Meeting Report

Spatio-temporal radiation biology: new insights and biomedical perspectives

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The ESF-EMBO Symposium *Spatio-Temporal Radiation Biology: Transdisciplinary Advances for Biomedical Applications*, 16–21 May 2009 Sant Feliu de Guixols, Spanish Costa Brava

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The ESF-EMBO Symposium *Spatio-Temporal Radiation Biology: Transdisciplinary Advances for Biomedical Applications*, 16–21 May 2009 was held in Sant Feliu de Guixols, 120 km from Barcelona, the capital city of Catalunya, Spanish Costa Brava. This 4-day conference created a timely forum for multidisciplinary discussions of recent researches on ionizing radiation effects in living organisms, from molecular to tissue scales. The symposium brought together 95 participants from 21 countries worldwide, such as physicists, chemists, biologists, clinicians and private companies with a common interest in using advanced radiation sources and techniques to explore modern radiation biology concepts and related biomedical applications.

Spatio-temporal radiation biology represents an emerging research domain driven in strong synergy with recent radiation source developments, evolving over several orders of magnitude from femtosecond and submicrometric scales. The conference was structured from seven transdisciplinary sessions, 45 posters and one forward look plenary discussion, taking advantage of the high research quality and technological environment in a European and international context. In the framework of experimental and theoretical developments, we report below some innovative aspects of spatio-temporal radiation biology that were presented during the symposium.

Molecular and Subcellular Imaging of Radiation Events

Exposure of living matter to radiation induces a broad range of modifications that cells remove to maintain functional integrity and prevent tumor formation. The first session focused on the molecular and subcellular identification of radiation events. Both improvements in imaging and theoretical simulations provide new insights on DNA damage and repair. Noël Lowndes (NUIG, Galway, Ireland) presented the DNA Damage Response (DDR) and DNA lesions signaling

pathways. Two models, including the budding yeast *Saccharomyces cerevisiae* and DT40 chicken B-lymphocytic cells, show how the DDR is crucial for each cell and allow the dissection of the involvement of each protein that participates in similar functions in such distant species. Marie-Pierre Gageot (Evry University, Evry, France) discussed semi-quantum simulations of primary radiation effects, considering the direct DNA damage dynamics and indirect effects with water molecules. Early physicochemical events were investigated in a few tens of femtoseconds, providing some aspects of real-time theoretical imaging of biomolecular radiation damage.

Subcellular imaging greatly improves our knowledge of DDR chronology, including H2AX histone phosphorylation. The resulting γ -H2AX focus formation assay is one of the most sensitive ways to investigate genome integrity. Foci formation peaking within 15–30 mn after irradiation and declining during several hours was observed in irradiated and nonirradiated cells. Olga Sedelnikova (NCI, Bethesda, NIH, USA) found that other stress factors such as untreated tumor cells induce γ -H2AX foci in bystander cells. The cell ability to repair DNA damage is not constant and decreases with age. Irradiation triggers other protein foci formation (53BP1 or RPA) as described by Burkhard Jakob, (GSI, Darmstadt, Germany). DNA repair proteins can be detected either as chromatin peaks or adjacent to the DNA maxima within nuclei. From living cell imaging, he showed that the motion is slow, likely driven by chromatin diffusion to migrate in 'repair factories'. The repair of high-LET radiation-induced DSBs is not coupled to the motional activity of lesions. Chromosome exchanges required the spatial proximity of DNA breaks. Ennio Prosperi (CNR, Pavia, Italy) discussed the common feature of checkpoint and DNA factors at DNA damage sites. Cell-cycle regulatory proteins such as p21^{CDKN1A} may be directly involved in DNA repair. The dynamics of p21 protein recruitment at damaged DNA sites suggest the existence of

a cross-talk between cell-cycle checkpoints and DNA repair systems.

