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Forum Article

REFLECTIONS ON BASIC SCIENCE STUDIES INVOLVING LOW DOSES OF IONIZING RADIATION

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Abstract—**Investigation of health effects of low doses of radiation as a field of study has been riddled with difficulties since its inception. In this document we will use 100 mGy as the cutoff upper limit for low-dose radiation, borrowing this definition from the U.S. Department of Energy, although other agencies and researchers sometimes include up to five-fold higher doses under the same title. Difficulties in this area of research are most often ascribed to the fact that effects of low doses of radiation are subtle and difficult to distinguish from the plethora of other low-grade stresses. Thus, for example, most epidemiological studies include hundreds of thousands of samples and generate risk estimates that are statistically meaningful only when they are considered on a scale of hundreds or thousands of people. A logical approach to remedy the situation for low-dose research was to conduct well-controlled animal studies with hundreds of animals; nevertheless, even after many such studies were completed, our understanding of the biological basis for risk from low-dose radiation exposure is still not conclusive. In this paper we argue that the problem lies in the fact that our approach to animal studies is not comprehensive but conceptually binary. While some researchers apply epidemiological models to animal data, others look into molecular and cellular biology only. Very few studies are conducted to bridge this gap and consider how a realistic model of DNA damage could be integrated into a realistic model of radiation carcinogenesis.**

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Key words: cancer; modeling, biological factors; radiation, low-level; radiobiology

INTRODUCTION

In this article, we argue that the current perception of what radiation carcinogenesis modeling is about is one of the core problems in radiation research focused on doses smaller than 100 mGy according to the U.S. Department of Energy's low-dose definition (U.S. DOE 2018). It is evident that a linear-quadratic formula has no place in describing DNA damage caused by low-dose radiation, but it is less clear that we should try to develop formulas rooted in molecular and cellular biology instead. It should be possible to conduct basic science research focused on a deeper level of understanding of low-dose radiation exposures (Fig. 1) and use it to develop models of radiation effects that would be based on concrete facts. To this day, many low-dose radiation carcinogenesis studies assume that the probability that one cell of a multicellular organism will acquire multiple mutations transforming it into cancer and the ability of that cancer to thrive can be described by a curve plotted against an axis that shows total radiation dose. At the same time, in all the many decades of radiation research no study has documented that radiation-induced cancers (even at high doses) have any unique feature

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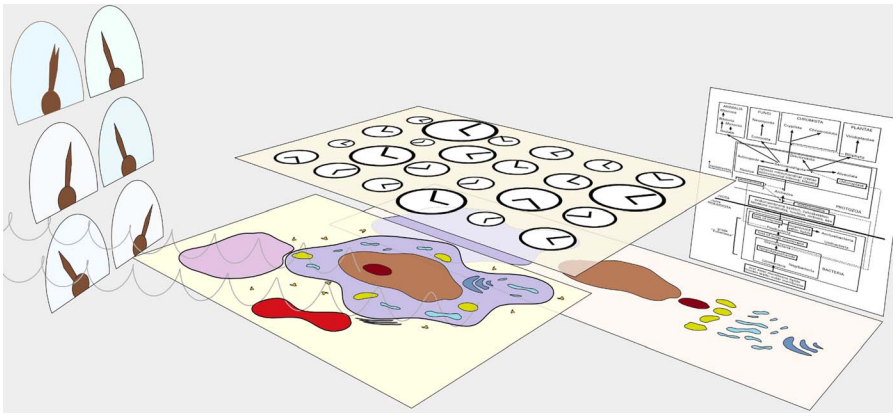

