Review Article

CHECKING THE FOUNDATION: RECENT RADIOBIOLOGY AND THE LINEAR NO-THRESHOLD THEORY

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Abstract—**The linear no-threshold (LNT) theory has been adopted as the foundation of radiation protection standards and risk estimation for several decades. The "microdosimetric argument" has been offered in support of the LNT theory. This argument postulates that energy is deposited in critical cellular targets by radiation in a linear fashion across all doses down to zero, and that this in turn implies a linear relationship between dose and biological effect across all doses. This paper examines whether the microdosimetric argument holds at the lowest levels of biological organization following low dose, low dose-rate exposures to ionizing radiation. The assumptions of the microdosimetric argument are evaluated in light of recent radiobiological studies on radiation damage in biological molecules and cellular and tissue level responses to radiation damage. There is strong evidence that radiation initially deposits energy in biological molecules (e.g., DNA) in a linear fashion, and that this energy deposition results in various forms of prompt DNA damage that may be produced in a pattern that is distinct from endogenous (e.g., oxidative) damage. However, a large and rapidly growing body of radiobiological evidence indicates that cell and tissue level responses to this damage, particularly at low doses and/or dose-rates, are nonlinear and may exhibit thresholds. To the extent that responses observed at lower levels of biological organization in vitro are predictive of carcinogenesis observed in vivo, this evidence directly contradicts the assumptions upon which the microdosimetric argument is based.**
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INTRODUCTION

THE CLASSICAL paradigm upon which radiation protection and conventional radiocarcinogenic risk estimation is based is the linear-no threshold (LNT) theory (Mossman 2006). The validity of the LNT theory is a subject of active debate and research in the radiation sciences. For example, the U.S. National Research Council recently concluded, "…current scientific evidence is consistent with the hypothesis that there is a linear, no-threshold dose-response relationship between exposure to ionizing radiation and the development of cancer in humans" (NRCNA 2005). Conversely, the French National Academies of Science and Medicine recently concluded, "…it is not justified to use the linear no-threshold relationship to assess the carcinogenic risk of low doses observations made for doses from 0.2 to 5 Sv since for the same dose increment the biological effectiveness varies as a function of total dose and dose rate" (Aurengo et al. 2005).

The LNT theory is predicated on the assumption that the initial event that leads to biological effects of ionizing radiation is the deposition of energy in the target cell. Radiation-associated energy deposition damages DNA molecules either directly or indirectly through radicals produced by hydrolysis of water molecules, which subsequently attack the DNA molecule (Hall and Giaccia 2006). It has been argued that since energy is deposited linearly with dose, the subsequent DNA damage and resulting biological effects are also characterized by a LNT dose-response. However, it is also explicitly recognized that this biophysical, or microdosimetric, argument is predicated on the direct proportionality between energy deposition in the target cell and biological effect (Brenner and Sachs 2006; NCRP 2001). In its examination of the LNT theory, the National Council on Radiation Protection and Measurements (NCRP) stated of the microdosimetric argument: "Application of this argument to complex endpoints such as radiation-induced carcinogenesis is, however, more uncertain. Based on these biophysical considerations about the shape of the dose-response relation for low-dose radiation-induced carcinogenesis, conclusions can be drawn if: (1) radiogenic cancer induction is causally related to radiation-induced damage in a single cell and (2) the ways in which other cells or cell systems subsequently modify the probability that any given initially radiation-damaged cell becomes the clonal origin of a cancer do not vary with dose in a nonlinear fashion."

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The purpose of this paper is to examine these two assumptions in light of recent radiobiological evidence and evaluate the relevance of the microdosimetric argument for estimating radiocarcinogenic risk. It is important to note that the LNT theory dictates that the response to ionizing radiation depends only on the dose, which means that responses differ only quantitatively, not qualitatively, with dose. In other words, the LNT theory predicts that only the risk or likelihood of the response (for stochastic effects) differs with dose. The LNT theory predicts that biological responses should be proportional to dose, and this implies that there should be no qualitative differences in response, for example, between high and low doses (in this paper, loosely defined as above or below 0.1 Gy respectively, although other papers, including some reviewed here, use different definitions), or between high and low dose-rates (in this paper, loosely defined as above or below $\sim$ a few mGy min^{-1}). The fact that this is not the case has already been widely recognized by the adoption of a dose and dose-rate effectiveness factor (DDREF) to improve the fit of linear risk models to observed data. If high and low doses produce qualitatively distinct responses in endpoints with relevance to carcinogenesis, it becomes increasingly implausible for the dose-response for radiation-induced cancer to remain linear across the entire range of doses from background to acute lethality. Preservation of linearity would require that qualitatively different processes combine to yield the same linear responses.

