillance, trauma, evaluation of inflammation, renal calculi, cardiac and vascular evaluation. Over the past several years, increasing attention has been focused on the potential for low-dose radiation exposure for inducing the development of cancers. As a result, an ongoing debate exists as to whether there is an increased risk of cancer from low-dose radiation exposure from a CT examination. Based on data from survivors of atomic bomb radiation exposure, studies sought to determine potential cancer risks of low-dose radiation, concluded that there may be a small increased risk of cancer from exposure to radiation from CT. One of the populations potentially vulnerable to adverse effects from CT is children. Studies have indicated that children are more sensitive than adults to the oncogenic effects of radiation, resulting in higher risks for acute leukemia and solid cancers.

Moreover, it is known that there is a molecular differently modulated response after low doses, like the ones delivered during a CT-scan, compared to the one after high doses of radiation, like radiotherapy treatment. However, it still remains unclear whether risks are present at low doses. In collaboration with multiple hospitals and medical centres throughout Belgium, blood samples will be collected from pediatric patients (age 0 to 12 years old) pre- and post-CT. We are interested, at the molecular level, to study the transient blood cells response to ionizing radiation, thus investigating the mechanism of this low dose hypersensitivity. On the other hand, we think that chemokines and cytokines play a vital role in communication between cells, thus investigating some of cytokine players may help us understanding the mechanism behind the low dose response.

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Mathematical Modelling and Experimental Studies on Adaptive Response

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A small 'priming' dose of ionizing radiation delivered to cells, tissues, or organisms prior to a larger, 'challenging' dose can modify the response of the system to the latter. In case of decreased damage, the effect is called adaptive response (AR). The main proposed mechanisms to explain AR are: increased efficiency of DNA repair, induction of anti-oxidant enzymes, alteration of cell cycle progression, changes in chromatin conformation.

The model proposed by Curtis in 1986 that considers a modulation of the efficiency of DNA repair activity and of the level of anti-oxidant enzymes, starting from the framework of lethal-potentially lethal (LPL) model, was extended with the inclusion of the dynamical variables representing the efficiency of repair, the levels of radiation induced radicals and of anti-oxidant enzymes. In the simulation code particular attention was devoted to the induction of anti-oxidant enzymes as adaptive response mechanism, even if the more relevant mechanism remains the modulation of the repair efficiency, by which the cells processes the initial radiation damage. Furthermore, in order to describe different radiation qualities, the weight of the two factors (i) production of direct damage and (ii) production of free radicals have been made variable in the model.

Our model is able to describe the protective effect of a priming dose. Moreover, in agreement with the literature data, the simulations show that the AR happens in a given priming dose and priming dose rate ranges only, and requires at least 4 hours to develop.

In order to get more insights on the role of cell-cell communication as factors affecting the AR, experimental studies have been performed using sparse or confluent AG01522 cell monolayer. The results obtained after gamma-irradiation suggest that cell density is a crucial factor for observing an AR. The possibility of an AR induced as bystander effects and as a function of radiation quality is under study.

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Micronuclei Induction by Carbon Ions in Directly Irradiated and in Bystander AG01522 Normal Human Primary Fibroblasts

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This study investigated the damage induced in AG01522 normal human primary fibroblasts directly irradiated with C-ions, using graded doses of 0.1, 0.25, 0.5, 1.0 and 1.5 Gy. Furthermore, an evaluation of the ability of medium taken from cultures of irradiated with C-ions to induce a response in non-irradiated AG01522 cells (medium-mediated bystander effect), was attempted.

The C-ions beam of the Superconducting Cyclotron radiobiology facility at the INFN-Laboratori Nazionali del Sud (LNS, Catania, Italy) was used, with E ~ 45 MeV/u at the cell entrance, corresponding to LET ~ 49 keV/μm.

The cell response was measured as chromosomal damage observed by the micronuclei (MN) formation in binucleate cells using the cytokinesis block assay. The same damage was measured in the presence of either DMSO or c-PTIO, scavengers of ROS and NO, respectively, in the medium of the irradiated cells. In the dose range 0.1 - 0.5 Gy they decreased the response by about 20-30 %, indicating that an indirect action, mediated by ROS and/or RNS, may contribute to the chromosomal damage induced by C-ions.

For bystander effect measurements, the medium was taken from cell cultures irradiated with C-ions at doses of 0.1 or 0.5 Gy, 1 or 5 h after irradiation, filtered and added to the non irradiated ("bystander") cells. A bystander effect around 40 % was observable only on addition of conditioned medium taken 5 h after irradiation, with no significant difference between the two doses used (0.1 or 0.5 Gy). However, a large variability was observed between different experiments.

To study the involvement of ROS and RNS as early mediators of the bystander signaling, DMSO or c-PTIO was added to the culture 1 h before irradiation. Comparison with sham-irradiated cells in the presence and in the absence of the scavengers showed that their presence weakens the bystander effect observed after addition of the 5 h conditioned medium. However, the large variability between experiments implies that the specific role of ROS and NO for the bystander effect needs further investigation.

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Mathematical Models of Leukaemogenesis for AML in Mice Based on Replicative Stress-Related Stem Cell Ageing

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Current risk estimation uses the LNT model and epidemiological data of the atomic bomb survivors in Hiroshima and Nagasaki for radiological protection. To clarify the dose rate effects to estimate the low-dose risk in carcinogenesis may be essential. Our approach is to construct a carcinogenesis model used for extrapolating high dose data into low doses and dose rates using both mice data and epidemiological data.

The essential aetiology of radiation-induced acute myeloid leukaemia (AML) in mice is the downregulation of the transcription factor PU.1. The causative mutation of the PU.1-endocing Sfpi1 gene consists mostly of C:G to T:A transitions at a CpG site and is likely to be of spontaneous origin. To work out a mechanism underlying the association between radiation exposure and the AML induction, we have hypothesised that replicative stress after irradiation accelerates the ageing of haematopoietic stem cells (HSCs), and the ageing-related decline in DNA repair could affect the spontaneous mutation rates.

Mathematical model analysis was conducted to examine whether and to what extent the cell kinetics of HSCs can be modified after irradiation. The haematopoietic differentiation process is expressed as a mathematical model and the cell-kinetics parameters were estimated by fitting the simulation result to the assay data. The analysis revealed that HSCs cycle vigourously for more than a few months after irradiation. The estimated number of cell divisions per surviving HSC in 3 Gy-exposed mice reached as high as ten times that of the unexposed. The mitotic load after 3Gy irradiation seems to be heavy enough to accelerate the ageing of HSCs and the hypothesis reasonably explains the leukaemogenic process. The mathematical model was developed for calculating AML incidence considering the effects of radiation on long-term reconstructing haematopoietic stem cells and multipotent progenitors(MPP).

The simple simulation suggested not only HSCs but also MPPs would be target cells of AML presuming the probability that AML specific Sfpi1 mutations spontaneously occurs.