Fig. 1. Many issues need to be considered in basic science exploration of low-dose radiation effects, and we propose that it should be possible to model them as well. This illustration is far from a complete view of the situation, nevertheless it conceptualizes some of the most important questions that require additional basic science research. A set of gauges at the left side of the image represents different aspects of radiation exposure such as radiation quality, dose and dose rate, fractionation and protraction, and other physical aspects of radiation exposure such as entire or partial volume of the cell, organ, or organism exposed. Images in the yellow (central) plane represent cellular communications in the physiological and molecular environment of an irradiated cell. Each cell is in constant communication with its environment as a part of a whole organism—with its immediate cell neighbors to which it is connected by tight junctions, with mobile cells such as red blood cells, as well as with foreign cells and agents such as bacteria. Cellular environment also includes extracellular matrix, exosomes, protein ligands, multitude of cytokines, chemokines, hormones, free ions, etc. Images in the orange (lower) plane represent different structural components of cellular organization, from organelles and their subparts to their component macromolecules and different structural aspects of their organization. In this context, DNA would be examined at all levels of organization—from its sequence to its three-dimensional organization within chromatin and numerous structural and chemical modifications. The white panel on the right side of the figure depicts six kingdoms of living organisms (adapted from Cavalier-Smith 1998) and the facts that organisms from all kingdoms of life cohabit the same space, respond to stresses in a complex manner, and in so doing, affect one another. In the beige-colored (upper) plane are many clocks. This drawing conceptually represents the importance of time and timing for all aspects of biological responses to radiation exposures. The time that the cell has for repair of radiation damage before it encounters another stress or responds to a physiological stimulus is critical for maintenance of cellular homeostasis. Equally important are age of a cell, age of the organism as a whole, etc. With the development of new computational approaches, we are nearing the situation where we may be able to integrate all of these different data (especially if we are using prospective wet bench and animal experiments) into multidimensional models.

attributable to the radiation exposure, let alone a biomarker that separates them from "regular" non-radiation-induced cancers. Thus, one notes the significant gulf between approaches used to discuss basics of "regular" cancer, such as those featured in articles about hallmarks of cancer (e.g., Hanahan and Weinberg 2011), and attempts to abandon consideration of nuances such as immune system responses and capacity to induce neoangiogenesis in order to reduce cancer into a single general concept that is plottable against the same type of stress across several orders of magnitude. Yet, it is also well established that low-dose radiation may activate cytotoxicity of natural killer cells (e.g., Luzhna and Kovalchuk 2014) as well as improve neovascularization (e.g., Ministro et al. 2017). In other words, radiation has many effects on an organism as a whole—some of them procancer, some anticancer. It even appears that in many cases effects on the same cellular function vary with changes in total dose and dose-delivery rate, while they also depend on genetic makeup of the organism as well (e.g., Ishida et al. 2010; Lemon et al. 2017).

This exposition of the situation in radiation biology should tell us that we may not be modeling what should be modeled; moreover, it appears that we are not even considering such an effort. Our computational powers are increasing, and yet we still consider whole organisms as compact units with permanent features. We will now mention a study to which we primarily contributed raw data—a recent paper by Tony Brooks (Puukila et al. 2017) as an example of how an altered approach to data interpretation may change the conclusions of a study. Data from long-concluded experiments with moderate and high doses of radiation delivered by internal emitters were significantly reinterpreted because the length of the cell cycle was used as one of the variables in the analysis.

In some ways, similar efforts were made in epidemiology studies as well where the two-stage clonal expansion model was used to reinterpret the solid cancer mortality data from the Techa River cohort (Eidemuller et al. 2008). In this study an existing biological model was used to inform statistical analysis; however, sample variability of epidemiological human data prevents us from using this data in a reverse manner to inform us about the validity of a biological model. In epidemiology there is no opportunity to fine tune the experiments and permit one to have true test and control populations, much less several similar but nonidentical test populations. It is precisely for this reason that complementary research with animal models is needed in radiation research in general and low-dose radiation research in particular.

