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RESEARCH CONFERENCES

ESF-EMBO Symposium

Spatio-Temporal Radiation Biology: Transdisciplinary Advances for Biomedical Applications

Hotel Eden Roc, Sant Feliu de Guixols (Costa Brava) • Spain
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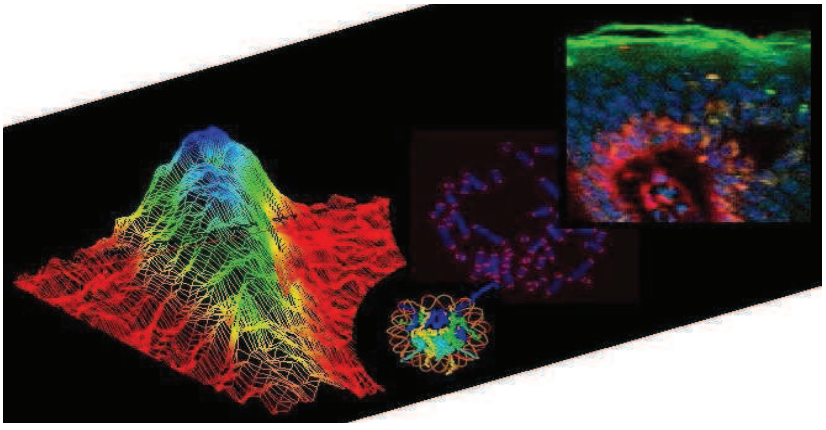
Highlights & Scientific Report



Conference Highlights

Please provide a brief summary of the conference and its highlights in non-specialist terms (especially for highly technical subjects) for communication and publicity purposes. (ca. 400-500 words)

The purpose of four days ESF-EMBO conference was to create a timely forum for multi-disciplinary discussions of recent developments in the ionising radiation effects on integrated living systems, from molecular to tissular scales, bringing together physicists, chemists, biochemists, biologists, genetics experts as well as physicians with a common interest in using different radiation sources and advanced techniques to explore transdisciplinary aspects of modern radiation biology and related biomedical applications. The participants included about 95 researchers and physicians, whose 35-40 % of young scientists from 21 different countries worldwide. The ESF-EMBO symposium would gather together actors from the academic research, applied and medical research as well as private companies and clinicians, to take advantage of the high research quality and technological environment developed in a European and international context.

Spatio-temporal radiation biology represents a newly emerging interdisciplinary research field driven nowadays in strong synergy with the most recent progresses of molecular biology, genomics and proteomics, biomarker detections, X-ray synchrotron micro-imaging, well-defined spatial irradiation with microbeams, pulsed relativistic particle sources, selective targeting radiopharmaceutical, advanced radiation therapies of cancers.

The format of this international symposium including plenary scientific sessions, flash poster presentations and poster sessions has permitted to share advanced knowledge, favouring intense and fruitful discussions between fundamental and clinical communities. The scientific program has addressed a number of highly topical aspects of spatio-temporal radiation biology, evolving over several orders of magnitude, typically from femtosecond (10^{-15} s) and sub-micrometric scales. Deeply understanding the basic mechanisms of radiation damage in vitro and on living cells, starting from the early induction of localized radical and molecular processes to mutagenic DNA lesions, protein recruitment to nucleic acids damages, cell signalling, genomic instability, apoptosis, microenvironment and Bystander effects, radio sensitivity should have, in the near future, many practical consequences like the customization of non-conventional radiotherapies of cancer (therapies with proton, ions beams or with high dose delivery profiles) and radioprotection protocols.

Innovating aspects of spatio-temporal radiation biology have been considered:

- Biological effects of spatial and temporal fractionated radiation using advanced microbeam and pulsed radiation beams technologies in the broad energy range eV - MeV. Complex living matter responses to radiation were considered at molecular, cellular and tissue levels, considering advanced 2 and 3D imaging of biopolymers damages (DNA, chromatine, proteins and chromosomes) in nuclear environment and sub-cellular environments (cytoplasm, mitochondria, membranes).
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- Recent progresses of semi-quantum simulations and classical modelling for investigating early processes of radiation events, ionisation tracks structures and biomolecular damages represent powerful tools. However, predictive computed data including numerous adjustable parameters must be confronted to experimental facts.

