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Review article

Concepts and challenges in cancer risk prediction for the space radiation environment

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ABSTRACT

Cancer is an important long-term risk for astronauts exposed to protons and high-energy charged particles during travel and residence on asteroids, the moon, and other planets. NASA's Biomedical Critical Path Roadmap defines the carcinogenic risks of radiation exposure as one of four type I risks. A type I risk represents a demonstrated, serious problem with no countermeasure concepts, and may be a potential "show-stopper" for long duration spaceflight. Estimating the carcinogenic risks for humans who will be exposed to heavy ions during deep space exploration has very large uncertainties at present. There are no human data that address risk from extended exposure to complex radiation fields. The overarching goal in this area to improve risk modeling is to provide biological insight and mechanistic analysis of radiation quality effects on carcinogenesis. Understanding mechanisms will provide routes to modeling and predicting risk and designing countermeasures. This white paper reviews broad issues related to experimental models and concepts in space radiation carcinogenesis as well as the current state of the field to place into context recent findings and concepts derived from the NASA Space Radiation Program.

1. Space radiation carcinogenesis and risk

The programmatic research goals of NASA's space radiation element are to assess the consequences of a complex space radiation environment using studies in experimental models, 'omics and systems biology to provide an improved new mechanistic basis for risk prediction. Since cell and tissue studies in humans are difficult to obtain, estimates of risk from human space travel need to be based on a mechanistic understanding of complex effects elicited by radiation exposure across time and level of organization. The radiation risks of concern are cancer and degenerative pathologies of the central nervous system and other tissues. Defining the radiobiology of space radiation is critical to understanding long-term risks, including how space radiation exposure at middle-age modulates diseases associated with increased age such as the development of invasive cancer and neurodegenerative diseases.

Astronauts traveling in space are exposed to several types and energies of ionizing radiation estimated to total approximately 50–2000 mSv, depending on whether they are spending 6 months on the International Space Station, or the surface of the moon or of Mars (Cucinotta and Durante, 2006; Cucinotta et al., 2008; Durante and Cucinotta, 2008). Concern is greater for deep-space

Abbreviations: Linear energy transfer, (LET); High-energy and charge, (HZE); Galactic cosmic rays, (GCR); Non-targeted effects, (NTE); Relative biologic effectiveness, (RBE); Life Span Study, (LSS); Solar particle event, (SPE).

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missions since radiation dose rates are higher than in low-Earth orbit and the duration of such missions will typically be longer (Zeitlin et al., 2013). However it is important to point out that these dose rates are considerably lower compared to those at which human populations show increased rates of cancer following γ-radiation or x-rays and that irradiation with particles is both qualitatively and quantitatively distinct.

The radiation encountered in space is encompassed by the term galactic cosmic rays (GCR), which include protons of low-, medium- and high-energy, particle nuclei of high energy and charge (HZE), and neutrons of diverse dose rates and energy levels produced in secondary radiation interactions within the spacecraft (Guo et al., 2015). Estimating the risk of fatal cancers induced by these chronic mixed radiation exposures, which is estimated to total 50 mSv, is the focus of current NASA radiation research using experimental models since no epidemiology of cancer exists for humans exposed to the GCR, secondary radiation from particle fragmentation within the spacecraft, or the radiation from SPE (Cucinotta, 2015).

Particle irradiation results in non-homogeneous dose distribution such that at low fluence some cells in a complex organism are traversed and some are not, and that particle traversed cells are essentially acutely irradiated, which means that dose and rate concepts used for sparsely ionizing radiation are misleading. The fluence rates during space travel correspond to tissue doses of about 0.3–0.6 mGy/d and effective dose-rates of 1–1.8 mSV/d, respectively (Cucinotta et al., 2008; Zeitlin et al., 2013). Over the course of a solar particle event (SPE), dose rates within the vehicle can fluctuate between 0–100 mGy/hr and can also differ between tissue sites because the variable energy spectra of particles have different depth distributions.

The current model of cancer risks used by NASA, NSCR 2012, scales cancer incidence and mortality rates estimated from epidemiology data using a dose and dose-rate effectiveness factor and radiation quality factor, to estimate the effects for the low dose-rates and radiation types in space respectively (Cucinotta et al., 2012). The large uncertainties, in order of decreasing importance, in this model are: the radiation quality factors, dose and dose-rate dependencies, the transfer or risk across populations, the determination of space radiation organ exposures, and the various errors in human data sources. In addition, there are uncertainties related to the underlying assumptions of the model due to possible qualitative differences between high- and low-linear energy transfer (LET) radiations, the validity of the assumptions of linearity and additivity of effects for different radiation components, and the possible synergistic risks from other flight factors on radiation risks.

The carcinogenic effects of radiation became apparent soon after Roentgen's discovery of radiation in 1896 (NCRP, 1993). Notably, neither morphologic nor biochemical characteristics uniquely identify a neoplasm as radiation-induced compared to sporadic cancer. Early studies of the pathogenesis of neoplasia using chemical carcinogens resulted in the description of four stages of the natural history of tumors: initiation, promotion, progression, and metastasis (Pitot, 1993). Initiation by genotoxic agents such as radiation is often ascribed to DNA damage, whose misrepair can generate oncogenic mutations. Advances in molecular oncology underscore the complexity of molecular events that create the genomic 'landscape' of cancers (Vogelstein et al., 2013). A myriad of genomic mutations, rearrangements, and deletions occur in an ongoing process during the molecular evolution of many cancers. The high frequency of genomic changes in some cancers argues for early mutations in surveillance mechanisms or genomic instability that allow the accumulation of a critical number of events.

Radiation is considered a "complete carcinogen", i.e. able to both initiate and promote (Fry et al., 1982). Radiation directly induces DNA damage, usually in proportion to dose, whose misrepair in epithelial cells may create oncogenic mutations Although radiation's promoting activity is less well characterized compared to experimental models using chemical carcinogens, radiation also affects phenotype and signaling that modifies the surrounding microenvironment; this class of actions is referred to as non-targeted effects (NTE) and often exhibit switch-like or threshold dose-responses (Barcellos-Hoff et al., 2014). Promotion of initiated cells may result from various radiation-induced molecular and cellular signaling events. Radiation rapidly triggers cell cycle checkpoints, initiates DNA damage processing cascades, and/or cell death programs. Long-term changes are also evident in cells, including well-documented stress and oxidative signaling that may be mediated by mitochondrial dysfunction (Spitz et al., 2004). Radiation exposure can perturb tissue homeostasis by activating innate immune system reactions, such as macrophage activation, that persist, leading to a cycle of sub-clinical tissue damage due to smoldering inflammation, which itself is capable of promoting cancer by altering cell interactions, as well as contributing to mutations due to oxidative processes (Mukherjee et al., 2014; Coates et al., 2008). Intestinal epithelial cells show pro-inflammatory markers and increased oxidative stress up to a year after irradiation (Datta et al., 2012). There is evidence for increased pro-inflammatory signaling in the survivors of the atomic bomb (A-bomb) cohort even decades later (Hayashi et al., 2012).