Synergistic Interaction of Cigarette Smoking and Radon Exposure

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Exposure to natural radiation and chemicals always happens in the environment. Radon and its decay products are considered as important sources of public exposure to the natural radioactivity, which is especially important in high background radiation areas. People are exposed to indoor radon combined usually with other agents including smoking. Epidemiological and toxicological studies indicate that the combined action of smoking and radon exposure can be synergistic. The authors proposed a simple model for synergism and tested it for describing cell inactivation after combined treatment with two factors [1]. This study was done to apply the proposed model to describe carcinogenic effects of radon exposure combined with cigarette smoking.

The model postulates that additional carcinogenic damage (N_{comb}) responsible for synergism is arisen from the interaction between putative precarcinogenic sublesions (N_1 and N_2) induced by radon (N_1) and smoking (N_2) with the frequency of the occurrence of these lesions being proportional to the number of cancer cases. The degree of synergism, called synergistic enhancement ratio (SER) *k,* can be defined by the ration $N_{comb}/(N_1+N_2)$. A periodic sputum cytology evaluation which was performed among 249 underground uranium miners and 123 controls [2] was used for validation of the model. Cytological sputum samples yielding moderate atypia, marked atypia, or the presence of cancer cells were classified as being abnormal. To estimate SER, data on both the combined action of the agents and on the separate action of each individual agent is required.

The frequencies of abnormal cytology were 5% after radon exposure alone (N_1), 7% after cigarette smoking alone (N_2) and 28% after combined action of these modalities (N_{comb}). Then the expected frequency should be 12% for independent action of these agents (N_1+ N_2) and in terms of the model discussed, the ratio N_2/N_1=1.4 and the synergistic enhancement ratio *k*=2.3. For people exposed to relatively low radon concentrations as compared to the affected miners, smoking was the main factor responsible for the lung cancer induction. This hypothesis was supported by the published data [3].

The model appears to be appropriate and the predictions are valid. And it enables us to predict the synergistic enhancement ratio for any ratio of the effective damages produced by a combination of smoking and radon exposure as well as the greatest value of the synergistic effect and the condition under which the maximum synergy is attained. The synergistic effect appeared to decline with any deviation from the optimal value of the ratio of the carcinogenic effective damage produced by each agent alone.

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Inhibitory Effects of Mercury on DNA Repair in Earthworms after Irradiation

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Organisms in the natural environment are always exposed to a number of physical and chemical factors. The risk assessment is usually performed on the basis of the simplest assumption that any harmful factor acts independently of others. However, the combined exposure to harmful multifactor might result in a higher effect than expected from addition of the effect produced by each factor. Earthworms are one of bioindicators for soil pollution, and have been identified by the ICRP as one of the reference animals to be used for environmental protection from ionizing radiation [1]. This study was performed to investigate the acute genotoxic effects of radiation and the synergistic effects between radiation and mercury in earthworm, *Eisenia fetida*.

The level of induced DNA damage was assessed by the single cell gel electrophoresis assay in the coelomocytes of *E. fetida* treated with radiation alone or with gamma rays after $HgCl_2$ treatment. Mercuric chloride was mixed to artificial soil for final treatment concentrations of 40 mg of $HgCl_2$ per soil weight (kg^{-1}). The worms were exposed to these soils for a period of 48 h in the climate-controlled room. After $HgCl_2$ treatment, the worms were irradiated with 0 - 20 Gy gamma rays. The coelomocytes were obtained by simple non-invasive technique. The SCGE assay was performed under alkaline conditions following Singh *et al*. [2]. The Olive tail moments (OTMs) were measured during 0 - 96 hours after irradiation.

The results showed that the increase in DNA damage was depending on the dose of radiation. The more the oxidative stress was induced by radiation, the longer the repair time was required. When combination of $HgCl_2$ and ionizing radiation was applied, the OTMs were much higher than those treated with radiation alone, which indicated genotoxic effect, was increased after combined treatment of radiation and mercury. The repair time in the animals exposed to $HgCl_2$ and radiation in combination was nearly five times longer than that in the animals treated with radiation alone.

As confirmed by our studies [3], mercury inhibits the repair of radiation-induced DNA damage, and synergistically exerts their genotoxic effect with radiation on DNA molecules of the cells. Synergism due to the combined action of deleterious factors, even in the low intensity or dose, should be taken into the risk assessment.

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EPI-CT: European Cohort Study of Cancer Risk after Paediatric Computed Tomography

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Background and Aims
The worldwide increasing use of paediatric computed tomography (CT) raised the question of possible late effects from exposure to ionising radiation. The European collaborative EPI-CT project aims studying the cancer risks and the underlying biological effects in a large international cohort study.

Material and Methods
Following recommendations of a pilot study (Child-Med-Rad) which demonstrated feasibility, an international cohort study of paediatric patients being examined by CT for various suspected conditions was launched in February 2011. Based on a common protocol, national cohorts will be extended or established in nine European countries: Belgium, Denmark, France, Germany, The Netherlands, Norway, Spain, Sweden and United Kingdom. Children who had no cancer diagnosis prior to their first CT scan are eligible. The cohort populations will be assembled both retrospectively and prospectively until 2013. We expect to include about 1 million individuals. For each child in the cohort, organ specific dose estimates will be derived based on Monte Carlo computer simulation of radiation transport in the human body and using hybrid mathematical phantoms of children of various ages. Linkage with national cancer registries will allow to calculate cancer incidence in the pooled cohort and to perform external comparisons (SIR-analysis). Association between estimated organ dose and cancer incidence will be evaluated. Based on the broad insights in European paediatric CT practice, optimisation strategies will be proposed. In parallel, biomarkers of CT exposure and sensitivity to radiation will be tested in blood and saliva.

Results
The study methodology and details of the biological studies will be presented. Patterns of use of CT scanning in different countries and over time will be assessed. Based on conservative assumptions, the analyses planned for the year 2015 will have sufficient statistical power to detect a SIR of 1.16 for leukaemia and 1.1 for all cancer respectively.

Conclusion
This project will provide direct epidemiological evidence on the potential cancer risk due to low doses of ionising radiation exposure in a large multinational European cohort. This will be the largest, and the most statistically powerful, study of paediatric CT scans undertaken until to date. Results will contribute to radiation protection, dose optimisation and low dose radiation research and are awaited 2015.

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Ionising Radiation Affects XRCC1 Gene Expression in Human Lymphocytes

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During evolution, mammals have evolved distinct pathways to repair their DNA thus preserving genome integrity and avoiding generating and fixing harmful mutations that could promote the onset of several diseases. Each of these pathways, specialized in removing or correcting different kinds of DNA lesions, are a finely regulated step-by-step process.

XRCC1 is a protein involved in several pathways which operate to repair radio-induced DNA lesions. It acts as a scaffold protein coordinating the recruitment of other proteins on DNA damaged sites. Moreover, XRCC1 physically interacts with DNA replicative apparatus to avoid fork collapse and perturbation of DNA replication.

Beyond investigations at the protein level, an important question is whether *XRCC1* gene expression could correlate with differential radiosensitivity. Although a low-abundance of *XRCC1* mRNA was found in most mammalian tissues, exposure to a wide range of DNA damaging agents, including IR, has not shown any convincing changes in *XRCC1* mRNA levels. So the *XRCC1* mRNA inducibility could represent an interesting field of research, especially in the light of recently developed techniques such as quantitative RT-PCR.