An efficient and concerted synergy between recent developments of radiation sources (spatial profile of microbeams, crosswise sequential irradiation, temporal properties of ultra-short laser and pulsed particles beams) on one hand and advanced progresses in genomic, proteomic, molecular and cellular biology, semi-quantum computed simulations on the other hand would favour the emerging of very innovative research programmes devoted to spatio-temporal radiation biology and medical physics for the understanding of molecular, cellular and tissue responses to fractionated or low doses and the improvement of cancer radiotherapy plans.

In this way interplay between spatio-temporal radiation biology and chemotherapy would represent an emerging domain of investigation leading to more predictive concepts of medical interests, such as selective radiation therapy of specific cancers. Advanced conceptual developments will be needed such as the real-time nanodosimetry of molecular probes within integrated biological systems.

Scientific Report

Executive Summary

(2 pages max)

The purpose of this 4 days ESF-EMBO conference was to create a timely forum for multi-disciplinary discussions of recent developments in the ionising radiation effects on integrated living systems, from molecular to tissular scales, bringing together physicists, chemists, biochemists, biologists, genetics experts as well as physicians with a common interest in using different radiation sources and advanced techniques to explore transdisciplinary aspects of modern radiation biology and related biomedical applications.

Deeply understanding the basic mechanisms of radiation damage *in vitro* and in integrated living systems (cells and tissues), starting from early radical and molecular processes to mutagenic DNA lesions, cell signalling, genomic instability, apoptosis, microenvironment and Bystander effects, radio sensitivity should have in the near future many practical consequences like the customization of radiation therapy or radioprotection protocols. In this context, spatio-temporal radiation biology represents a newly emerging interdisciplinary field of studies driven nowadays in strong synergy with the most recent progresses of molecular biology, genomics and proteomics, biomarker detections, X-ray synchrotron micro-imaging, microbeams, pulsed relativistic particle sources, selective targeting radiopharmaceutical, advanced radiation cancer therapies.

This ESF-EMBO symposium has brought together actors from the academic research, applied and medical research as well as private companies and clinicians, to take advantage of the high research quality and technological environment developed in a European and international context. The participants included about 95 researchers and physicians, whose 35-40 % of young scientists and a broad geographical representation with 21 countries worldwide. The expertise of seven members of an international Scientific Committee contributes to a high scientific program quality, as well as for oral than poster contributions.

The conference has been structured from trans-disciplinary advanced contributions, including 17 invited oral presentations and 22 selected short talks. The fruitful synergy between participating scientists has also been encouraged during four flash poster presentations and two poster sessions (42 selected posters). Significant times of discussion (15 and 5 minutes for long and short talks respectively) have favored enlightenments between speakers and participants The selection of participants has been based on merit, considering a good balanced geographical equilibrium between Europe (70%) and the rest of the

world (USA, Canada, Japan, China). A large majority of selected applicants, including young scientists, has benefited of conference grantees via reduced conference fees or travel support. Eight additional sponsorships, representing 30% of available conference funds, have largely contributed to support the attendance of selected scientists. The high research quality of several young scientists has been recognized in the framework of an exciting EMBO poster prize competition.

The scientific program of the ESF-EMBO conference was conceived around seven transdisciplinary sessions. They have addressed a number of highly topical aspects of spatio-temporal radiation biology, evolving over several orders of magnitude. In each session, invited lectures and selected short talks deal with fundamental aspects of radiation biology, considering experimental and theoretical developments, mixing high spatial and/or temporal resolutions. Several complementary domains have concerned molecular, sub-cellular and cellular imaging of radiation effects, prethermal and thermal radiation processes, Induction and amplification of molecular and cellular damages, microbeam radiation and microenvironments responses, innovative approaches for radiotherapies.