Significant dose-dependent evidence for the human risk of cancer from radiation exposures has come from the A-bomb survivors and a select number of medical or accidental high-dose/dose-rate exposures. Data from these cohorts indicate tissue-specific radiation-induced cancer mortality (Shimizu et al., 1990). For example, epidemiology suggests excess leukemia, breast and skin cancer among U.S. radiological technologists working before 1950 under high-dose limits (Mohan et al., 2003; Sigurdson and Jones, 2003).

In contrast, there is no evidence of increased cancer risk with chronic occupational exposures to conventional radiation under current occupational limits in medical workers (Yoshinaga et al., 2004), in nuclear workers in the U.S. (Berrington de Gonzalez et al., 2009), in a combined study of nuclear workers in the U.S., United Kingdom and Canada (Cardis et al., 1995), or European airline flight crews (designated as radiation workers since 1996) (Langner et al., 2004; Sigurdson and Ron, 2004; Zeeb et al., 2003). Cancer mortality among U.S. nuclear power industry workers after chronic low dose exposure to ionizing radiation actually shows a significant "healthy worker" effect with a reduced mortality compared to the general population (Howe et al., 2004).

The occupational exposures incurred by astronauts in deep space have no terrestrial parallels on which to base risk estimates. Radiotherapy with neutrons and charged particle beams has provided only a few reports of particle-radiation-induced tumors with limited follow-up times, and case reports with inadequate statistical evaluations (Chung et al., 2013; Marta et al., 2015). Charged particles are used to treat cancer because their physical characteristics of dose distribution allows more sparing of normal tissues than conventional radiation therapy, but to date neither the use of proton nor carbon radiation therapy is associated with an increased risk of second malignancy compared with photon therapy, however the number of studies with long-term follow up times is limited.

Laboratory animal studies with neutrons and charged particle beams have provided important information on dose-, dose-rate, and dose-fractionation-dependent cancer latency, incidence, genetic susceptibilities, and aggressiveness. Compared to low-LET radiation, such as γ-rays, early animal model studies using neutrons or HZE ions have raised the troubling concern that their relative biologic effectiveness (RBE) for tumorigenesis may well

be more than a magnitude greater (Cucinotta et al., 2013; Fry et al., 1983). Unique aspects of the particle-induced DNA lesions, including gene-specific susceptibilities, and immune responses are discussed below. Carcinogenesis studies in animal models have begun to elucidate underlying mechanisms of action involved in the generation of space radiogenic tumors, which may provide leads to effective countermeasures. Therefore, we need to address the following questions as we move forward experimentally:

1. What mechanisms underlie radiation carcinogenesis for the major tumor types that threaten astronauts on long duration missions?
2. Do high-LET and low-LET radiation exposures induce cancers by the same mechanisms?
3. How efficient are low dose rate exposures for carcinogenesis?

The goal of this paper is to provide an expert's review of the current status of the field of space radiation carcinogenesis, and a summary of the research questions and challenges requiring further study. Documenting radiation quality differences in experimental systems and defining the underlying biological mechanisms are necessary for understanding the potential risk of space travel.

2. Cancer paradigm

For more than half a century, the multi-stage model for carcinogenesis has been the paradigm for carcinogenesis (Armitage, 1985; Armitage and Doll, 1954). This model states that initiation, promotion, and progression are sequential stages occurring within cells that result in a malignant cancer. More recently, the importance of concomitant cell-extrinsic events that include the establishing a tumor microenvironment and evading immune responses during carcinogenesis has come to be more appreciated (Barcellos-Hoff et al., 2013; Dunn et al., 2004). There are no human data available to estimate the cancer risk on the effects of high LET particles in the space radiation environment, thus, current risk models of space radiation-induced carcinogenesis depend mainly on low-LET human data and animal experiments. Many mathematical models of radiation-induced carcinogenesis focus either on short-term processes involved in initiation of carcinogenesis or longer term processes involved in promotion; more recently, models have been developed that integrate the short and long-term processes (Shuryak et al., 2006, 2009, 2010a; Little, 2000; Mothersill and Seymour, 2003).

2.1. Initiation

Carcinogenesis is a chronic, complicated process that involves gene mutation, genomic instability, over-activated oncogenes, inactivation of tumor suppressors, epigenetic changes, abnormal metabolism and changes in microenvironment (Hanahan and Weinberg, 2011). However, the mechanisms underlying carcinogenesis have yet to be completely understood. The Life Span Study (LSS) cohort of atomic-bomb survivors is a primary source for quantitative risk estimates that underlie radiation protection (Preston et al., 2003). The data derived from A-bomb survivors suggests that a single exposure to radiation tends to increase the incidence across the spectrum of cancers that are common in a population rather than increase the frequency of specific tumor types. Such information suggests that radiation may promote some cancers, thereby via shortening the overall latent period and subsequently speeding up the general carcinogenesis process. Notably, shortened latency is not seen in all experimental models in which cancer incidence is increased, suggesting that different mechanisms likely contribute.

Initiation of carcinogenesis is defined as the induction of genetic changes, such as mutations, that create a premalignant state. HZE particles induce clustered DNA damage in which multiple complex double-strand breaks occur within one or two helical turns of the DNA, which is more challenging to repair than individual, widely dispersed lesions (Georgakilas et al., 2013). In fact it is difficult to completely separate initiation from promotion and progression since radiation-induced DNA damage can activate myriad pathways that result in genomic instability and may be involved in multiple stages of carcinogenesis. The initial complexity of HZE damage contributes in as yet poorly understood manner to the phenotypes observed in irradiated cells that include induction of reactive oxygen species, DNA damage signaling and inflammation (Sridharan et al., 2015).