Biological systems are organized hierarchically (Feinendegen et al. 2007; Trosko 1998). Ionizing radiation acts at the atomic level, and effects may (or may not be) propagated up through the hierarchy to molecules (e.g., DNA), cells, tissues, and organisms. Molecular and cellular responses to radiation damage are the first steps in a complex, holistic response to radiation injury. At each level of organization, there are mechanisms that respond to disturbances in homeostasis (Fig. 1). This review focuses on radiobiological studies at the lower levels of the biological hierarchy—atoms, molecules, cells, and tissues. The initial deposition of energy in molecules of the cell is considered, followed by an examination of the biological responses to this initiating event at progressively higher levels of organization.

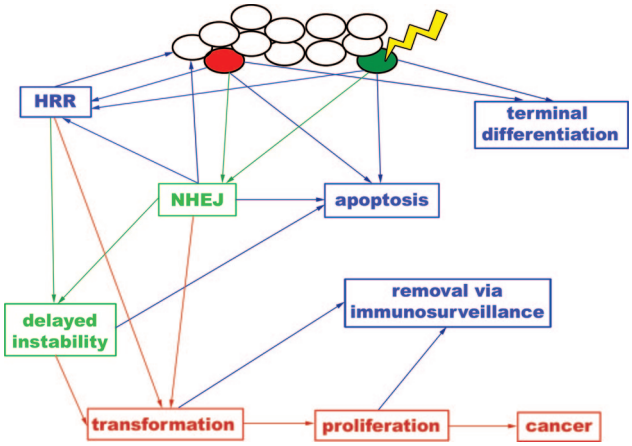

Fig. 1. The web of biological responses to radiation damage occurring across several levels of biological organization. The initiating event is the damage of cellular targets (e.g., DNA) by ionizing radiation, depicted in this figure as a lightning bolt impacting a cell (green circle). It is important to note that the population of cells includes normal cells (unfilled circles) as well as cells carrying potentially carcinogenic damage from endogenous and other exogenous processes and agents (orange filled circle). In this figure, processes that are most likely associated with low or decreasing risk are shown in blue. Processes that are most likely associated with higher or increasing risk are shown in red. Processes with uncertain risk consequences are shown in green. HRR = homologous recombinational repair, NHEJ = non-homologous end joining.

DAMAGE DEPOSITION

Exposure to ionizing radiation causes a variety of types of damage to DNA, either directly or indirectly through the production of radical species. It is widely accepted that the deposition of energy in biological molecules (e.g., DNA) and the resulting damage are the events that initiate a biological response. This damage includes DNA single- and double-strand breaks, base damage, and DNA-protein cross-links, among others. It is generally accepted that DNA double-strand breaks are the most serious type of damage as they can lead to chromosome aberrations. Some researchers have suggested that chromosome aberrations are correlated with cancer risk (Hagmar et al. 2004; Norppa et al. 2006), although a causative relationship has not been directly demonstrated. Opinions are mixed on the question of whether or not chromosome aberrations resulting from misrepair of radiation-induced double-strand breaks immediately following radiation exposure directly lead to eventual carcinogenesis.