- **Major themes** have been considered and largely discussed:

- Real-time clustered DNA damages and sub-cellular responses
- Spatial focies distribution due to radiation damages in function of track structure
- Molecular repair processes and adaptative responses
- Chromatine remodeling, chromosomal aberrations and gene amplification
- Spatial amplification of molecular damages and modulation of radiosensitivity
- Microbeam irradiation on unique cell and tissue
- Nanodosimetry and dosimetry for microbeam radiation therapy
- Non targeted cellular radiation responses
- Fractionated dose delivery for clinical treatments
- Relativistic particles and ion sources for cancer radiation therapy

Invited lectures, short talks and posters have significantly contributed to fruitful discussions between scientists and clinicians. A forward look plenary discussion has been planned for identifying some open questions and scientific challenges for spatio-temporal radiation biology. Advances for more selective and predictive cancer radiation therapy, using microbeams, pulse particle beams or ion sources have been considered too. The identification of hot topics would provide guidance for the future development of transdisciplinary researches in a European and international context.

Scientific Content of the Conference

(1 page min.)

- *Summary of the conference sessions focusing on the scientific highlights*
- *Assessment of the results and their potential impact on future research or applications*

The scientific programme has addressed a number of highly topical aspects of spatio-temporal radiation biology, evolving over several orders of magnitude, typically from femtosecond and sub-micrometric scales. In the framework of spatial and temporal considerations, the scientific content was focussed focus on the understanding of basic mechanisms of radiation damage in vitro and on living cells, starting from the early induction of localized radical and molecular processes to mutagenic DNA lesions, protein recruitment to nucleic acids damages, cell signalling, genomic instability, apoptosis, microenvironment effects on non-irradiated living matter (Bystander effect). Innovative radiation therapy methods have been presented in the framework of advanced ions beams or pulsed relativistic particles beams technologies and spatially fractionated radiation therapy (GRID) of large tumors.

- The different scientific aspects of the conference have been organized around seven trans-disciplinary sessions:

Molecular and sub-cellular imaging of radiation effects

Sensing and responding to DNA damage in model systems
Early quantum steps of water-trigger biomolecular damages
Applications of γ-H2AX foci, a biomarker of DNA damages, for aging , cancer and bystander effect
Spatio-temporal cell response to DNA damage: role of checkpoint and DNA repair proteins
Spatio-temporal organisation of damage recognition and processing: Double strand break motion in function of track structure

Prethermal and thermal radiation processes

Ultrafast UV damage of DNA: complex cascade of electronic events
Importance of short-lived unrelaxed hydrated electon in radiation biology and radiotherapy
Monte Carlo calculation of track structure based DNA repair
Infrared spectrocopic and chemometric analysis of radiobiological effects

Induction, amplification of damages

Femtosecond and submicrometric resolution of cell DNA damage, signalling and repair
Amplification of molecular damages by photo-activation of heavy elements
Chromosome aberrations and gene amplification
Radiosensitizer effects on DNA interstrand crosslinks
Multiscale physical approach of ion beam cancer therapy

Microbeam radiation

Microbeam based probing radiation responses at cellular and tissue levels
Spatially fractionated irradiation of *in vitro* models (cell populations and tissues)
Synchrotron microbeam radiation therapy (MRT): dosimetric challenges of MRT

Cellular imaging for radiation biology

Metabolism of radiation-induced DNA breaks
Long term cell tracking using synchrotron radiation and imaging of nanoparticles
2 and 3D dynamical analysis of DSB-related protein focies
Chromosome organization in cancer cells
Spatially fractionated radiation for radiotherapy

Microenvironments and radiation responses

Time dependence of cellular response to ionizing radiation
Signalling factors involved during bystander response
Cell cycle and control of DNA repair processes in normal cells and tumors
Nanodosimetry of mixed LET ionizing radiations
Iso-effects for radiotherapy modalities at nanometric level

Innovation approaches for radiotherapies

New laser accelerated particle source in the MeV energy domain
Carbon ion radiotherapy: state of the art
Importance of secondary electrons in tissue-like media
time dependent cellular senescence in response to carbon-ions
Optimization of spatially fractionated radiation therapy
Infrared radiation for cancer diagnosis and therapy

• In the framework of molecular, cellular and tissue radiation biology, significant results have been presented and discussed during the conference:

- The complex role of Rad 9 cell-cycle-dependent phosporylation for prokaryote in sensing and responding to DNA damage is more and more understood.