2.2. Promotion and progression

Classically, promotion and progression describe the conversion of premalignant cells to cancer cells and their expansion and acquisition of additional traits (Hanahan and Weinberg, 2000). Importantly, it is now recognized that cancer involves the co-option of normal cellular pathways (Hanahan and Weinberg, 2011). Again, radiation is thought to be a "complete carcinogen" as it is both an initiator and a promoter (Fry et al., 1982; Shuryak et al., 2009). With increasing age at radiation exposure, cancer promotion and progression assume more importance due to the larger number of already-initiated, premalignant cells in older individuals. This concept was recently demonstrated in treatment-related acute myeloid leukemia occurring after chemotherapy and/or chemotherapy combined with radiation therapy for other cancers. The authors showed that the same *TP*53 mutations found in some leukemias were present in blood or bone marrow samples obtained more than 3 years prior to diagnosis and in two cases prior to receiving chemotherapy (Wong et al., 2015). Moreover, nearly half of peripheral blood samples from healthy elderly individuals harbored low frequencies of functional *TP*53 mutations (Wong et al., 2015).

Long latency between exposure and solid tumor development is considered evidence of relative importance of promotion and progression, in contrast to hematopoietic cancers with short latencies ascribed to the primacy of radiation-induced initiation. Long latency is characteristic of ovarian tumors induced by radiation (Jeng et al., 2007; Furth and Boon, 1947; Gardner, 1950; Clapp, 2016). Interestingly, depletion of germ cells in mice by several methods, including administration of ovotoxic chemicals and radiation exposure, leads to the development of epithelial and/or stromal ovarian cancers (Capen et al., 1995; Vanderhyden et al., 2003). Germ cell depletion results in loss of ovarian negative feedback, leading to elevated levels of the pituitary gonadotropin hormones, follicle stimulating hormone and luteinizing hormone. Chronic gonadotropin stimulation of the ovarian surface epithelial cells, which possess both hormone receptors, has been proposed as the common pathway by which experimental paradigms that result in germ cell depletion cause ovarian tumors (Capen et al., 1995; Vanderhyden et al., 2003). These data support the importance of ovarian tumor promotion and progression as a result of chronic gonadotropin hyperstimulation.

2.3. Extrinsic processes

It is generally accepted that carcinogenesis involves cell intrinsic and extrinsic processes. Radiation-induced effects that manifest away from the irradiated target (e.g. bystander or abscopal effects) are evidence of systemic consequences of irradiation (Kadhim et al., 1994; Lorimore et al., 2003; Wright and Coates, 2006; Hei et al., 1997; Wu et al., 1999; Zhou et al., 2000, 2005). An

important example is the immune response. How the interplay between inflammatory cells and mutated neoplastic cells promotes cancer development and progression remains a subject of intense investigation. Several important pathways have been identified. Among them, IL-6 signaling pathways play a major role (Naugler and Karin, 2008). Macrophages are the main source of IL-6 during acute inflammation, and T cells during chronic inflammation. Importantly, IL-6 orchestrates the transition from acute inflammation, dominated by granulocytes, to chronic inflammation, dominated by monocytes/macrophages and regulates, together with TGFβ, the differentiation of naive T cells to the Th17 pro-inflammatory phenotype, thus influencing the type of adaptive immune response (Bettelli et al., 2006).

At ionizing radiation doses of 0.5 Gy or less there is minimal cell death in most tissues. However, irradiated cells release oxygen and nitrogen free radicals that activate both normal cells and innate immune cells (such as macrophages) to release cytokines and chemokines (Barcellos-Hoff, 1998). This can lead to chronic inflammation initiating a pro-tumorigenic role of the immune system (Wright and Coates, 2006). At higher doses of radiation leading to significant cell death, radiation induces signals that are sensed by the innate immune cells (e.g. dendritic cells), resulting in an adaptive immune response. Immune-modulating effects of radiation are influenced by many factors including total doses, dose-rates and cell autonomous and micro-environmental cellular responses to both normal and tumor initiating cells (Hall, 2006). The complex relationships among DNA damage, cell death, the microenvironment and the host immunological responses, and their dependence on radiation exposure dose, dose-rate and quality continue to be areas of intense research.

Recent studies of radiation carcinogenesis have implemented methods to isolate tissue-mediated processes from initiation *per se*. For example, mammary cancers that developed after transplantation of *Trp53*-/- mammary gland tissue into hosts irradiated before transplantation with densely ionizing charged particles had shorter latencies to appearance of palpable tumors, grew more rapidly and had different immunohistochemical and gene expression profiles compared to tumors arising in sham or γ-irradiated mice (Illa-Bochaca et al., 2014b).

Epidemiological studies have associated obesity and psychological effects of environmental enrichment or stress with increased cancer risk. Experimental studies have demonstrated that these associations may be largely due to effects on promotion and progression mediated by neuroendocrine and immune systems. Obesity is associated with systemic inflammation, as well as local inflammation in white adipose tissue, and there is evidence that both of these enhance progression of breast cancer (Howe et al., 2013; Arendt et al., 2013). Provision of environmental enrichment to mice decreases tumor growth in implanted and spontaneous tumor models via brain-derived neurotrophic factor activation of sympathetic nervous system signaling to decrease leptin and increase adiponectin production in adipocytes (Cao et al., 2010). On the other hand, chronic psychological stress and depression have been found to correlate with increased risk of metastasis of several types of cancer and with increased tumor angiogenesis, growth, adhesion, and invasion (Moreno-Smith et al., 2010).

What is currently lacking is a broad understanding of the effect of microgravity, stress, and the different types of radiations to be encountered during space travel despite the adequate spacecraft shielding from low energy protons (85% of the space radiation field). It is generally believed these combinatorial effects can lower immune responses but little is known about the reversibility, if any, of these effects upon return to earth. Moreover, on a long-term flight to Mars and back there are likely to be significant effects of HZE particles that cannot be protected against by shielding. Based on the measurements from the radiation assessment detector aboard the spacecraft that carried Curiosity to Mars, it was estimated that an astronaut would likely have an exposure close to the 3% REID limit of exposure now permissible for an astronaut's entire career, even with shielding (Kerr, 2013).