Prethermal and Thermal Radiation Processes

Spatio-temporal aspects of radiation effects on biomolecular systems are investigated more extensively in the framework of secondary electron excited states (prethermal regime) and of fully relaxed electronic states in tracks. Using double helix models with one type of base-pairs (dA)n.(dT)n, Dimitra Markovitsi (CEA, Saclay, France) explained how early direct damages triggered by femtosecond UV radiation involve the complex cascade of electronic events. The existence of collective behaviors limits the pertinence of oversimplified conclusions on radiation damage in DNA. The importance of very short-lived excited electrons in biomolecular damage was discussed by Quing-Bin Lu (Waterloo University, Waterloo, Canada). A dissipative electron transfer from a short-lived prehydrated electron state has a key role in the activation of radiosensitizers (halopyrimidines and *cis*-platinum).

The effect of ionization track structure on molecular damage and repair represents an important aspect of the thermal radiation processes. Werner Friedland (GSF, Neuherberg, Germany) illustrated this key point showing Monte Carlo simulations of DNA repair via nonhomologous end-joining pathways. On the basis of PARTRAC track model structure calculation, spatio-temporal studies require some improvements. As reported by Vaclav Stepan (NPI, Prague, Czech Republic), future refinements concern the charge transfer on DNA, DNA-protein complex interactions, DNA chemical fixation for oxygen effect and subsequent repair processes. Thermal radiation effects on living cells can be investigated using modern vibrational techniques. Indeed, Aidan Meade (FRI, Dublin, Ireland) discussed noninvasive studies on human skin cells, mixing Fourier-transform infrared and Raman microspectroscopy with parallel measurement of physiological functions within 6–96 h after γ -irradiation.

Induction and Amplification of Damages

A complete understanding of the processes involved in the induction and amplification of radiation-induced biological damage is required for improving the methods of cancer treatments and radioprotection. It aims at a better preservation of healthy tissues, higher efficiency and individualization of treatments. This session focused on the time and genetic dependences of radiation-induced DNA malfunction, as well as the effect of drug associations in DNA. Stanley Botchway (Rutherford Appleton Lab, Didcot, UK) presented the femto-second near-infrared submicrometric laser irradiation of single cell nucleus permitting a high-resolution imaging of DNA damage, signaling and repair processes. A multiphotonic 3D UV radiation in a femtoliter volume allows the real-time probing of protein fixation. Homologous recombination and nonhomologous end-joining present distinct kinetics. Elena Giuletto (Università Di Pavia, Pavia, Italy) discussed the role of chromosomal aberrations and genetic background effect on gene amplification, a process involved in oncogene activation. Considering radiation-induced DNA defects, amplified DNA is highly radiosensitive. A single double-strand

break may induce a dysfunction in gene expression (overexpression or inactivation). Short telomeres enhance cell sensitivity to radiations and alter the genome contributing to tumor progression after successive cell divisions.

Different aspects of DNA damage amplification by chemical radiosensitizers were considered. Darel Hunting (Sherbrooke Medical Faculty, Sherbrooke, Canada) discussed the toxic effect of bromouracil in mismatched bases of oligonucleotides. This radiosensitizer triggers potentially deleterious interstrand cross-links. Erika Porcel (Paris-Sud University, Paris, France) presented a new method based on platinum incorporation in nanoparticles for the spatial control of radiation effects in well-defined tumor volume. Andrey V Solov'yov (Goethe University, Frankfurt am Main, Germany) underlined that multiscale simulations promote the spatio-temporal understanding of molecular and tissue radiation effects. He discussed fast ion interactions with DNA, considering the low-energy electron contribution in tracks.