- DNA stand exchange in homologous recombinations are driven by structural changes in protein-DNA complexes. These processes are controlled by ATP hydrolysis and DNA tension.

- From *in vivo* micro-irradiation and on eukaryote fixed cells, spatio-temporal p21protein localization to DNA damage sites depends on interaction with PCNA, an important DNA repair factor.

- 2D and 3D dynamics of γ-H2AX focies, a biomarker of DNA damages and other double strand breaks-related protein focies (ATM, MDC1, NBS1, Rad51 etc) can be investigated by ion or micrometric irradiation beams, small angle irradiation, 3D microscopy and image analysis. Several important parameters whose average volumes of focies, micrometric foci track length or kinetics of focus size give some quantitative information on clusters of protein foci after well-defined irradiation conditions.

- The cell nuclear architecture, gene density and chromatin structure (condensed vs de-condensed chromatin) significantly influence the formation of double-strand breaks of DNA (serious DNA lesions) and subsequent DSB repair processes. These processes are closely related to the mechanism of chromosomal translocations, cell death or carcinogenesis.

- New strategic approaches are developed to lure DNA damage signalling with mimetic DNA damages (siDNA molecules) in tumors by modifying the spatio-temporal organization of the DNA damage response in the cell and inhibiting DNA repair.

- Quantum, semi-quantum and classical simulations of water/DNA clustered damages in ionization tracks and particle track structure contribute to a precise understanding of radiation effects in the temporal window 10^{-14} – 10^{-12} s, with predictive approaches in the classical domain of stochastic and diffusion processes. The real-time probing of low energy secondary electron biological effects becomes feasible in *the non-classical prethermal regime of ionization tracks*.

- The local control of crosswise sequential irradiation, dose fragmentation and high dose rate induced by micro-beam radiation or ultra-short sources are dependent on dosimetric concepts developed at the nanometric and sub-micrometric levels. The biological consequences of such sub-micrometric dose delivery profiles are in progress.

- New irradiation sources such as laser, ion and synchrotron X-ray micro-beams, with a micrometric resolution within a broad energy range 0.5 eV- 5 MeV, contribute to targeted studies in individual fibroblast or tumor cells and different tissue models whose resistant tumor tissue (neuroblastome for instance).

Forward Look

(1 page min.)

- *Assessment of the results*
- *Contribution to the future direction of the field – identification of issues in the 5-10 years & timeframe*
- *Identification of emerging topics*

A two hours forward look plenary discussion has taken place during the last conference evening. In a first step, a synthesis of highlights presented at the conference was performed by session chairs and conference chair. Unresolved points, key aspects and open questions were underlined and subject to a general discussion.

- Regarding the state of the art, significant advances would be observed in the near future, considering hot topics as follow:

  - *Spatial and temporal characteristics of new irradiation sources (sub-micrometric synchrotron beams in the keV energy domain), ultra-short laser-accelerated particle beams in the relativistic regime (Me):* some of these irradiation sources permit temporal spectroscopic resolution from femtosecond (10^{-15} s) to sub-nanosecond (10^{-9} s).

  - *Micro-irradiation on unique cell, mono-cellular layer and thin tissue:* sequential micro-irradiation with different dose rates become feasible with specific ion, particles and X-ray sources whose the linear energy transfer value (LET) changes over several order of magnitude.