Excess relative risks are higher for those exposed earlier in life, with attained age-specific risks changing by about 20% per decade, but tend to decrease with increasing attained age, roughly in proportion to for any age at exposure (Preston et al., 2003). However, the risk models incorporate this prediction based on the assumption that HZE carcinogenesis is not qualitatively different for the now-older astronaut (on average, 45). Recent data from experimental models suggest this may not be correct and that promotional processes become increasingly important as the age at exposure increases (Shuryak et al., 2010b). As older astronauts are likely to have some initiated precancerous lesions and an ageing immune system, they may have increased (not decreased) risk of life threatening cancer from exposure to space radiation. On the other hand, that the aging host can have a powerful net suppressive effect on cancer progression was demonstrated in one study (Beheshti et al., 2015), and is in line with findings by Preston (Preston et al., 2007) for non-lung solid cancers, to the extent such suppression may delay diagnosis for greater ages at exposure. Noting the possible exception reported by Preston et al. for lung cancer, it may be important to distinguish between cancer diagnosis and mortality due to lung cancer, given that death from cancer, rather than incidence, is the risk endpoint. Understanding the combinatorial effects of space flights in which GCR exposure, as well as physiological and psychological effects of confined quarters and microgravity, contribute to cancer risk will require additional experimental data and modeling.

3. Evaluating carcinogenesis in experimental models

Animal models provide an opportunity to examine risks and mechanisms of HZE particle exposure to inform carcinogenesis risks in astronauts. Radiation carcinogenesis studies with HZE ions have been performed with rat strains and stocks, inbred mouse strains and their F1 hybrids, genetically diverse stock, and genetically engineered mouse models. Here we describe important recent findings from studies with conventional and genetically engineered rodents, discuss the limitations of these models, and outline challenges for future research.

3.1. Conventional mice and rats

Carcinogenesis studies using conventional, non-engineered mice and rats have focused on radiation quality effects and to a lesser extent on dose-rate/fractionation effects. The poorly understood impact of different radiation qualities (e.g., different LETs and track structures) on radiation carcinogenesis is a major source of uncertainty in risk calculations. Animal studies comparing the carcinogenic efficacies of radiation qualities encountered in space with γ-rays or X-rays, for which the risk of carcinogenesis is better understood, are used to generate relative RBE values for radiation carcinogenesis. These RBE values strongly influence the determination of quality factors used in risk estimation models. The advantage of such comparative studies is that information regarding the risk of carcinogenesis from HZE particles can be developed without a mechanistic understanding of how HZE particles cause cancer.

There have been relatively few large-scale carcinogenesis studies with HZE ions as described in recent reviews (Bielefeldt-Ohmann et al., 2012; Rivina and Schiestl, 2013). HZE ions have been shown to be highly effective in inducing mammary tumors, lung adenocarcinoma, Harderian gland tumors and hepatocellular carcinoma, but are no more effective than γ-rays for inducing acute myeloid leukemia or increasing lung cancer in certain genetically modified

mice, in rodent models that have known susceptibility to certain malignancies. The disparity in RBE for leukemia and solid tumors has been interpreted to indicate different underlying mechanisms for induction of these tumor types. It also underscores the importance of microdosimetry in HZE ion carcinogenesis (Peng et al., 2009; Weil et al., 2014).

Patterns are beginning to emerge from carcinogenesis studies. In the rat mammary tumor model, the radiation quality effect appears to be on latency. Almost all female Sprague Dawley rats develop spontaneous mammary tumors, generally more than one tumor per rat. Rats irradiated with either γ-rays or Fe ions develop them sooner. In the mouse hepatocellular carcinoma model, the radiation quality effect is on tumor incidence relative to genetic background. CBA or C3H mice have about a 12% incidence of spontaneous hepatocellular carcinoma. Irradiation with iron or silicon ions greatly increases the background tumor incidence compared to γ-rays, but does not decrease latency. In the mouse model of acute myeloid leukemia, the spontaneous incidence is very low (essentially zero), but leukemia is induced with roughly equal efficacies by HZE ion or γ-ray radiation.

Molecular and cytogenetic characterizations of radiation-induced acute myeloid leukemia suggest that similar lesions occur in leukemia from HZE ion and γ-ray irradiated mice, but the results are based on small numbers of cases. Similar characterizations have not yet been reported for solid tumors (with the exception of minimal data on mammary cancer Imaoka et al., 2007; Illa-Bochaca et al., 2014a), but may reveal radiation signatures or even radiation quality signatures. For example the gene expression profile of *Trp53* null mammary tumors arising in irradiated mice compared to controls can cluster not only human breast cancers into prognostic subtypes but can also cluster radiation-preceded from spontaneous human thyroid cancers and sarcomas (Nguyen et al., 2013). Such signatures could have profound implications on human radiation epidemiology studies because they may be used to distinguish radiogenic tumors from spontaneous tumors of the same histology.

Another benefit of characterizing tumors at the molecular level is that it may allow for better extrapolation of results between tumor types, something now based on histopathology. For example, the Harderian gland tumor model provides the largest data set for the LET to RBE relationship, but Harderian gland tumors do not occur in humans (humans lack Harderian glands). However, if molecular characterization of Harderian gland tumors revealed a fusion protein or an activated pathway associated with a human tumor, the LET to RBE data may also be applicable to that tumor type.

Results from fractionation studies have varied from no effect on tumor incidence, to a decrease in incidence, and a potential increase in incidence (Bielefeldt-Ohmann et al., 2012; Weil et al., 2014). The inconsistencies may be due to the different model systems and fractionation schedules employed and to small group sizes in some experiments.

A number of insights have emerged from carcinogenesis studies using conventional rodents. Two of the more important ones are that differences in genetic susceptibility to radiation carcinogenesis observed in low LET exposures extend to HZE ion exposures and that the proportion of malignant tumors induced may be greater for HZE ions than γ-rays. Murine strain differences in susceptibility to radiation-induced cancers have been known at least since the 1950s (Kaplan et al., 1956) and were highlighted in a large-scale survey of four strains reported 30 years later (Storer et al., 1988). In a few cases sequence polymorphisms responsible for the strain differences have been identified (Mori et al., 2001; Perez-Losada et al., 2012; Rosemann et al., 2014; Yu et al., 2001).