Here we present data on the time course of *XRCC1* mRNA expression and the repair kinetics after IR exposure in G0 PBMCs isolated from buffy coat of healthy donors. After the isolation, the quiescent peripheral blood mononuclear cells were irradiated with 2 Gy of X-rays and at different time points (0, 15, 30, 60, 90 and 120 min) total RNA was extracted and radio-induced DNA damage repair was measured through alkaline Comet assay. Furthermore the expression of XRCC1 protein was measured at different times from irradiation through western blotting.

Radiation Response of Murine Splenic CD4+ Regulatory and Effector T Cells

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Regulatory T cells (Treg) play a key role in maintaining peripheral immune tolerance, by which they can interphere with the development of an efficient antitumor immune response.

The aim of the study was to evaluate radiation induced quantitative and functional changes in the splenic Treg pool, as well as mechanisms responsible for Treg radiation response. Mice were total body irradiated with 2 Gy and splenic cellular fractions analyzed at different time points following irradiation. A 1.6 fold increase in the proportion of Treg cells within the total CD4 cells was seen as soon as 1 day after irradiation, pointing to an increased radioresistance of the Treg population. Foxp3+ Treg cells were less prone to irradiation induced apoptosis than Foxp3- effector T cells, as shown by TUNEL assay. Also, the proliferation index of Treg cells (as evaluated by their Ki67 positivity) was more pronounced than that of effector T cells. Irradiation induced a moderate upregulation of the CTLA4 antigen on the Treg cell surface showing a spontaneous activation of Treg cells in the irradiated animals. However, the degree of Treg cell activation induced by non-specific activation stimuli was impaired by irradiation. The suppressive capacity of Treg cells was also impaired, which was evident both through their interaction with effector T cells and also with dendritic cells. IL-10 and TGF-beta RNA expression levels were moderately changed in the Treg cells of irradiated animals but were significantly increased in the effector T cells.

In conclusion, we showed that Treg cells are more radioresistant than effector T cells, which leads to their enrichment within the splenic CD4 pool of irradiated animals. A reduction in their apoptotic potential and an increase in their proliferation index are responsible for this enrichment. Functionally, Treg cells from irradiated animals are impaired, which is evident both in their reduced suppressive capacity and also in their altered response to activation stimuli.

Radiation Bystander Signaling *In Vivo*: Implications for Tumorigenesis

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Radiation is a well-known genotoxic agent and human carcinogen that gives rise to a variety of short- and long-term effects. The paradigm for the biological effects of exposure to ionizing radiation has been that radiation traversal through the nucleus of cells is a requirement for genetic damage and biological responses. However, this view is now challenged by observations of radiation-induced bystander effects in non-directly irradiated cells. Bystander effects of radiation have been shown in single-cell systems *in vitro* for several biological endpoints related to cancer development, including cell killing, mutations, changes in gene expression, genomic instability, and have also been shown in more complex 3-D human tissue model systems.

To date, evidence that radiation-associated bystander responses are effectual *in vivo* has been limited, and only recently our laboratory has provided the first direct evidence that genetic damage caused by bystander responses contributes to cancer risk in mouse CNS, with drastic acceleration of medulloblastoma in radiosensitive *Ptch1*$^{+/-}$ mice irradiated with shielded brains. We have also recently shown that gap junction intercellular communication (GJIC) is critical for transmission of oncogenic radiation damage to the non-targeted cerebellum, and that a mechanism involving ATP release and upregulation of Cx43, the major GJIC constituent, regulates transduction of oncogenic damage to unirradiated tissues. However, the spatial and temporal dependence of bystander responses and the dose-effect relationship remain poorly understood issues in the *in vivo* context.

We therefore irradiated *Ptch1*$^{+/-}$ mice with different doses (1, 2, 3 Gy) of X rays adopting different shielding geometries to protect approximately two thirds of the mouse body. Genetic damage was measured in bystander cerebellum at different times after irradiation, while tumor development was monitored in lifetime groups. Groups of *Ptch1*$^{+/-}$ mice were also whole-body exposed to X rays (1, 2 and 3 Gy) and the same end-points were evaluated. Results obtained show a clear dose dependence of *in vivo* radiation bystander signaling in the external granule layer (EGL) of the developing cerebella. Moreover, we show a dependence of bystander damage on the amount of irradiated tissues, regardless of the distance of irradiated parts from the shielded cerebellum. In the lifetime groups, tumor development was abrogated by shielding more than 1/3 of the mouse body regardless of the shielding geometry, indicating that bystander-related tumor induction is strongly dependent on the amount of irradiated tissue. To better understand the interplay of different factors in oncogenic bystander effects (*i.e.*, dose, amount of irradiated tissue, distance from target tissue), new groups of mice have been irradiated with a higher dose of radiation (10 Gy) adopting the same shields. Results confirm that tumor development in bystander tissue is dependent on dose and amount of irradiated field, suggesting that a threshold of genetic damage is required for bystander tumorigenesis.

Effects of Smoking Habits on Individual Sensitivity to Ionizing Radiation: Evidences of Adaptive Response in a Study on Monozygotic Twins

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Recently, twin studies have provided evidences on the genetic basis of mutagen sensitivity, a possible cancer predisposing factor in human population, proposing that heredity would explain up to 60% of inter-individual variation in ionizing radiation (IR) sensitivity. A substantial contribution has also been attributed to environmental factors (i.e. smoke, alcohol, diet), even if the relative role of those elements has not yet been clarified.

The present study has been performed to specifically evaluate the effects of tobacco smoke on IR sensitivity. Monozygotic twins with discordant smoking habits have been selected as experimental model to minimize the contribution of genetic variation. Isolated lymphocytes from 22 pairs of healthy twins were challenged in G_0 with 2 Gy gamma-rays and the individual response to IR was evaluated through the analysis of i) the kinetic of phosphorylation of the histone H2AX by immunofluorescence; ii) the kinetic of DNA damage repair by the Comet assay; iii) the induction of apoptosis by flow cytometry; iv) the induction of chromosome damage by the cytokinesis-block micronucleus assay.

The direct comparison between smoking and non-smoking twins did not highlight significant differences in the end-points of DNA damage response analysed. However, when subjects were stratified by years of smoking, a significant inverse correlation, age-corrected, between the induced micronuclei and the duration of exposure was observed ($p=0.04$). Moreover, significant differences were observed between heavy (HS) and light smokers (LS) in the kinetic of DNA damage repair, HS showing a faster repair than LS (Residual Damage 15 min after irradiation: HS=1.3%; LS=18.7%; $p=0.04$).

These results suggest that tobacco smoke would induce both a short-term adaptive-like response as well as modifications arising along smoker lifetime, modulating individual sensitivity to IR. In view of these indications, the experimental model consisting of monozygotic twins with discordant smoking habits could represent a suitable approach to evaluate the effects of low doses of ionizing radiation by the analysis of the induction of an adaptive response.

