Radiation-perturbed signalling and systems radiation biology

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INTRODUCTION

In deep space astronauts are usually exposed to doses of ~ 1 mSv/day of charged particles, including HZE. Due to the exposure to GCR (e.g. during a mission to Mars), each cell nucleus of an astronaut would be traversed by a proton or by a secondary electron every few days, but only by an HZE ion every few months [1]. For this reason, besides the clustered properties of the incoming radiation on the target cells, also the complex cellular interplay between hit and non hit cells (through the modulation of the surrounding microenvironment) is mandatory to understand the biological response of the irradiated tissues. Thus, extensive research is needed aimed at understanding the mechanisms driving the collective global response which could be relevant in risk estimates for both cancer induction and non-cancer diseases. This complex perturbation must be investigated through a systematic multi-scale approach, that takes account of the multiplicity of biochemical networks and signaling pathways activated by the radiation exposure.

SYSTEMS BIOLOGY

Systems Biology has been defined as a “movement” [2], an “approach” [3], merely “new wording, more fashionable, for physiology” [4] and in many other ways. Systems theory – in which systems biology is framed - is a multidisciplinary study that aims to elucidate the dynamic behavior of a generic complex system [5]. Rather than reducing a complex object (e.g. the human body) to the properties of its parts or elements (e.g. organs or cells), systems theory focuses on the arrangement of and relations

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between the parts which connect them into a whole [6]. The need for a systems biology approach rose in the ’50s, when systems theory was born and it was for the first time applied to the biology of living organisms. One of the most famous introductory books to systems biology (“An introduction to systems biology, Uri Alon” [6]), trying to frame the systems-like problems in biology, considers cells as “matter that dances”, referring to their spontaneous ability to re-arrange and self-organize in response to external stimuli. Indeed cells are able to “encode and process information virtually without errors, despite the fact that they are under strong thermal noise and embedded in a dense molecular soup” [6]. The emergent phenomena characterizing life, that rise from the complex environmental background noise, cannot be explained purely from the sum of the characteristics of the components. For these reasons, a holistic approach[1], looking at the global features of the system (such as the interrelationship networks, modularity, robustness, etc.) is mandatory to provide information about a complex systems dynamics and perturbations.

SYSTEMS RADIATION BIOLOGY

With the increasing efficiency of the experimental biological techniques (and irradiation technology), more mechanistic understanding of the induction and response to radiation damage has been discovered, starting – just to refer to the most famous one – from the analysis of gene expression in irradiated cells. In the past few years, with the evolution of experimental detection techniques, the vision of the biological damage has also 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 insult is seen as a broader response of the system (single cell, tissue, organ, etc.) to the perturbation induced by the radiation exposure. It has become clear that the paradigm of DNA damage alone that held sway for the last part of the 20th century, was overly simplified, and that the response to radiation is more than induction and resolution of DNA damage [8].

Amongst all the mechanisms studied, new attention has been devoted to the role of cell communication in the induction of the radiation effects at a multicellular level. One of the main techniques to evaluate the *supra*-cellular radiation effect is to look at the damage in cells “in contact” – i.e. physically, through gap junction, through soluble factors, etc. - with irradiated cells (*bystander effect*) [9, 10].

[1] A holistic/systemic approach concerns relational biology rather than reductionist biology. Relational Biology deals with the study of general, relational connections of complex biological systems, usually abstracting out any specific structures (morphological, anatomical, etc.) [7].

Unlike the investigation of DNA damage induced by radiation (especially complex/clustered lesions), the study of the radiation effects on cell signaling examines processes which are always present, that regulate cell homeostasis, and where radiation acts only to modulate or perturb already activated processes. The cell is constantly receiving, processing and responding to signals received by its neighbours in order to activate molecular pathways and biochemical signalling networks needed to maintain the regular activity of the cell machinery. It is in this frame that radiation acts. Besides the well-known initial effect of disrupting the integrity of the DNA molecule, radiation can also affect the microenvironment of the cells, leading to a different gene expression pattern, and possibly to a different transcription of signalling proteins. Besides the obvious complexity related to the potentially high number of molecules (e.g. reactive oxygen species and proteins) involved in signalling, another "layer of complexity" resides in the complicated and non-linear (*complex*) ways in which these proteins interact (often through the coordinated activation of self-sustaining feedback loops after transient stimuli). Further peculiar features of cell communication are the very large ranges of time/spatial scale and the common lack of separation between responses to external stimuli versus internal programs [11, 12]. This makes any tentative mechanistic modelling very hard and even more problematic the quantification and evaluation of the perturbation induced by radiation.