The studies of genetic susceptibility thus far have involved low LET radiation (and alpha particles in the case of osteosarcoma Rosemann et al., 2002) and it is by no means a foregone conclusion that strain differences also exist in susceptibility to HZE ion-induced tumors. However, two recent reports, one showing rat strain and stock differences in carbon ion-induced mammary tumorigenesis (Imaoka et al., 2007) and the other showing murine strain differences in iron ion-induced hepatocellular carcinoma (Bielefeldt-Ohmann et al., 2012), suggest that susceptibility to HZE ion carcinogenesis is under genetic control. Research to determine if the same genetic polymorphisms that determine susceptibility to spontaneous or gamma ray-induced tumors also determine susceptibility to HZE ion-induced tumors is ongoing. This is of interest because some risk estimates assume that the incidence of HZE ion-induced tumors will be a multiple of spontaneous or gamma ray-induced tumors. That is a reasonable assumption if gamma ray and HZE-ion induced tumors arise through substantially the same processes, in which case they should be controlled by the same susceptibility loci.

The carcinogenic effects of high LET radiation may be distinct in terms of magnitude, duration or quality compared to those following low LET radiation. In the Harderian gland model, pituitary isographs are used to increase incidence and decrease latency, but omitting this procedure doesn't appear to affect HZE ion-induced Harderian gland tumors, suggesting that HZE irradiation has promotional effects not elicited by low LET radiation. Compared to spontaneous or γ-ray-induced, HZE-induced hepatocellular carcinoma metastasizes to the lung more frequently (Weil et al., 2014), a finding presaged by observations of increased metastasis of Harderian gland tumors induced by neutron irradiation and earlier lethality of neutron-induced tumors (Fry et al., 1983; Grahn et al., 1992). There is, however, a caveat. Higher radiation doses are also associated with increased frequencies of Harderian gland tumor metastases and radiation dose effects have not yet been unraveled from radiation quality effects for HZE ion-induced tumors.

3.2. Genetically engineered mice

Genetically engineered mouse models are powerful tools to unravel the mechanistic underpinnings of radiation carcinogenesis, tumor aggressiveness and metastatic propensity. These models have recently been employed in studies of space radiation carcinogenesis to define the roles of specific proteins, such as the tumor suppressor p53 and pathophysiological pathways, such as inflammation. They are also being used to study the effects of space radiation on specific steps in carcinogenic pathways in lung cancer, the role of NTE in breast carcinogenesis and to identify the "cell of origin" for malignant transformation in glioblastoma and lung cancer. Evidence is also emerging from studies of engineered mice for enhanced aggressiveness following HZE exposure as measured by the proportion of carcinomas over total tumors (i.e. including adenomas) (Datta et al., 2013). The spectrum of mammary carcinomas arising from *Trp53* null epithelium in HZE irradiated mice shifts to more aggressive cancers as estimated by gene signatures and rapid growth rate compared to those arising in γ-irradiated mice or controls (Illa-Bochaca et al., 2014a).

Genetically engineered mouse models introduce mutation(s) that make the mice susceptible to carcinogenesis (Moding and Kirsch, 2012). In some cases, the mutation causes the activation of an oncogene and is sufficient to initiate tumor development by itself. For example, $Kras^{LA1}$ mice develop a large number of lung adenomas even in the absence of radiation. This mouse model can therefore be used to study the impact of HZE particles on lung tumor progression (Delgado et al., 2014). Lung cancer accounts for the most cancer related deaths worldwide, estimated at 1.3 million deaths each year. The large surface area/size of the lung makes it a prominent target for terrestrial and space radiation ex-

posure (Doll and Peto, 1978; Darby et al., 2006; Shay et al., 2006; Bruske-Hohlfeld et al., 2006).

The $Kras^{LA1}$ mouse model is a well characterized genetically engineered mouse that is susceptible to lung adenomas and adenocarcinomas similar to the major type of lung cancer common in humans, non-small cell carcinoma (Johnson et al., 2001). This model has been used to examine the effects of heavy ion (^{56}Fe) and simulated solar particle events (SPE) on cancer progression (Kim et al., 2014). The long term goal of this study was to assess the risk of developing invasive cancers in this mouse model and to extrapolate the data to human risk projections. The murine model ($Kras^{LA1}$) randomly expresses mutated KRAS in a subset of lung cells resulting in initiation and formation of lesions that mimic lung cancer progression in humans. Greater than 50% of the mice with oncogenic K-ras expression die in less than a year with a small percent of mutant mice living to a maximum of ~600 days (Delgado et al., 2014). About 9% of $Kras^{LA1}$ mice on a 129 strain background spontaneously develop invasive non-small cell lung adenocarcinomas. While the risk of normal mice to tumorigenesis upon exposure to low and high-LET radiation has been studied in the past, genetically engineered models have been helpful in that very limited data are available on progression of cancer susceptible mice to more advanced, perhaps fatal, invasive cancers.

Initial studies in this area included administrating whole body proton irradiation as a simulated SPE, of 2.0 Gy over 2 hours with a wide range of energies (50 MeV–150 MeV) compared to single dose protons and x-rays (Kim et al., 2014). Histopathological analysis of the irradiated $Kras^{LA1}$ mice 70–100 days post-radiation revealed an increase in both the number and size of lung hyperplastic lesions and adenomas. In addition, histopathological analysis of the irradiated $Kras^{LA1}$ mice one year post-radiation demonstrated an increase in tumor grade to invasive adenocarcinomas (Delgado et al., 2014). Thus, 2.0 Gy of SPE spectrum protons demonstrated a significant increase in occurrence of invasive adenocarcinomas compared to x-ray. The $Kras^{LA1}$ mouse model 70 days post fractionated (0.2 Gy × 5) exposure to 1 GeV/n ^{56}Fe increased expression of inflammatory factors within the lung about 200 days prior to the observation of invasive cancer. This suggests that chronic inflammation may be important in the progression of invasive cancer in the lung. Interestingly, the microarray signature of the lungs in these irradiated mice was compared to transcriptomic analysis of early stage lung cancer in patients and was predictive of human lung and breast cancer patient survival across multiple datasets (Delgado et al., 2014).

Alternatively, "sensitized" animal models with deletions of tumor suppressor genes can be used to expeditiously study the tumor promoting effects of HZE particles relative to low LET radiation. For example, *Apc*(*Min*/+) mice, which carry one mutant copy of the Apc tumor suppressor gene are predisposed to develop gastrointestinal tract tumors and develop an increased number and higher grade of intestinal neoplasias after HZE ion exposure (Datta et al., 2013).