Spatial and Temporal DNA Repair Response for Mammalian Cells Irradiated with Low and High LET Radiations

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During the last decade [1] ionising radiation-induced foci (IRIF) have become primary tools for studying radiation induced DNA damage, especially at low doses. IRIF are microscopically visible nuclear spots showing the localization of DNA damage sensing or repairing proteins (e.g γ-H2AX) to the sites of double strand breaks (DSB) within seconds or minutes after radiation exposure.

Several studies [2] showed that IRIF formation is closely related to the presence of DSB, but it is important not to equate *a priori* IRIF number with DSB number, since the dependence of IRIF frequency on time, dose and radiation quality challenges a purely one-to-one equivalence [3]. The objective of this work was to derive quantitative information about DSB induction and repair from IRIF measurements for a given quality of radiation. The work focuses on *γ-H2AX* foci.

We studied the evolution of DNA damage (in terms of protein recruitment), investigating the phosphorylation/de-phosphorylation processes of IRIF's appearance, in particular focusing on the kinetics of the protein recruitment leading to the induction of a visible focus.

The study was carried out with 2 different radiation types (e.g. gamma rays, alpha particles), starting with a qualitative study on the size and the properties of the induced foci. A tentative analytical approach was developed to quantify the parameters involved in the foci induction (such as kinetics of phosphorylation, residual damage) as a function of the radiation adopted, and for different exposure scenario (acute *vs.* fractionated doses)

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Effects of Low Dose Ionizing Radiation on the DNA Repair System in Human Primary Keratinocytes

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Introduction

The effects of low-doses of ionizing radiation in humans are of growing concern, especially in the context of current radiation techniques such as medical imaging. The biological response of healthy tissue to low dose of 1-10 cGy *in vivo* is largely unknown. Moreover, knowledge of cellular responses in tissue microenvironment is crucial for the accurate prediction of human health risks following chronic or acute exposure to ionizing radiation. Because skin is the first target of the body upon exposure to radiation, we propose to explore the potential biological effect of low-doses of ionizing radiation first on isolated human skin cells in monolayered culture and for the first time in three-dimensional (3D) artificial human skin tissue then in different skin models

Objectives

In this project, we propose firstly to study the effects (long and short-term) of low-doses on cell proliferation, apoptosis, and capacity to obtain a cohesive and stratified epidermis after irradiation. Secondly, we will evaluate the carcinogenesis risk by measuring the modulation of the DNA repair/damage systems after low-dose exposure.

Methods

For short-term radiosensitivity, cell viability was determined by MTT assay after 24, 48 and 72 h post irradiation; we also performed an *in vivo* colony-forming assay, which measures the radiation toxicity after 2 weeks. DNA repair system and damage was assessed by different techniques available in our laboratory (DNA repair chips, modified comet assay …). Finally, organogenesis potential was determined by the capacity of normal exposed keratinocytes to form a pluristratified epithelium in 3D organotypic cultures

Results and Conclusions

We showed that low-dose of ionizing radiation increases 2 fold the oxidative DNA damage without any activation of the base excision repair pathway, an important pathway to repair oxidative DNA damage. Moreover, we showed that low-dose affects the organogenesis potential of keratinocytes and impairs the proliferation-differentiation balance in the reconstructed skin. We postulate that when the dose or dose rate is very low the radiation damage sensors (ATM or ATR) are not activated, and the repair machinery is not induced. Hence damage could be accumulated in the genome of a cell until eventually it become malignant.

Inhibition of Cellular Growth of Melanoma Cells Line by Heterogeneous Beta Chronic Irradiation at a Very Low-Dose Rate

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Introduction. Radioisotopes that decay via beta emission are widely used in science and medicine. The main advantage of beta-emitters is the relatively long path length in biological tissue (in the mm range). The objective of this work was to determine the inhibition of cellular growth of melanoma cells (melanoma cells are one of the most radioresistant tumor cells) by beta irradiation at very low dose rate using a simple and economic device. We compare it with the high dose rate gamma irradiation.

Materials and methods.
Cell culture**:** Human melanoma cell lines, FON and M8 were used. The cells were seeded 1 day before the start of the irradiation on 25 cm^2 tissue culture flasks and the final cell number was determined at the end of irradiation period (4 to 6 days).
Beta irradiation system: The system is composed of two identical tissue culture flasks superposed. The bottom flask (irradiation flask) was loaded by adding ^{32}P orthophosphate solution. The absorbed dose in the upper culture flask and the isodose curves were calculated applying MCNPX 2.5f Monte Carlo code coupled to photon and electron cross sections from ENDF/B-VI library and validated by Gafchromic EBT2 film dosimetry. The irradiation and cell culture system was kept in incubator at 37 °C and with 5% CO_2 until the total dose was delivered. The final dose was 2 Gy and the initial dose rate was 12-15 mGy/h. The dose ranged from 100% in the centre of the flask, to 67% at the external limit.
Gamma irradiation**:** Both cells line were irradiated with 2 Gy at 30.000 mGy/h with Gamma cell 220 at room temperature and 6 days post irradiation the number of viable cells was determined.

Results. The diminution for the survival of FON and M8 melanoma cells respect to the control by beta irradiation was 28.61±7.6 % and 40.75±5.8% and for gamma irradiation was 28.33±9.5% and 45.22±7% respectively.

Discussion. We determine the effectiveness in producing a significant cell death of two melanoma cell lines at very low dose rate beta irradiation (12-15 mGy/h) and we compare this effect with high dose rate gamma irradiation (30.000 mGy/h). This last dose rate is in the range of the dose rate used in radiotherapy. Gamma irradiation was employed because the relative biological efficiency is similar to beta irradiation. For FON and M8 cell lines, cell survival diminution respect to controls was significant and independent of the dose rate.

Studies are in progress to determine if the mechanisms that influence cell killing for low and high dose rate are different and if the bystander effect is involved in this response at very low dose rate. This irradiation system, which represents a similar radiodiagnostic, radio-immunotherapy and brachytherapy situation because there is a continuous emission of exponentially decreasing low-dose-rate irradiation with heterogeneous dose deposition, permitted us to study in very simple and economic mode, different dose rate only changing the activity of beta emitter.

Telomere Dysfunctions and Chromosomal Instability Induced in Human Fibroblasts by Low-doses of Radiations with Different Quality

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Several endpoints have been evaluated for the comparative study of low- and high-LET radiations in the high-dose range. On the other hand, biological effects produced by low-doses of radiations, though relevant for the risk assessment, have been less investigated.