Microbeam Radiation

The knowledge of living matter responses to spatially fractionated irradiation requires *in vitro* and *in vivo* researches using advanced microbeam technologies. Kevin M Prise (Queen's University, Belfast, UK) showed that X-ray and charged particle microbeams allow a precise energy deposition at subcellular and cellular levels, with spatial accuracy less than 1 μ m. He explained how targeted radiation in the nucleus and mitochondria permits the probing of subcellular radiation signaling responses and bystander process. In the framework of intensity-modulated radiotherapy, Stéphanie Blockhuys (Ghent University Hospital, Ghent, Belgium) showed spatially fractionated irradiation effects on cell metabolic activities, using MTT conversion by cellular dehydrogenases. She discussed an induced metabolic activity coupled with an increased cell mobility and invasive capacity. Guido A Drexler (Munich University, Munich, Germany) observed competition effects in DDR during a cross-wise sequential carbon ion microirradiation of live HeLa cells. Phospho-ATM, γ -H2AX and MDC1 foci being higher at second microirradiation sites, the accumulations of 53BP1 and recombination protein Rad51 are strongly inhibited. He concluded that the time course of damage load influences DNA repair at individual nuclear sites. Spatio-temporal characteristics of microbeams allow the 3D investigation of irradiated tissues. Elke Bräuer-Krisch (ESRF, Grenoble, France) discussed the interest of microbeam radiation therapy (MRT) performed with quasi-parallel X-ray microbeams generated from the third synchrotron generation. A high dose rate microplanar radiation (4000 Gy s⁻¹) offers promising treatments for specific brain malignant tumors. Erik A Siegbahn (Karolinska Institute, Stockholm, Sweden) underlined that Monte Carlo calculations of spatially fractionated dose profiles in the microbeam peak and valley represent a dosimetry challenge for further preclinical MRT trials with normal and cancer tissues.

Cellular Imaging for Radiation Biology

Major advances in cell imaging techniques including fluorescent marked proteins (GFP tagged) allow a satisfying

spatio-temporal analysis of the DSB repair mechanism. Roland Kanaar (Erasmus MC, Rotterdam, The Netherlands) explained how optical tweezers and single molecule fluorescent microscopy were used successfully to complement the findings in living cells. He revealed that a 10–20 DSB caused by an α -irradiation recruits within 30 s the necessary DNA repair enzyme to fit the gap. In his presentation, Martin Falk (Biophysics Institute, Brno, Czech Republic) showed that chromosome regions having various densities are not repaired at the same level. Heterochromatin is protected by bound proteins and is less susceptible to DSB. Recent 2 and 3D dynamics studies of DSB foci in individual cells as a response to ion irradiation were presented by Guanghua Du (Munich University). The correlation between 53BP1/ γ -H2AX foci disappearance and DSB repair was questioned by Igor Belyaev (Academy of Science, Moscow, Russia). He raised the awareness that other marker procedures should be considered to prevent model bias. Using the 3D-FISH technique, Claire Heride (CEA, Fontenay-aux-Roses, France) observed that chromosomal territories are radially organized in the nuclei and concluded that precise localizations of chromosome territories permit to investigate their role in the occurrence of radiation-induced rearrangements and long-term chromosomal instability. Two additional contributions underlined the interest of advanced imaging for radiation biology. As illustrated by Alberto Astolfo (Sincrotron Trieste, Trieste, Italy), tumor cell tracking using bright synchrotron sources and phase contrast microtomography are feasible techniques producing high-resolution 3D images. José A Penagaricano (Arkansas University, Arkansas, USA) presented the curative interest of spatially fractionated radiotherapy for cell carcinoma in the head and neck.

Microenvironments and Radiation Responses

Six lectures highlighted microenvironment effects in the framework of DNA repair, modified phenotype expression and nanodosimetry. Sylvain Costes (Berkeley National Lab, Berkeley, USA) discussed how new modeling tools provide a better understanding of early and delayed radiation responses. Image analysis of living cells comparing patterns of DSB with predicted biophysical models showed that foci largely occur in euchromatin even if breaks must occur all along the ion track. An open question exists: What is the role of chromatin environment in foci formation and repair? The understanding of cell population behavior in tissue is challenged by cell type heterogeneity. Ghida Harfouche (CEA, Evry, France) described the special features of stem cells, generally believed to be the source of tumors. Keratinocyte stem cells exhibit an activated DNA-DSB repair with the induction of autocrine FGF2 signaling. It is not understood yet if the DNA repair in stem cells is efficient or if a misrepair is more frequent than in progenitors. DNA repair activation can be used against the cell as proposed by Marie Dutreix (Institut Curie, Orsay, France). She tested a new strategy to increase cell killing in response to irradiation with the aim of improving the therapeutic efficiency of radiations. Transfected short DNA molecules with stable blunt ends (false DSB, Dbait, DNA Therapeutics, Paris, France) lure cells that activate the DNA repair process (massive ectopic