In contrast, genetically engineered mice with brain-targeted deletions of Ink4a, Ink4b, and Arf (Nestin-Cre; Ink4a/$b^{-/-}$; $Arf^{f/f}$) do not present with spontaneous gliomas throughout their lifespan but readily develop radiogenic gliomas following irradiation with ^{56}Fe ions (Camacho et al., 2015). Ionizing radiation has been shown to increase the risk of GBM development in humans exposed to doses as low as 50 mGy of X-rays (from CT scans) making the study of HZE-driven gliomagenesis particularly relevant for extraterrestrial exploration (Pearce et al., 2012). The histopathology of the radiogenic tumors in the Ink4/$b^{-/-}$; $Arf^{f/f}$ mice closely resemble that of high grade human gliomas, and the mouse tumors are driven by oncogenic activations seen in human GBM. This clinical relevance together with the lack of background lesions and short latency makes such mouse models highly suitable for comparative evaluation of HZE particles with a range of LETs. Preliminary studies with the Ink4/$b^{-/-}$; $Arf^{f/f}$ mouse comparing the tumorigenic potential of charged particles of increasing Z with that of X-rays suggest a strong radiation quality effect on glioma frequency but with no effect on latency or clinical progression (Burma, personal communication). Another advantage of using transgenic mouse models is that complementary mouse models with different genotypes can be used to corroborate results obtained for a particular cancer, thereby bolstering confidence in the risk estimates being made.

In yet other genetically engineered mouse models, the timing of activating an oncogene and/or mutating a tumor suppressor gene can be controlled by delivering a virus expressing a recombinase, such as Cre (Kirsch et al., 2007), or by delivering a ligand such as tamoxifen to activate Cre-ER (Blum et al., 2013). Such models could be used to study the non-targeted effects of HZE particles, i.e., the effects on the tissue microenvironment rather than on the incipient cancer cell. Thus far, non-targeted effects of HZE particles have been studied in a mammary chimera model in which p53-null epithelia are transplanted into an irradiated host (Illa-Bochaca et al., 2014b). In the chimera model, the irradiated host stroma has been shown to accelerate tumor development, indicating that HZE particles can also indirectly promote carcinogenesis by altering the tumor microenvironment. Such non-targeted effects could be more directly examined in genetically engineered mouse models by exposing the mice to HZE particles before the gene mutation is initiated.

Genetically engineered mouse models can also be used to investigate the impact of "cell-of-origin" on HZE particle carcinogenesis. For this purpose, the use of cell type specific promoters allows for the introduction of mutations only in a desired cell population within the target organ rendering them susceptible for transformation by radiation. For example, one way to address the question of "cell-of-origin" of radiation induced gliomas is to use brain cell-type specific promoters driving Cre-ER^{T2}/Rosa26-YFP expression. Upon induction, e.g. by administration of tamoxifen, depending on the promoter of choice (for example, Nestin to target neural stem cells and Cystatin C to target astrocytes), tumor suppressor genes of interest are deleted only in the targeted cell population (Balordi and Fishell, 2007; Niu et al., 2013). The inclusion of the Rosa26-YFP reporter allows for the permanent labeling of the cells that have undergone Cre-driven recombination such that tumors arising from the targeted population would be YFP-positive. Glioblastomas are postulated to arise both from stem cells as well as from mature astrocytes (Dunn et al., 2012; Chen et al., 2012), so it will be important to elucidate which cell type is most susceptible to malignant transformation by HZE particles. For instance, tumors arising from a stem cell population may turn out to be similar to cancer stem cells and thus more resistant to therapy (Adorno-Cruz et al., 2015). In sum, addressing radiation-induced carcinogenesis at the cellular level and identifying the susceptible populations within a specific organ may be crucial for the ultimate development of preventive or therapeutic strategies.

Recent experiments with engineered models have focused on identifying the type and means by which normal cells mediate the development of cancer (Kuperwasser et al., 2004; Bhowmick et al., 2001; Maffini et al., 2004; de Visser et al., 2006). In a *Ptch-1* heterozygous mouse model, studies by Saran and colleagues found that partial body irradiation at a young age promotes *Ptch* mutant meduloblastoma (Mancuso et al., 2008). A radiation-chimera mammary model was developed to evaluate NTE by irradiating only the mice before orthotopically transplanting *Trp53* null mammary epithelium (Illa-Bochaca et al., 2014a; Nguyen et al., 2011). The absence of p53 from the epithelium primes the cells to undergo high efficiency transformation and gives rise to diverse types of carci-

nomas over the course of 12–18 months. Unexpectedly not only the timing and frequency but the types of cancers are affected by irradiating the host; moreover there is a distinct radiation quality effect increasing that result in more aggressive tumors. This model demonstrates that the response of normal cells to radiation acts to promote carcinogenesis. A recent study using a low (10 cGy) dose of γ-radiation to irradiate genetically diverse host mice identified two genetic loci that associate with cancer latency in controls and an additional 13 loci that affect latency in irradiated mice (Zhang et al., 2015). Further mapping will provide both important insight into mechanisms by which radiation perturbs systemic controls and enable comparison to genetics of human susceptibility.

3.3. Limitations

The NASA Radiation Program Element supports translational research to evaluate risk in healthy astronauts. As discussed above, engineered mice provide good models for determining the gene functions for tumor suppressors or oncogenes but must be used with caution for evaluating the risk of radiation-induced cancer unless the results can be shown to be relevant to risk in human populations. The engineered gene mutations that increase spontaneous cancer, thereby increasing the 'signal', can also change the biological response to radiation, which may result in a different stress response as compared to wild type mice.