The aim of the present work is to evaluate telomere dysfunctions and chromosome instability in the low-dose range, and as a function of radiation quality. In particular, we reported here results aimed to analyse whether in the low-dose range the metabolism of telomere is modulated, as we observed previously for higher doses, and if so, the radiobiological meaning of telomere alterations. For this purpose AG01522 human primary fibroblasts were irradiated with 0.1, 0.25, 0.5 and 1 Gy of X-rays, 0.8 MeV low-energy protons (28.5 keV/μm; INFN-LNN), and 8.4 MeV $4He^{2+}$ ions (62 keV/μm; INFN-LNL). Cells were irradiated and harvested 24, 48 and 72 hours later for the analysis of telomere "stickness". Such telomeric alterations have been measured in anaphases in terms of frequency of chromosome bridges and bridges carrying telomeric signals (FISH analysis of PNA telomeric sequences). Furthermore, by means of Q-FISH analysis the length of telomeres at 24 and 48 h was evaluated. Chromosome instability was measured by M-FISH (Multicolour FISH), which allows the detection of chromosome aberrations on the whole genome.

Results so far collected evidenced a LET- and dose-dependent response in the frequency of anaphase bridges induced and in their persistence as a function of time. We found that irrespective of LET and dose, telomeres play only a minor role in the generation of the radiation-induced chromosome bridges, which are therefore the product of unstable chromosomal aberrations. Chromosome damage induction followed a LET-dependence showing a higher extent of complex aberrations in helium-ion-irradiated cell cultures.

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Production of Cytokines by Splenocytes and Macrophages after Single or Fractionated Low-Level Irradiations with X-Rays

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Natural killer lymphocytes and activated cytotoxic macrophages are primary effectors of the anti-tumor surveillance system. Tumoricidal activity of these cells consists in non-specific recognition and killing of neoplastic cells through secretion of cytolytic factors and cytokines which either directly induce apoptotic cell death or stimulate cytotoxic function of other effector cells. In view of this, the aim of the present study was to assess the effects of irradiations with low and higher doses of X-rays on the production of the selected cytokines involved in cytotoxicity mediated by lymphocytes and/or macrophages.

For the investigation, splenocytes and peritoneal macrophages were collected from BALB/c mice pre-exposed to single irradiations with 0.1, 0.2, or 1.0 Gy X-rays, or irradiated for five days per week for 2 weeks at 0.01, 0.02, or 0.1 Gy X-rays (total absorbed doses of 0.1, 0.2, and 1.0 Gy, respectively). Production of IL-1β, IL-2, IL-12, IFN-γ, and TNF-α by these cells in culture was examined using the respective ELISA assays.

The result demonstrate that both single and 10-day irradiations with total doses of 0.1, 0.2, or 1.0 Gy markedly stimulate production of IL-2 by splenocytes and IFN-γ by the NK cell-enriched splenocytes. Likewise, secretion of IL-1β, TNF-α, and IL-12 by macrophages co-cultured with tumor target cells markedly increased after single or fractionated exposure of mice to all the three total doses of X-rays.

Collectively, these results indicate that both single and 10-day exposures of mice to low (0.1 and 0.2) and higher (1.0 Gy) total doses of X-rays stimulate synthesis of cytokines responsible for anti-tumor functions of lymphocytes (IL-2, IFN-γ) and activated macrophages (IL-1β, IL-12, TNF-α).

The EURATOM Project ALLEGRO: Early and Late Health Risks to Normal/Healthy Tissues from the Use of Existing and Emerging Techniques for Radiation Therapy

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The ALLEGRO Project was funded by EURATOM for two years within the VII framework to provide some initial answers and more importantly pointers for where the efforts should be put into new research. As well as helping with important decisions for radiotherapy, research into the normal tissue risk from radiotherapy has the potential to complement and contribute to current EURATOM initiatives in low-dose radiation risk research (e.g. DoReMi). The project has now completed the second year and has successfully finished each of the planned tasks.

The ALLEGRO consortium is made up of 13 partners from 8 European countries. The partners are leading research institutes, hospitals, and university medical departments, all active in the development of new radiotherapy modalities, or optimising current modalities. The project was divided into seven work packages, four of which comprised research and technological development (RTD) tasks. These four work packages focussed respectively on:

1. measurement and modelling of the normal tissues doses from conventional and emerging radiotherapy;
2. assessment of the accuracy with which the normal tissue doses can be estimated for treatment planning optimisation and research purposes;
3. investigation of state of the art NTCP modelling and investigation of the suitability of NTCP models validated on conventional treatments to predict normal tissue risk from novel modalities;
4. investigation of the possibility of using existing clinical databases for deriving a dose response model for the incidence of second cancer following radiotherapy.

A further work package provided an expert forum to review the results of the RTD tasks and to produce a series of reports defining the current state of the art, providing recommendations to the radiotherapy community on how the current knowledge can best be exploited to optimise radiotherapy treatment planning, and to give priorities and directions for the future normal tissue risk research effort.

The most important results and messages that come from the project will be presented and discussed.

Influence of Radiation Dose on Cancer Risk Associated with HR and NHEJ Deficiency in *Ptc1*$^{+/-}$ Mouse Cerebellum

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In the central nervous system (CNS) correct responses to DNA damage are necessary to maintain homeostasis and avoid disease. DNA double-strand breaks (DSBs) are the most biologically significant lesions that occur in cells. The repair of DSBs in mammalian cells is carried out by two pathways: homologous recombination (HR) and nonhomologous end joining (NHEJ). These two pathways appear to compete for DSBs but the factors determining pathway choice are only partially defined.

Our group, using a well established animal model of medulloblastoma (MB), the *Ptc1* heterozygous knockout mice (*Ptc1*$^{+/-}$), has recently shown that although HR and NHEJ collaborate in protecting neural precursors of the cerebellum from DNA damage and apoptosis, they have opposite roles in tumorigenesis. In fact, while loss of *Rad54* function promotes, spontaneous and radiation-induced (2 Gy) brain tumorigenesis, *DNA-PKcs* deficiency is associated with low brain cancer levels.

However, as differences in biological responses to high- and low-dose radiation are starting to emerge, we set up a new study to clarify whether radiation dose is a factor influencing the relative contribution of HR and NHEJ to radiation responses. *Ptc1*$^{+/-}$ mice with two, one or no functional *Rad54* or *DNA-PKcs* alleles were irradiated at postnatal day 1 with 0.25 Gy of X-rays and monitored for tumor development.

We also examined the effects of *Rad54 or DNA-PKcs* deletion on the processing of endogenous and radiation-induced DSBs in neural precursors of the developing cerebellum, the cells of origin of MB. Understanding the impact of inefficient HR/NHEJ DNA repair mechanisms on cancer risk after low dose irradiation is of paramount importance because radiation exposures associated with human activity are almost always at very low dose.

Low Dose Research on Cardiovascular Risks at SCK•CEN

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In recent years, epidemiological data support the fact that lower radiation doses (< 5 Gy) increase the risk of cardiovascular diseases, and this after much longer intervals than previously expected (*cfr.* A-bomb survivors). However for doses below 0.5 Gy, these epidemiological findings are unclear and a better understanding of the underlying biological and molecular mechanisms is needed.