We propose that is possible to go much further and envision new ways of modeling that would be synergistic with

animal research and capitalize on defined biological variation as the long-established and most fruitful source of all biological knowledge. Just as any wet bench scientist knows that it is not possible to obtain good data without positive and negative controls, new computational approaches can be imagined that would use biological positive and negative controls in inventive ways; for example, we could try to include a portion of the data that is deliberately selected to be skewed in a predetermined direction and include test data that are expected to fall within or outside of a certain expected domain. At the very least we could manage to plot the course of radiation carcinogenesis using probabilistic mathematics, starting from the number of cells that may have sustained DNA damage in an organism at the time of its exposure, factoring in cellular turnover, the size of the genome, and an overall probability of procancer mutation per cell per cell cycle per sievert. These numbers would be extremely small, but we could employ them and then widen the study by varying the volume of the body that was irradiated (and with it the number of cells exposed) or by varying the repair capacity of the organism (using either genetically modified organisms or altering their radiation resistance by chemical means).

As a matter of fact, many such studies were already done but nobody has attempted to do a comprehensive crisscross modeling using primary data or even a meta-analysis of the published data. In many cases the laboratories that have conducted such studies have collected much data but selected for publication only some of it; at other times only some of the data was recorded. This attrition of data (and possible data) is sometimes driven by the interest of funding agencies (e.g., if a lab has a grant to do lung research they may dispose of the nonlung data); at other times, data that gets published is chosen because of input from peers. For example, generally low interest in obesity has led to difficulties with publishing low-dose radiation findings concerning it. Thus, even though increased incidence of obesity in animals exposed to chronic low doses of low linear energy transfer (LET) radiation was noted in the early 2000s, these data were not published until 10 y later (Uehara et al. 2010). Moreover, even though others found association between neutron exposures and leptin (Cestelli Guidi et al. 2012), no direct research into the relationship of obesity and low doses has yet been done. It is worthwhile to note that our understanding of carcinogenesis in general is still far from comprehensive—additional low-dose induced carcinogenesis research could open up the doors to a new understanding of this process. It is interesting to note for example, that the list of hallmarks of cancer (Hanahan and Weinberg 2011) increased to include immune system modulation after high-dose radiation work with animals demonstrated that ionizing radiation inhibits growth of distant untreated tumors due to immune system response (Demaria et al. 2004).

TIME, RADIATION QUALITY, AND VOLUME OF IRRADIATED TISSUE

Understanding of biological aspects of radiation risk is integrated into radiation protection policies that are mindful of exposure-associated time periods. For example, astronauts have prescribed maximal monthly, yearly, and career exposures (Nelson 2016). It is clear from this practice of radiation protection that we have a strong understanding about the importance of dose protraction, which in turn means that resilience of biological organisms to radiation depends on the opportunity for DNA and cell repair to take place. Many intrinsic and extrinsic factors influence the repair (e.g., Bladen et al. 2007; Jeggo and Downs 2014); experimental in vivo approaches to dissect this concept are difficult yet very informative (Tanaka et al. 2017) but still are not used for extensive modeling. The situation is slightly simpler with regard to in vitro modeling in single cells (Ou et al. 2017) where extensive optical and electron microscopy aid in development of models that describe ultrastructure and three-dimensional organization of chromatin in interphase and mitotic cells.

In addition to everything mentioned above it is also important to remember that DNA damage induced by radiation has a slightly different "flavor" depending on radiation quality. Thus, while any radiation damage is considered to be more complex than a DNA cut caused by intrinsic reactive oxygen species (ROS) production, abundance of unrepaired, clustered DNA lesions following irradiation is higher after high-LET radiation exposure (e.g., Mavragani et al. 2017). In addition, in high-LET irradiation DNA damage increases with atomic Z number for the same LET value (Jezkova et al. 2018), while for materials with the same Z number, lower-energy particles cause more damage than high-energy particles (Sridharan et al. 2015). As a consequence, different DNA repair mechanisms are engaged after different types of radiation exposures, at least in cancer cells (Fontana et al. 2015). Therefore, a thorough model for low-dose-caused carcinogenesis associated with high-LET exposures would need to consider chromatin structure on one hand and then focus on the volume of specific type of DNA damage per cell, the likelihood of engagement of a given DNA repair mechanism, and conversely, the likelihood of its failure.