The use of mutant mouse models is usually based on an underlying hypothesis in which the genetic manipulation is meant to mimic that observed in human predisposition (e.g. APC) or frequently found in human tumors (e.g. K-Ras) or has a fundamental role in cancer suppression (e.g. P53). Using such models for estimating radiation risks in humans is not one-to-one, particularly for evaluating the risk of HZE particle radiation for which there are no human data on cancer incidence. In some instances, mutant mouse models may provide unexpected results, indicative of more complex mechanisms or unknown biology. For example, miR-21 (an oncogene) is induced by radiation, so a deletion model was used to test its role in radiation carcinogenesis. Consistent with an oncogenic role, miR-21 knock-in mice have a high incidence ($\sim$40%) of spontaneous lung adenocarcinoma, which radiation exposure decreased (Chen et al., 2015). Surprisingly, irradiation decreased miR-21 expression in these mice, in contrast to the effect in wildtype mice. Another example is that of mice in which a lung tumor suppressor, Gprc5a, is deleted. The spontaneous incidence (10%) of lung adenocarcinoma is increased by radiation to $\sim$35%, however there was no difference in the lung tumorigenesis incidence between high and low-LET irradiated Gprc5a null mice. In contrast, wild type mice show $>$6 fold greater lung tumorigenesis at 1 Gy after exposure to HZE particles (iron, silicon and oxygen) compared to X-ray (Wang et al., 2015). These results suggest that though transgenic mouse models can be used to explain the functions of these modified genes in spontaneous tumorigenesis, radiation effects may be more complex. Such considerations may support studies in wild type mouse models, but a very low cancer incidence or rate requires significantly more time and mice. This limits the numbers of ions and doses that can be assayed.

The endpoint, a malignant cancer, is usually given greater weight than a surrogate endpoint such as a change in gene expression. Nevertheless, the tumor is a rodent tumor, not a human tumor, and for most tumor types the extent of the similarities between the rodent tumor and the human tumor it is meant to mimic is not fully established. Moreover, risk of mortality from cancer is a critical barrier to space flight; thus it is important to distinguish in experimental models between the broad class of tumors, which may include benign tumors such as adenomas, versus carcinomas, and those cancers with characteristics that associate with poor prognosis (e.g. invasion, metastasis). Better characterizations of radiogenic tumors in humans and mice (and potentially other species) are needed to ensure extrapolation of results from rodents to human is appropriate for key tumor types. Humanized mice (mice bearing human tissues) are another likely bridge between the species.

3.4. Challenges

Astronauts on deep space explorations will be exposed to radiation over many months, with a Mars mission envisioned that could extend to three years. The consequences of protracted low fluence exposure during travel in deep space on carcinogenesis are not well understood. Most cellular traversals involving protons would not be more frequent than daily occurrences (Cucinotta and Durante, 2006; Cucinotta et al., 2013). Thus, the reality of the space radiation environment suggests the need to focus studies at lower total doses and protracted exposure to provide more meaningful information relevant to risk estimates. It is possible that the relatively high fluences employed for animal experiments may not accurately capture the risk from more protracted radiation exposure in space, but both the technical limitations for protracting HZE exposure and the relatively short life spans of rodents currently complicate risk extrapolation to humans. Once a means to deliver low fluence HZE particles over a long period is feasible, research may need to be undertaken in a second, longer lived, non-rodent species to address issues of scaling to lifespan in highly protracted exposures.

4. Current challenges to reconciling the biology with risk modeling

Cancer is an important long-term risk for astronauts exposed to protons and HZE particles during deep space travel and residence on other planets and the moon. There are no human data on the risk from the extended exposure to complex radiation fields that will occur during space travel. Although the prevailing radiation health paradigm focuses on radiation-induced DNA damage leading to mutations, numerous studies over the last 50 years have provided evidence that radiation carcinogenesis is more complex than generally appreciated (reviewed Barcellos-Hoff, 2005).

As discussed above, models of cancer risk and mitigation are focused on 'targets', i.e. the cell that will undergo neoplastic transformation or the genetic alterations that initiate and promote cancers via four interdependent stages. The first stage, *initiation*, is typically irreversible and heritable and alters the cell genome resulting in an enhanced growth potential. This potential is only realized, however, if the cell later undergoes *promotion*, the second stage of carcinogenesis. *Promotion* is often thought to be the rate-limiting step in carcinogenesis since it has been shown that initiation alone is not sufficient to induce cancer (Berenblum and Shubik, 1949). Integration of mutations in a specific tumor suppressor gene was originally introduced by Knudson (Knudson, 1971). In order to account for the observed power of age dependence in carcinomas, a multi-stage theory of carcinogenesis was introduced (Armitage and Doll, 1954; Armitage and Doll, 1957). However this model suggested 5 to 7 rate-limiting stages, in contradiction with biological data. Some approaches addressed this contradiction by introducing the two-stage clonal expansion model where a cell leads to a tumor by two separate mutations and clonal expansion (Moolgavkar et al., 1988; Moolgavkar and Knudson, 1981; Moolgavkar and Luebeck, 1990).

Carcinogenesis initiated by aberrant cells may continuously evolve fitness-improving properties under the selective constraints imposed by the host. As a consequence of the tendency of the host

to restore homeostasis, multiple clonal populations compete to resist the suppression, advancing their fitness against this challenge to varying degrees. Malignancy usually fails at this point through an extinction event (e.g. barrier phase). If a population succeeds in traversing this barrier, clonal competition continues, with cell numbers now advancing to a level where extinction is only a remote possibility. These populations continue to compete to find host weaknesses that positively intersect with their features (e.g. selection model). At this point, suppressive influences from the host paradoxically serve to refine overall population fitness as the less fit clones are pruned away. In so doing, tissue-level effects operate to predispose the relative successes of clones, eventually driving reciprocal interactions amongst the clones and host that sub serve tumor expansion (e.g. coercion model). In these ways, the cancer population demonstrates the property of "emergence" – the expression of population-level capacities that exceed those attributable to individual cell changes. Consequently, the importance of individual cell contributions is soon overshadowed by the tumor/host interaction network as cancer progresses.

Cancers produce unique biology that is co-determined by context and components. Emergence is thus more than the quest to identify the parts, but is rather an effort to understand how interactions result in novel behaviors. By modeling the irradiated tissue/organ/organism as a system rather than a collection of non-interacting or minimally interacting cells, cancer can result as an emergent phenomenon of a perturbed system (Barcellos-Hoff, 2007). A biological model in which radiation risk is the sum of dynamic and interacting processes could provide the impetus to reassess assumptions about radiation health effects in a healthy astronaut population and spur new approaches to countermeasures.