The endothelium is believed to be a critical target in the development of radiation-related cardiovascular diseases because of its pivotal role in normal vascular functioning. Hence, we used the immortalized endothelial cell line EA.hy926 and the primary cell line HUVEC as models, to characterize the endothelial response to low and medium doses of X-irradiation. In particular, we analyzed the influence of radiation on morphology, DNA damage, cell cycle changes and associated apoptosis. Morphology was studied after May-Grünwald Giemsa staining. DNA damage was assessed at several time points post irradiation (p.i.) (15, 30 and 120 min) using a range of X-ray doses (0.05, 0.1, 0.25, 0.5, 2 and 5 Gy) by means of immunostaining for γH2AX-foci and quantitative fluorescence microscopy. All doses induced DNA damage as observed by a significant increase in γH2AX-foci number. Apoptosis was assessed by means of Annexin-V/PI costaining and flow cytometry. After exposure to 5 Gy, the percentage of apoptotic cells was significantly higher compared to the controls, which was most manifest after 48 and 72 h p.i. Cell cycle changes were studied 24 h p.i. via PI staining and flow cytometry. Preliminary results indicate a G0/G1 or G2 arrest after exposure to 2 or 5 Gy, respectively. The results mentioned above are obtained in EA.hy926 cells. Similar experiments with HUVEC are ongoing and results are projected for the near future.

On the basis of these results, it can be concluded that in EA.hy926 cells, cell cycle changes and apoptosis are effects limited to higher dose irradiation (2, 5 Gy), at least within the time range considered in the present study. However, more subtle effects such as DNA damage could be observed down to the lowest dose of 0.05 Gy. To elaborate more on the subtle changes at the level of the cell, we will include in future research, assessment of ROS production, the inflammatory response, adhesion of leukocytes and cell-cell integrity, in both EA.hy926 and HUVEC cells. Moreover, to get a better understanding of the underlying signaling pathways, the endothelial response will be analyzed in further detail at the (epi)genetic level by means of gene expression profiling and analysis of alternative splicing.

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Low Dose Research in Neurobiology at SCK•CEN

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An increase in severe mental and/or cognitive disorders has been observed among children who were exposed *in utero* (week 8 to 15 during pregnancy) to ionizing radiation during the Hiroshima and Nagasaki A-bombings. The 95% lower dose limit for these effects ranged from 0.06 to 0.31 Gy. This indicates the particular sensitivity of the brain to radiation at early stages of its development, during which differentiation and migration of neuronal cells is taking place. Exposure to ionizing radiation at this period could therefore selectively damage neuronal cells and lead to brain dysfunction.

In vitro cell cultures of primary neurons isolated from the hippocampus and/or cortex of the brain of 17 day old foetuses (E17) have been used to study the effect of ionizing radiation on neurite outgrowth and cell survival.

We first focused on the morphological changes of differentiating neurons after X-irradiation exposure. Immuno-stained neurons were compared (control versus irradiated conditions with low and moderate doses of 0.1, 0.2 and 0.5 Gy), by measuring the length and counting the number of neurites per neuron as well as the axonal branching. At early stages of neuronal maturation we observed a reduction in the length and the number of neurites per cell, as well as an inhibition of the axonal branching. Our results indicate a possible recovery of this inhibition 72 h after exposure. Later on, during the crucial step of synapse formation, the same doses caused neuronal degeneration, which is probably due to the loss of cell-to-cell contact. Taken together, these radiation-induced effects might lead to a defect in neural network formation and consequently, to possible cognitive disorders at the adult stage.

Molecular and biochemical events are currently being investigated to confirm the observed effects.

Acknowledgements: This work is supported by the FP7 Euratom Network of Excellence DoReMi (Low Dose Research towards Multidisciplinary Integration, grant n°: 249689).

The Underground Laboratory for Biological Experiments Set Up at the National Laboratory of Gran Sasso (LNGS) of the National Institute of Nuclear Physics (INFN): Influence of Environmental Radiation on the Metabolism of Living Organisms and on their Response to Genotoxic Agents

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In low dose radiobiology there is a strong interest in phenomena that could imply a deviation from the linear-no-threshold (LNT) model, which is currently used to estimate the excess relative cancer risk at low doses of ionizing radiation (IR) via a linear extrapolation from high- and intermediate-dose epidemiology risk data. Such phenomena include the adaptive response, i.e. the modification of the cell response to a challenging dose by a small priming dose delivered few hours before, as well as bystander effects, i.e. those occurring in cells which do not experience direct radiation exposure. These phenomena suggest that biological response to harmful agents such as ionizing radiation may be more complex than what is assumed by the LNT model.

Environmental background radiation represents a source of chronic low dose-rate exposure which may condition the response of living systems to acute exposure to genotoxic agents, also including IR itself. Reduction of background environmental IR dose rate exposure is achieved at the National Laboratories at Gran Sasso (LNGS) of the Italian Institute for Nuclear Physics (INFN). Located underneath the Gran Sasso mountain range in central Italy, these laboratories offer an excellent opportunity to continuously maintain living systems at extremely reduced levels of environmental background radiation.

After the pionieristic experimental studies conducted in S. Cerevisiae [1], that indicated a progressive sensitization to mutagenic drugs in yeasts kept under reduced environmental background IR conditions, a cell culture laboratory has been set up at the LNGS to study the response of more complex biological systems, such as rodent and mammaliam cells. Data obtained using V79 Chinese hamster fibroblasts [2] as well as human TK6 lymphoblastoid cells [3] indicated that environmental radiation may be acting as a chronic priming dose, being the cells grown underground more susceptible to IR induced damage.

Other underground laboratories in the world are starting with biological experiments and strong attention has been manifested by the international community to the results already obtained at the LNGS. Low dose research activity is still in progress using *in vitro* biological systems and an increasing interest is growing in the possibility of performing *in vivo* studies.

The cell culture facility and the most relevant data so far obtained at the LNGS will be described.

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Establishment of a Multi and Inter-Disciplinary Network to Investigate the Effects of Low Dose Ionizing Radiation at the Istituto Superiore di Sanità

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The Istituto Superiore di Sanità (ISS) is the leading technical and scientific body of the Italian National Health Service. Its activities include research, control, training and consultation in public health. In addition, the ISS is the main public advisory body in the field of radiation protection regulation.

At the ISS consolidated expertise in radiation research involving epidemiology, modeling, radiation protection, radiation biology and biophysics, radiation dosimetry and environment radioactivity are present. Moreover, the presence of consolidated expertise in other fields, such as toxicology, immunology, cell biology, neuroscience and nanotechnologies, represents a great opportunity for developing synergisms able to fulfill the research priorities identified by MELODI. Consequently, an inter- and multi-disciplinary network was created that may act to investigate the impact that low dose and low dose rate radiation have in cancer and non cancer diseases.

In the framework of the inter-Department collaboration, both *in vitro* and *ex vivo* as well as *in vivo* studies will be conducted aimed at investigation low dose radiation risk. Experimental studies will pertain to radiation induced cancer and non cancer disease investigations. The former will be conducted primarily on wild-type and mutant primary human cell lines, the latter will concern in particular investigation on neurological and cognitive functions and will be carried out in murine models and tissue explants. Peripheral blood lymphocytes will be also used for investigation on immune functions and for biological dosimetry.