Furthermore, in the *in vitro* systems used to analyze the bystander effect, the "net" effect of radiation on cell communication does not seem to show an emergent phenomenon induced by a collective behavior (paradigm of systems biology) generated from the cultured cells used [13,14,154]. In fact, in this frame, the whole process moderately affects the behavior of a small number of cells, with a resulting final effect often comparable with the intrinsic basal fluctuation of the chosen experimental systems. It is not by chance that the most robust results obtained in this field are found in experimental models where the collective response of the elements comprising the system, i.e. cell communication, are strongly enhanced by the tissue architecture such as animal models and histiotypic culture models in which multicellular organization is enabled [16,17,187]. Therefore, in order to explore bystander effect mechanisms, it is mandatory to study the peculiar features of the intercellular signaling and of its perturbation induced by radiation.

Systems biology can be of help in obtaining this objective.

CELL COMMUNICATION PERTURBATION: THE EXAMPLE OF IL-6 SIGNALING

The objective of systems radiation biology is an accurate and predictive understanding of the entire system after radiation exposure. Nevertheless, with the current experimental and theoretical techniques, it is almost unfeasible to completely describe the late biological effects perturbed by low dose of radiation exposure, on the whole cell signaling system. In order to evaluate the complex response of the system, we performed a series of experiments to establish protocols for reliable studies of the mediators that regulate the bystander processes. The study focused in particular on protein mediated signals, including cytokines and growth factors. These experiments gave quantitative information of the perturbation induced by irradiation and were coupled with different modeling approaches in order to control for the different possible scenarios involved: a Monte Carlo code and an analytical model were developed to quantify both the local mechanisms and the average quantity dynamics, respectively, that regulate the transmission of the signals [19].

Our findings showed a key modulation of IL-6 (Interluekine-6) induced by radiation for up to 20 hours after irradiation, suggesting a possible involvement of this molecule for the long-term induction of bystander effects [13].

In order to quantify the perturbing role of radiation on this system it is necessary to evaluate the peculiar features of the systems without radiation (sham irradiated cells). The experimental results illustrated a major role of IL-6 release for unexposed cells induced by a change of the cell culture medium (one of the common techniques developed to investigate the bystander phenomena), indicating that these messengers could also be part of the response of the cells to generic stimuli (stress response). Interestingly, the examination of the irradiated cells pointed out that the radiation-induced response in this context is approximately one-third of the response induced by a change of the medium.

From the analysis of these data, the perturbation induced by radiation seems to modulate the already perturbed signaling generated by change of the cellular culture medium. According to Kitano's definition [20] of robustness ("*a property that allows a system to maintain its functions against internal and external perturbations"*), this specific experimental system (fibroblasts cultured *in vitro*) does not appear very robust in terms of the stability of the system processes, i.e. cell-to-cell signaling, after the external perturbation (i.e. a change of medium and irradiation). In this context, the usual linear cause–effect model (i.e. an isolated and unique signal able to induce a single effect), although very useful to

frame the theoretical and experimental work, is an oversimplified (and possibly also a misleading) view of cell-to-cell communication.

To validate this approach and to extend the model, the general systems theory (See Section 1) can be of great help: indeed, the reported analysis was a 'purely' mechanistic, additive theoretical perspective. In this study, it has been demonstrated that it is difficult (or even not possible) to investigate the role of radiation in a 'totally isolated' system. One needs to move the focus from the pure linear interpretation of cause–effect of cell response, to a more 'circular' interpretation which is more sensitive to feedback and self-corrective changes typical of complex systems.

IS THE POOR ROBUSTNESS OF THE EXPERIMENTAL MODEL THE ORIGIN OF CONFLICTING BYSTANDER RESULTS?

In vitro inquiry of biological effects represents an important step in research since it allows us to perform investigations in situations "as controlled as possible", with a limited set of parameters usually accessible with elementary experiments. However, the idea to separate a complex system (biological model) into simpler units, though useful for picturing the possible processes, can be very misleading. In fact, the behavior of the *whole* is usually driven from the complicated and non-linear interaction networks of biological pathways of the elementary units. This leads to an overall situation often complex and counterintuitive.

Despite that, the *in vitro* investigation represents a unique tool and probably (due to its relative simplicity) the most common technique used in most of the laboratories around the world. In order to interpret and possibly translate the data resulting from these investigations to the *in vivo* situation, we have to be aware of how far we are from the real situation, performing, whenever possible, key experiments able to quantify the features of the two systems.

The results mentioned above [13] helped us to obtain information on the systems associated with cellular communication involved in the bystander phenomena including the quantification of the key processes involved in signaling (release, diffusion and decay of the molecules involved in medium-mediated damage). It turned out that the system adopted – signaling via IL-6 molecules for cells grown in 2-d culture in vitro - is far from the robustness seen in a more structured model (*in vivo* or 3D

tissues), elucidating one of the possible reasons for the plethora of conflicting experimental results reported in the literature.

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