Systems radiation biology seeks to integrate information across time and scale that are determined by experimentation. For example, the application of systems biology uncovered an expected central hub for inflammation in skin cancer. While a positive association exists between chronic inflammation and cancer, the innate immune system is itself a network that can be disrupted by both positive and negative stimuli. Anti-inflammatory drugs can have contradictory effects on skin tumor development (Viaje et al., 1977; Fischer et al., 1980) and over-expression of pro-inflammatory cytokines such as IL-1 can prevent skin tumor formation in mouse models of chemically induced skin cancer (Murphy et al., 2003). In contrast, germline deletion of TNF-α, another potent pro-inflammatory cytokine, also confers resistance to skin tumor formation (Moore et al., 1999). The role of inflammation in cancer is therefore very complex, with different consequences associated with acute or chronic inflammatory conditions.

4.1. Modeling in context

The concept that inflammatory responses are necessary components of cancer development has recently been formalized by Mantovani et al. (2008) in a two-pathway model: the intrinsic versus extrinsic. In the intrinsic pathway, genetic mutations lead to release by the transformed cells of pro-inflammatory factors recruiting innate immune cells. For example, oncogenic *Ras* activates the transcription of the inflammatory cytokine interleukin-8 (IL-8). Other oncogenes such as *Bcl2* inhibit apoptosis leading to necrotic tumor cell death and release of damage associated molecular pattern molecules that activate innate immune cells via toll-like receptors (Mantovani et al., 2008; Sparmann and Bar-Sagi, 2004). In both circumstances, the resulting host response is a chronic inflammation that promotes tumor growth and invasion (Mantovani et al., 2008; Zeh et al., 2005). In the extrinsic pathway, the chronic inflammation results from inability of the immune system to resolve an infection (e.g., hepatitis B) or from a dysregulated immune response as in autoimmune diseases (e.g., inflammatory bowel disease). The persistent inflammation cooperates with pre-existing oncogenic mutations by providing the microenvironment that promotes cancer progression, but it may also induce DNA damage resulting in the acquisition of new mutations (Guerra et al., 2007; Farber et al., 1990).

Wright and colleagues demonstrate that radiation-induced genomic instability in hematopoietic stem cells can results from specific cell interactions (reviewed in Lorimore et al., 2003). A recent study shows that macrophages from irradiated mice can induce chromosomal instability in non-irradiated hematopoietic cells and that production of TNFα and reactive oxygen and nitrogen species by the macrophages are responsible for this effect (Lorimore et al., 2008). Furthermore, Coates et al. showed that the mouse genotype affects macrophage phenotype, designated as M1 or M2 and that radiation exposure further amplifies the differential effect of genotype (Coates et al., 2008). Together, these data support the hypothesis that certain radiogenic cancer risk may be augmented by alterations in a network of cellular interactions, at the center of which is the innate immune system. The aging process has been shown to be associated with increased levels of chronic inflammation, which are thought to contribute to many age-associated diseases, including cancer, and increased serum levels of IL-6 have been reported in older individuals (Sarkar and Fisher, 2006). Interestingly, the LSS of A-bomb survivors has also been associated with significant increases in serum IL-6 levels that are still detectable after many years (Hayashi et al., 2003). Studies in experimental models suggest that diet is a significant player in radiation carcinogenesis. Burns and colleagues have shown that chronic dietary exposure to vitamin A acetate can prevent 90% of the malignant and benign tumors that occur in rat skin exposed to electron radiation and 50% of ^{56}Fe ion induced tumors (Burns et al., 2007). Gene expression analysis suggested that ^{56}Fe ion radiation significantly induced inflammation-related genes, many represented in the categories of 'immune response', 'response to stress', 'signal transduction' and 'response to biotic stress', which vitamin A reduced or blocked (Zhang et al., 2006). These data are consistent with the hypothesis that HZE NTE induce inflammatory processes that contribute to carcinogenesis.

4.2. Limitations and challenges

Cancer is really a multi-scale problem, in which corruption of normal cell and tissue function is a gradual but unstable process of self-amplification via recruitment of reinforcing tissue and systemic cell interactions across a long duration (Hlatky and Hahnfeldt, 2014). The contribution of each process may vary in magnitude, timing and composition, making each tumor type and, indeed, each tumor unique. Modeling carcinogenesis is a significant challenge while modeling space radiation carcinogenesis must take into account the complexity of radiation effects across these scales. The NASA Space Radiation Program Element has successfully generated a critical mass of physical and radiobiological research over the last 20 years. Delineation of operational signaling pathways and definition of cellular and tissue level effects are supported by various high throughput data in response to simulated space radiation exposure. Events in radiobiology can be integrated with epidemiologic evidence from irradiated humans and detailed genomic and molecular knowledge of human cancers using systems biology to provide mechanistic models of how cancer is increased following exposure. Combining a multi-scale systems biology model of epithelial carcinogenesis with risk modeling to generate predictions is now feasible.

Chronic exposure to HZE ions at a space relevant dose rate over the course of months is not technically feasible. A very large and meticulously conducted study with low LET gamma rays found no increase in cancer incidence in mice irradiated at

0.05 mGy/d (20 mGy total dose) or 1.1 mGy/d (400 mGy total dose), but increased incidences of several tumor types affecting both sexes at 21 mGy/d (8000 mGy total dose) (Tanaka et al., 2007). Mice have been exposed to chronic neutron irradiation, which has high LET effects, at dose rates about 10 fold higher than those in space. The results varied by tumor type ranging from tumor sparing to an increased tumor incidence as compared to acute exposures (Ullrich et al., 1977; Ullrich et al., 1976; Ullrich, 1984). While chronic exposures to HZE ions over weeks or months isn't possible, attempts have been made to simulate these exposures by delivering fractionated doses of HZE ions to rats and mice. As with neutron exposures, the results are not consistent across tumor types (Burns et al., 2007; Burns et al., 1989; Alpen et al., 1994; Dicello et al., 2004).

Ultimately risk estimates are limited by theoretical understanding of both radiation quality effects (Kim et al., 2015), and the carcinogenic process (Shay et al., 2006; Barcellos-Hoff, 2007). Since epidemiology in humans is not in the foreseeable future, estimates of risk from human travel in space need to be based on a mechanistic understanding of complex effects elicited radiation exposure across time and level of organization. Defining the radiobiology of space radiation is critical to understanding these long-term risks that include the malignant and degenerative diseases associated with age itself. Thus, NASA programmatic research goals to assess the consequences of a complex space radiation environment using 'omics and systems biology should identify clinically-relevant cellular and molecular networks underlying carcinogenesis. It is hoped that this information will inform cancer risk regulatory models and provide the means to mitigate health consequences of space flight in the future.

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