The experiments will benefit from the infrastructures present at the ISS. Besides irradiation facilities, namely the Cs-137 irradiator (for γ-rays exposure of cells and mice) and the α-particles irradiator (especially suitable for long term cell culture experiments), a wide spectrum of microscopes (Apotome, Confocal, SEM and TEM) are available together with other relevant facilities such as EPR and NMR spectroscopy, Mass Spectrometry, Flow Cytometry. An Animal Housing Facility is also present and biological material deriving from a cohort of twins is deposited in the tissue bank at the CNEPS.

This interdisciplinary collaboration, that integrates the different expertise, will likely be the ideal framework to achieve increasing knowledge on the impact of genetic, epigenetic and individual factors to the low dose/dose rate radiation risk.

Radiological Study of Soils in Oil and Gas Producing Areas in Delta State, Nigeria

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Measurement of the average radioactivity concentration in soils around the oil and gas producing areas of the Delta state of Nigeria was made using a high purity germanium, (HPGe) detector based, low-level, passive gamma-counting system. In all, twenty samples from the study area were collected and analyzed. With the exception of ^{40}K and the anthropogenic ^{137}Cs, all the radionuclides detected are traceable to the pre-medial series of ^{238}U and ^{232}Th. The specific activity value obtained varied from 6.97 to 60.12 Bq kg^{-1} with a mean value of 24.12 ± 2.45 Bq kg^{-1} for ^{238}U; 7.04 to 73.48 Bq kg^{-1} with an average of 29.32 ± 3.07 Bq kg^{-1} for ^{232}Th; 15.39 to 696.54 Bq kg^{-1} with an average of 256.46 ± 36.56 Bq kg^{-1}; while that of the fallout ^{137}Cs vary between 1.09 and 24.73 Bq kg^{-1} with an average of 7.03 ± 0.73 Bq kg^{-1}. The mean value obtained for the Representative levels index (I_γ), the radium equivalent (Ra_{eq}), the total absorbed dose rate (ADR) were 0.62, 85.80 Bq kg^{-1} and 40.62 nGy h^{-1} respectively.

The discrepancies of our data can be attributed to several factors such as oil exploitation and exploration, geological formation, transport process, etc. Although our results are just some fractions of the international standard limit, but still within the same ranges when compared with those obtained elsewhere. These results also will serve as a baseline data for future investigations.

Radioactivity in Some Foodstuffs in Oils and Gas Producing Area in Delta State, Nigeria

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The concentrations of ^{137}Cs, ^{238}U, ^{232}Th and ^{40}K in some Nigerian foodstuffs in oils and gas areas have been measured by mean of a high purity germanium, (HPGe) detector based, low-level, passive gamma-counting system. In all, twenty five samples from the study area were collected and analyzed. The results of this study can be considered as a first step towards calculating the baseline levels of radioactivity in foodstuffs in Nigeria.

Furthermore, the data presented herein can be used as a reference level for future food radioactivity monitoring during the possible uses of radioactive materials for oil and gas production, as well as to screen imported foodstuffs that are suspected of being contaminated. The overall intake is low and no significant radionuclide.

Study of Ionizing Radiation-Induced Adaptive Response in Bone Marrow and Splenocytes of Wild Type and PARP-1 Knockout Mice

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Radiation adaptive response is a biological event in which a low priming dose decreases the biological effectiveness of a larger subsequent dose. This phenomenon has been broadly observed in mammalian systems but its underlying cellular mechanisms remain to be clarified. A possible mechanism is thought to be the production of protective proteins and repair enzymes induced by low-dose ionizing radiation. Poly(ADP-ribose) polymerase 1 (PARP-1), is a chromatin associated nuclear enzyme involved in DNA repair, transcriptional control, genomic stability, cell death and transformation. It is activated by DNA strand breaks to participate in DNA repair.

In this study we examined the induction of radio-adaptive response in mouse bone marrow cells and splenocytes from wild type and PARP-1 knockout mice. Cells were subjected to a priming irradiation of 0.05 Gy X-ray 4 hours before a challenging dose of 3 Gy X-ray. DNA damage and repair capability were assessed by comet assay. Preliminary data show a lower induction of damage in pre-irradiated cells indicating an adaptive response both in bone marrow and in splenocytes. The decrease of damage was less evident in cells from PARP-1 knockout than in wild type mice suggesting the involvement of PARP-1 in the adaptive response.

Further experiments are ongoing to confirm these data and to investigate possible mechanisms involving PARP-1 in radio-adaptive response.

The Route of Carcinogenesis by the Low Dose Radiation May Be Same as that Natural Carcinogenesis - An Important Viewpoint in Study of the Low Dose Radiation

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It has been believed that the first target of radiation carcinogenesis is DNA. However, this is not proved for radiation carcinogenesis yet. We discovered that frequency of aneuploid cell was closely related to that of radiation-induced cell transformation and natural cell transformation by high-density cultivation, but gene mutation was not. Cell with p53 gene becomes tetraploid, but does not get tumorigenicity. On the other hand, cells without p53 gene function easily become a triploid, and acquire tumorigenicity. Both radiation exposure and high-density cultivation elevated the level of intracellular oxidative radicals. These radicals induced centrosome destabilization and produced cells carrying extra centrosome, which promote merotelic attachment of chromosome by altering spindle geometry. Unresolved merotelic attachments can give rise to lagging chromosomes at anaphase. Aneuploidy was seen in high frequency in early process of cell transformation. These results strongly suggest that a main target of carcinogenesis by low dose radiation is not DNA, but is centrosome, which are the proteins to constitute chromosomal homeostasis maintenance mechanism. In addition, this route may be the same as that of natural carcinogenesis.

These serial results support necessity of a review of a LNT hypothesis at a radioprotective point of view, and also suggest that we have to elucidate a mechanism of the spontaneous carcinogenesis to understand a carcinogenic mechanism of the low dose radiation.

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MULTIBIODOSE: Multi-Disciplinary Biodosimetric Tools to Manage High Scale Radiological Casualties

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In the event of a large scale radiological emergency biological dosimetry is an essential tool that can provide timely assessment of radiation exposure to the general population and enable the identification of those exposed people, who should receive immediate medical treatment. A number of biodosimetric tools are potentially available, but they must be adapted and tested for a large-scale emergency scenario. These methods differ in their specificity and sensitivity to radiation, the stability of signal and speed of performance. A large scale radiological emergency can take different forms. Based on the emergency scenario different biodosimetric tools should be applied so that the dosimetric information can be made available with optimal speed and precision.

The aim of this multi-disciplinary collaborative project is to analyse a variety of biodosimetric tools and adapt them to different mass casualty scenarios. The following biodosimetric tools will be established, improved and/or validated: the dicentric assay, the micronucleus assay, the gamma-H2AX assay, the skin speckle assay and the blood serum protein expression assay. In addition EPR/OSL dosimetry in components of pocket electronic devises will also be investigated. The assays were chosen because they complement each other with respect to sensitivity, specificity to radiation and the exposure scenario as well as speed of performance.

The project will involve the key European players with extensive experience in biological dosimetry. Training will be carried out and automation and commercialisation pursued. An operational guide will be developed and disseminated among emergency preparedness and radiation protection organisations.