activation of DNA-PK) by inhibiting breaks repair. DNA lesion quantity can also be modulated by radiation quality. Hans Rabus (PTB, Braunschweig, Germany) explained how nanodosimetry investigates ionizing particle interactions with biomolecular target volumes through the influence of ionization cluster size distributions. The development of a nanodosimetry-based model for cell survival by Chris K Wang (Georgia Institute of Technology, Georgia, USA) estimates the iso-effect for mixed LET radiotherapy modalities. From nano- to millimetric scales, Chulin Sha (Fudan University, Shanghai, China) showed that nonirradiated cells respond to irradiated neighbors, including nitric oxide and TGF- β 1. A growing effort is given for the spatio-temporal investigation of bystander effect and its cancer-prone or protective actions.

Innovating Approaches for Radiotherapies

Technical innovations and new concepts for cancer radiotherapy are proposed as alternative protocols to overcome the therapeutic index of conventional radiation sources. These advances contribute to improved treatments and to lowering the costs of sophisticated radiation treatments. Victor Malka (Ecole polytechnique-ENSTA, Palaiseau, France) exposed a new laser-plasma accelerator providing femtosecond electron beams in the range of 5 – a few 100 MeV. Electron beam properties are relevant for innovative advances in bioradical femtochemistry, spatio-temporal radiation biology and pulsed radiotherapy. Robert J Griffin (Arkansas University) proposed an alternative treatment based on spatially fractionated radiotherapy with a millimeter beam positioning. Successful tests on mice showed that a single shot irradiation induces a significant tumor cell survival reduction. Janusz M Dabrowski (Krakow, Poland) discussed about near-infrared radiation for cancer diagnosis and therapy. For improving photodynamic therapy, he considered a new class of selective halogenated bacteriochlorins. The past, present and future of the carbon ion therapy at NIRS-HIMAC was presented by Tadashi Kamada (NIRS, Chiba-Shi, Japan). Taking the ballistic advantages of the Bragg peak into consideration, this therapy represents an alternative cancer treatment that started in the late 1980s in Japan, namely, for cancers localized in head, neck, prostate, bone and soft tissues. Short-course therapy and new combinations with chemotherapy were discussed in the framework of clinical trials involving 4500 patients during the 1994–2009 periods. It is fundamentally accepted that fast ion effects are mediated by secondary electrons along a primary track. Using Monte Carlo simulations to model the Bragg peak, Emanuele Scifoni (Goethe University) discussed a good relationship between ion-beam damage and secondary electron spectra in tissue-like media. In response to carbon ion irradiation, Lorenzo Manti (Naples University, Naples, Italy) presented a time-dependent onset of senescence on human umbilical vein-endothelial cells. A stress-induced premature senescence revealed by telomere reduction below 0.5 Gy could have some implication on normal tissue stability after hadrontherapy.

Concluding Remarks

For the first time, scientists and clinicians working in very different fields of radiation biology have mutually exchanged

their experiences by communicating in a very comprehensive way with each other. Transdisciplinary discussions have highlighted some key points of the innovative approach of *Spatio-Temporal Radiation Biology*. A profound understanding of the mechanisms induced in radiation damage within living cells and tissues include early induction of localized radical processes, their amplification up to mutagenic DNA lesions, protein recruitments, cell signaling and repair processes, genomic instability, apoptosis, bystander effects and, last but not the least, radio sensitivity. All attendees agreed that insights into these addressed issues will have, in the near future, many practical consequences, such as customization of nonconventional and selective cancer radiotherapies and radioprotection protocols.

Conflict of interest

The authors declare no conflict of interest.

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