Partial-body irradiation was infrequently used in low-dose radiation research; this is not surprising considering that even total-body irradiation experiments with low doses of radiation caused only relatively subtle effects. Nevertheless, with better modeling efforts it may be possible that moderate doses delivered to a portion of the body could be used to understand and predict effects of low doses of radiation. In many cases, partial-body irradiation with high doses was used to study bystander effects either with regard to off-site tumor induction or localized DNA damage investigation (Buonanno et al. 2015). In any event, a good model

for radiation effects would have to include partial-body irradiation effects.

BIG DATA COLLECTION AND ANALYSIS HOPES—ARE THEY JUSTIFIED IN LOW-DOSE RADIATION?

There is a general perception in recent years that science may be accelerated by utilizing "big data" analysis approaches on one hand and on the other, by collecting as much in-depth data about a given subject of inquiry as possible and making it publicly available in raw as well as relatively digested formats (e.g., NCI 2018). There are serious concerns about direct adoption of either one of these approaches without specific adaptations that would make them more suitable for radiation biology, and yet, if interest in low-dose radiation research is limited, its researchers are unlikely to obtain funding needed for extensive genome-wide association studies. It is necessary to think about biology and biological processes first and then try to engage the interest of computational scientists who may be willing to conceptualize new—low-dose radiation compatible—ways to analyze data. It is equally essential that all low-dose, in-depth analyses be done with numerous positive and negative controls—not just with regard to radiation exposures but also with regard to genetic and environmental variation. Most importantly, it is necessary to develop data depositories that would permit cross comparisons and analysis of primary data from different laboratories. Low-dose radiation effects are subtle but numerous, and the likelihood of overlooking possible key findings may be greater in this field than in many other spheres of biology. Websites supported by the National Institutes of Health (NIH) provide support for gene expression analysis—it is when we dip into this source to extract radiation data that we find how limited that kind of research has been. It is therefore that much more important to deposit results of low-dose research into these databases and perhaps create analogous databases dedicated to radiation research on animal models.

It is important to appreciate that computational exploration of cancer is primarily focused on specific cancer types and specific genes and pathways that can be associated with them (Constantinescu et al. 2016) or on in-depth sequence comparisons to estimate differences between germ line and somatic mutations (e.g., Bareke et al. 2013). No model that disregards exact mutations yet focuses on cell duplication time and radiation dose per cell has been developed so far, yet such a model would best serve the low-dose radiation research community.

Existence of databases devoted to radiation research could be of tremendous help in accelerating this specific area of research by reducing duplications and providing guidance about potentially unexpected effects of radiation. An example from high-dose radiation research is a good illustration of this point. A recent study by Ramirez-Fort and others (Ramirez-Fort et al. 2018) discusses the need to deal with viral reactivation in irradiated patients. At the same time, viral gene expression was noted in irradiated cells in culture as early as the 1980s (Silver and Dales 1982). Similarly, Dubrova and others (Dubrova et al. 2000) found an increase in minisatellite mutations in mice exposed either to gamma rays or neutrons irrespective of direct damage to minisatellite DNA. To the best of our knowledge, nobody has studied Alu (repetitive DNA) sequence changes in humans associated with ionizing radiation, although some have attempted to use Alu pyrimidine dimers as biomarkers of ultraviolet (UV) exposure (Englander and Howard 1997). When considering either viral or repetitive DNAs, one need only to think about their great capacity to induce other mutations and the possible connection that these DNA changes could have with cancer induction.

CONCLUSION

A recent study by Demetriou and others (Demetriou et al. 2018) discusses the temporal sequence of acquisition of hallmarks of cancer in the course of exposure to chemical carcinogens, based on mutations in biopsies. Radiation is much more complex in its effects than chemical mutagens, and it modulates all hallmarks of cancer, with different doses and radiation-delivery approaches often having contradictory effects, sometimes supporting and sometimes opposing cancer growth and metastasis. It is also clear that certain doses and dose rates can be associated with different cancer hallmarks. In all, a better approach to model the low-dose radiation–cancer connection may be to plot each individual cancer hallmark separately against a spectra of low doses of radiation considering all other relevant aspects of radiation exposure (dose rate, LET, volume of tissue irradiated, etc.).

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