The final deliverable of this project will be establishment of a biodosimetric network that is fully functional and ready to respond in case of a mass casualty situation. Thus, the project will strengthen the European security capabilities by achieving tangible results.

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URL: http://www.multibiodose.eu.

Low Dose Ionising Radiation Leads to a Decreased Secretion of IL-1 beta and a Decreased Expression of p38 and p65 in Activated Macrophages in a Discontinuous Dose-Dependency

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Macrophages are cells of the innate immune system that control essential steps in the development, propagation, and damping of inflammatory processes. Activated macrophages are key components of the inflammatory microenvironment in the tissue. They secrete cytokines leading to the recruitment of further immune cells from the blood into the tissue. For processing and secretion of the pro-inflammatory cytokines IL-1 beta and IL-18, an activation of the macrophage's inflammasome, being a cytoplasmic multi-protein complex that controls activation of caspase-1, is mandatory.

The activation of the inflammasome was determined by analysing the secretion of active IL-1 beta by activated macrophages. We analysed how low and intermediate dose of X-radiation (LD-X-ray) modulates the secretion IL-1 beta by activated macrophages and influences the expression of p38 kinase and NFkB-p65. p38 MAPK regulates the production of key inflammatory mediators including IL-1 beta. p65 is a subunit of the NF-kappa-B transcription complex and plays a crucial role in inflammatory immune responses.

LD-X-ray did not significantly alter the intracellular protein amount of caspase 1. However, single dose of 0.1, 0.3, 0.5, and 0.7 Gy all led to a significant decreased secretion of IL-1 beta, compared to only activated, by not irradiated macrophages. Importantly, 0.5 Gy or 0.7 Gy of radiation resulted in a significant lower amount of IL-1 beta in the supernatant of the activated macrophages, compared to 0.1 or 0.3 Gy. In addition, p38 MAPK protein expression was reduced in macrophages after LD-X-ray with 0.5 or 0.7 Gy. The expression of p65 was significant reduced after 0.7 Gy of LD-X-ray. A single dose of 1.0 Gy did neither diminish the amount of extracellular IL-1 beta nor that of intracellular p38 and p65.

We conclude that LD-X-ray induces an anti-inflammatory phenotype of activated macrophages. The modulation of MAPK pathways, transcription factors and the inflammasome by LD-X-ray might contribute to an anti-inflammatory microenvironment.

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Effects of H^+-, γ- and α-Irradiation on Transmembrane Potassium Currents Recorded from Mammalians Cell Lines

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Most mammalian cells express a wide range of potassium (K^+) channels. In non-excitable cells, K^+ channels are involved in volume regulation, hormonal secretion, cell proliferation and apoptosis [1,2]. The ability of ion channels to act as modulators of conductance is due to their innate property of rapid conformational alterations to initiate or respond to signal transduction [3]. A previous study performed in human cell lines showed that both normal and tumor cells exhibit an increase in K^+ currents (I_K) after treatment with low-dose radiation suggesting that I_K modifications are acting as a component of a signal transduction pathway(s) in response to stress [4]. Several lines of evidence from molecular, biochemical, and biological studies outlined that different mechanisms are operating in cells and organisms at low and high radiation doses.

We evaluated effects of protons (H^+), γ rays, helium-4 ion (α) radiations at different doses (from 0.25 to 6.5 Gy) on V79 cells and effect of H+ on T98G cells. We selected these tumoral cell lines to compare radiobiological effect in different organisms; V79 are widely used in radiobiological studies and are of rodent origin, while T98G are derived from human glioblastome. The effects of ionizing radiations are studied by measuring the K^+ total current of the cell membrane, using whole-cell configuration of the patch-clamp technique. H^+ and α particles irradiations have been performed at the Radiobiology facility at the INFN-LNL 7MV Van de Graaff CN accelerator. Protons of 3.0 MeV (0.8 MeV energy at the cell entrance surface, corresponding to LET values of 28.5 keV/μm) and helium-4 ions of 12 MeV (8.4 MeV energy at the cell entrance surface, corresponding to LET values of 62.3 keV/μm) have been used. Sham irradiated cells were used in all the experiments as control (un-irradiated) cells.

The amplitude of K^+ currents recorded from V79 cells (irradiated by γ rays and H^+) and T98G cells (irradiated by H^+) showed as a function of the applied voltages, a general decrease compared to control cells. In particular: a) the V79 cells showed a decrement of K^+ current amplitude from 1 to 6.5 Gy while for lower doses such as 0.25 and 0.5 Gy, the irradiation seems to be more effective *(low dose hypersensitivity)*; b) the T98G cells showed a significant decrease of K^+ current amplitude for the doses of 0.25 and 2 Gy and not for 0.5 Gy (non-linear effects). Interestingly, V79 irradiated by α particles showed a reduction of K^+ current amplitude in the range of doses between 0.5 and 2 Gy with a surprising effect of current enhancement at 0.25 and 4 Gy. In conclusion we showed for the first time, to our knowledge, the feasibility of I_K recording from V79 and T98G cells. We observed that the amplitude of I_K recorded after irradiation is strongly dose- and radiation type-dependent, allowing to hypothesize a different mechanism for α particles, while $H^+$and γ-rays seems to underline similar electrophysiological responses.

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Micronuclei in Lymphocytes of Czech Uranium Miners

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Micronuclei can be used as markers of past radiation exposure, but few pertinent studies have dealt with alpha radiation. Here we report on micronuclei in lymphocytes from uranium miners, comparing some that are currently active and others that retired 15-20 years ago.
The last working uranium mine in the European Union is the one at Rožná, Western Moravia, Czech Republic. Less than 100 miners are currently active there. Thousands of former miners, however, are living elsewhere in the country, where mines were closed at the beginning of the 1990s, mainly around Příbram, Central Bohemia.

Currently active miners had a higher frequency of micronucleus-containing lymphocytes and a higher percentage of micronuclei without centromers (presumably caused by radiation) than former miners, and the latter had higher values than individuals in a control group.

When the age dependence of the occurrence of micronuclei was taken into consideration, the differences between currently active miners and controls becomes even more pronounced, while the differences between former miners and controls disappeared.

For the currently active miners, there was a significant correlation between the occurrence of micronuclei and the “retrievable” dose, i.e. the cumulative dose whose effects in terms of micronuclei should still be recognizable, calculated under the assumption that lymphocyte have a half-life of 3 years. The “retrievable” dose at which a doubling of the micronucleus frequency was observed was around 30 mSv.

Our observations, in particular the higher frequency of micronucleus- containing lymphocytes and the higher percentage of micronuclei without centromere in currently active miners, are in agreement with the assumption that their micronuclei are mostly the direct result of recent radiation exposure. In former miners, who have not had any (occupational) radiation exposure for almost 20 years, all observable micronuclei would seem to be unrelated to radiation.

That no difference was seen between former miners and unexposed control persons speaks against the earlier suggestion of a persistent genomic instability a decade or more after exposure to alpha radiation.

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