These types of DNA damage are not unique to ionizing radiation. Rather, ionizing radiation adds to the background level of damage from endogenous oxidative processes and other exogenous sources. Many studies have been conducted to compare the level of radiation-induced DNA damage to background DNA damage (Pollycove and Feinendegen 2003). These studies frequently argue that the level of damage produced by low to moderate doses of ionizing radiation is trivial in comparison to that produced by endogenous oxidative sources. A counterargument has been raised based on a qualitative rather than quantitative analysis of DNA damage. Specifically, it has been asserted that ionizing

radiation frequently results in damage clusters or localized multiply damaged sites (LMDS) (Ward 1994), and induction of LMDS by ionizing radiation has been observed (Georgakilas et al. 2004; Hada and Sutherland 2006; Sutherland et al. 2002). On this basis, it has been argued that LMDS represent a form of damage that (1) is distinct from endogenous oxidative damage, (2) is refractory to repair, and (3) may present greater risk than endogenous oxidative damage. The first part of this argument is examined in the next section. The second and third parts of the argument are examined in later sections.

Is radiation-induced damage different from endogenous damage?

Overall, the first part of this argument appears sound. Ionizing radiation does indeed appear to produce *distributions* of damage that are distinct from endogenous oxidative damage, if not unique *types* of damage. However, cells are also exposed to natural background radiation, and there is no reason to believe that radiation from anthropogenic sources (occupational, medical, etc.) produce initial damage distributions that are qualitatively different than those produced by natural radiation. It is logical to presume that whatever mechanisms cells use to respond to damage from natural background radiation would also be brought to bear on damage from similar anthropogenic exposures. However, it must be noted that while exposures from anthropogenic radiation sources may be qualitatively similar to natural background, the doses and dose-rates from some sources (e.g., medical) are much higher than natural sources. As discussed elsewhere in this paper, both the dose and the dose-rate can qualitatively affect the biological response to radiation exposure.

GENE EXPRESSION

While ionizing radiation damages DNA at the atomic and molecular levels, the initial biological response is mediated at the level of genes. Cells respond to radiation damage by modulating the expression of particular genes, which in turn produce proteins with specific functions (e.g., DNA repair, apoptosis initiation or suppression, etc.). It is at this lowest biological level of organization that the biological response to ionizing radiation exposure has been demonstrated to depart from linearity.

Does gene expression qualitatively depend on dose?

Several studies of gene expression profiles detected using microarrays have observed qualitative differences in gene expression at low doses and/or dose-rates compared to high doses and/or dose-rates. A study of a human myeloid leukemia cell line using cDNA microarrays to examine the expression of 6,727 genes (Amundson et al. 2003) found that most of the genes which showed a dose-response were upregulated. However a dose-rate effect was observed in the expression profiles of a group of genes involved in apoptosis (0.5 Gy delivered at dose rates of 0.28, 2.4, and 29 cGy min^{-1} led to less apoptosis per unit dose than did 0.5 Gy delivered at 290 cGy min^{-1}), while expression of genes involved in cell-cycle regulation/proliferation tended to be independent of dose-rate. The authors concluded that this pattern represents a qualitative difference in response between high and low dose-rates, and could not have been predicted from extrapolation from high dose, high dose-rate results.

In a second study, the gene expression profile in G_1 normal human fibroblasts following a 2 cGy dose delivered at 20 cGy min^{-1} was also qualitatively distinct from that observed following a 4 Gy dose delivered at 2 Gy min^{-1} (Ding et al. 2005). This study examined the expression of 7,458 genes. An analysis of the known functions of the genes studied revealed that those coding for proteins involved in cell-cell signaling, signal transduction, and response to DNA damage were upregulated after the low dose, low dose-rate exposure, while those coding for proteins involved in apoptosis and cell proliferation were upregulated after high dose, high dose-rate exposure. The increase in cell-cell signaling and signal transduction gene expression has also been observed in another study of G_1 normal human fibroblasts following a 1 cGy dose delivered at 6 cGy min^{-1} (Fujimori et al. 2005). However, doses between 2–5 cGy (0.1 or 0.44 Gy min^{-1}), but not between 10 cGy and 1 Gy, have been observed to induce proliferation in exponentially growing normal human fibroblasts through phosphorylation of proteins that induce growth-related genes (Suzuki et al. 2001). These apparently conflicting results may be the result of using cell cultures in G_1, which failed to show induction of genes associated with proliferation, vs. exponentially growing cells, which did show an increase in proliferation.