  - *2D and 3D sub-micrometric imaging of living cells, for well-defined identification of molecular damages and cell morphology after ionizing irradiation:* advanced imaging analysis would include immunofluorescence technics, nanoparticle detection, quantum dots characterization in nuclear or sub-cellular environments.

  - *Monte Carlo simulations of radiation transport codes and early track structure codes do not follow low energy particle and secondary electrons (E < 100 eV):* new developments would be obtained, including quantum or semi-quantum modeling of molecular damage and radical formation, statistical physics of cell population dynamics.

  - *Carbon ion therapy with gantry system allowing irradiation from all directions with high accuracy represents an interesting way for curing cancers that remain incurable with other treatments*; indeed, during the least fifteen years, more than 4500 patients had been treated with carbon beams at Chiba (Japan). Several ion beam therapy centers are presently developed in Europe.

- **Major goals and scientific challenges** have clearly been identified for future research programmes. The synergy between experimental and modelling developments would be the “driving force” for getting significant advances in spatio-temporal radiation biology.

- *Sub-molecular structure and real-time dynamics of focies*. Does γ-H2AX /53BPA really represent double strand breaks of DNA? Although there is a considerable body of knowledge about DNA lesions formation and repair, identification of repair proteins, protein interactions and involvements in signaling pathway, little is known about the spatio-temporal aspects of DNA lesions in native focies.

- *Spatio-temporal description of processes cascade leading to cell DNA damage response and multiple gene amplification* after an initial radiation induce DNA damage (double strand break of DNA)

- *Biological effects of secondary low energy electrons in the prethermal regime.* These major effects concern the different types of radiation characterized either by low (X and γ rays, electron, proton) or high (ions) LET values.

- *Spatial and temporal probing of concerted molecular/sub-cellular processes and signaling factors involved in radiation induced Bystander effect.* The modulation of non-irradiated cell response in the vicinity of irradiated cells represents a real challenge for an optimization of dose profile delivery (biological dose) in normal tissue/cancer tumor and for more predictive responses of biological matter.

- *Radiotherapy dose fractionating from spatial radiation configurations* (C^{12} ion with Bragg peak characteristics, synchrotron microbeams) or temporal radiation techniques (pulsed radiation source characterized by high dose rate) represent complex challenges for optimizing physical and biological doses in function of experimental cellular and tissue responses. Classical Monte Carlo dose calculations delivered by spread Bragg peak, short-pulsed radiation beams or activated radiosensitizers would be completed by quantum calculations of secondary low energy bio-effects at multi temporal and spatial scales.

▪ Is there a need for a foresight-type initiative?

Regarding transdisciplinary aspects of spatio-temporal radiation biology, continuous scientific contacts and discussions must be amplified via European networks and cooperative programs whose COST actions. These contacts would involve broad scientific communities working in the vicinity of large infrastructures in Europe (ion beam accelerators, synchrotron sources, high intensity laser center…), scientists form different institutes conducting researches in specified area (theoretical and calculated simulations, molecular physical chemistry, molecular and cellular biology) and medical people (medical physics and radiation thepary).

This ESF-EMBO symposium had favored contacts and discussions between international actors from the academic, applied and medical researches as well as private companies and clinicians, to take advantage of the high research quality and technological environment developed in Europe and all over the world.

Specific actions such as exploratory workshops and international conferences would be organized in future, focusing on advanced transdisicplinary aspects of spatio-temporal radiation biology.

Atmosphere and Infrastructure

▪ *The reaction of the participants to the location and the organization, including networking, and any other relevant comments*

The conference location and organization have been greatly appreciated by the participants. Hotel infrastructures were well adapted to the ESF-EMBO symposium, except the restricted space devoted to posters sessions, inside the conference room. From a general point of view, the qualities of Eden Roc‘s welcome and infrastructure have favored an enjoyable and very fruitful atmosphere between young and senior scientists. Transdisciplinary discussions during scientific sessions, coffee breaks, lunches or dinners have permitted to establish fruitful links between physicists, physico-chemists, molecular or cellular biologists and medical community.