A similar study (Franco et al. 2005) examined the expression of ~8,000 genes in differentiated normal human keratinocytes exposed to 1 cGy (0.215 cGy min^{-1}) or 2 Gy (28 cGy min^{-1}). This study identified 269 genes modulated at both dose treatments, but there were also 214 genes uniquely modulated at the low dose/dose-rate treatment and 370 genes uniquely modulated at the high dose/dose-rate treatment. Again, this suggests a qualitative difference in gene expression between high and low dose/dose-rate exposures; however, a limitation of this study was the inability to link differences in gene expression with coherent functional significance.

A recent large study examined the expression profile of 23,040 genes, which represents a majority of the upper bound estimate of 25,000 genes in the human genome (IHGSC 2004), in normal human fibroblasts after acute (1 Gy min^{-1}) radiation doses ranging from 0.5 Gy to 50 Gy (Tachiiri et al. 2006). This study observed 238 genes upregulated after 0.5 Gy but not after 50 Gy, and 89 genes upregulated after 50 Gy but not after 0.5 Gy. The genes uniquely upregulated by the lower dose (0.5 Gy) included genes that encode proteins with a pro-survival effect (e.g., heat-shock and cell cycle proteins), while the genes uniquely upregulated by the high dose (50 Gy) included those that code for proteins with a pro-apoptotic effect (e.g., several targets of p53). More genes tended to be down-regulated with increasing dose. The advantage of this study is the comprehensive set of genes examined. However, some caution must be exercised in generalizing these results, as the dose-rate used was about eight orders of magnitude higher than those more typical of environmental or occupational exposures, and even the lowest dose examined was about two orders of magnitude higher than annual background.

Taken together, these studies of gene expression suggest that there are qualitative differences between gene expression responses to high doses and dose-rates compared to low doses and dose-rates. Some degree of caution is in order in translating these in vitro results to functional significance in vivo, and even more so for making inferences about radiation carcinogenesis. Differences in gene expression profiles in response to high vs. low doses and/or high vs. low dose-rates may or may not necessarily have relevance for carcinogenic risk. It is critically important that these differences be linked to subsequent biological endpoints with relevance to carcinogenesis. To the extent that these patterns are representative of relevant, in vivo biological responses, they call into question the fundamental assumption of qualitative similarity in biological response across all doses, as predicted by the LNT theory.

DNA REPAIR

Is radiation-induced damage difficult to repair?

The argument that radiation-induced LMDS are refractory to repair appears plausible, but the evidence for this is limited. There is some evidence that at least some LMDS are repaired slowly (Georgakilas et al. 2004) (but note that this study employed a very high dose-rate, 1–2 Gy min^{-1} and high doses on the order of a Gy or more), and it has also been demonstrated that LMDS can be converted to DNA double-strand breaks (DSBs) during repair (Yang et al. 2004). It is also intriguing that a fast and a slow pathway have been identified for at least one of the mechanisms available to the cell to repair DSBs, nonhomologous end joining (NHEJ). It is tempting to speculate that the slow pathway would be preferentially relied upon for particularly challenging types of damage, like LMDS; however, this has yet to be demonstrated.

Are radiation-induced DSBs repaired in G_0/G_1 via NHEJ?

It is frequently asserted that radiation induced DSBs occurring in mammalian G_0/G_1cells are repaired via NHEJ, and that homologous recombinational repair (HRR) is only involved to a significant extent in repairing DSBs that occur during G_2 or S phases of the cell cycle. Several studies have demonstrated high levels of NHEJ relative to HRR in G_0/G_1; however, these observations are almost always made after exposure to high doses of ionizing radiation (on the order of one or even tens of Gy) (Essers et al. 2000; Hinz et al. 2005; Jeggo and Lobrich 2006; Rothkamm et al. 2003; Wang et al. 2001a and b). These irradiation protocols involve doses that are orders of magnitude higher than those normally experienced in occupational or environmental settings. To conclude that NHEJ is the primary DSB repair pathway operating after typical occupational or environmental exposures, it must be assumed that patterns observed at acute doses of one or more Gy can be extrapolated down to chronic exposures on the order of mGy or less.

There is ample reason to be suspicious of this assumption. First, it has been conclusively demonstrated that mammalian cells are capable of repairing DSBs through HRR, using either a sister chromatid (preferred), when available (in G_2/S), or homologous sequences near the break site or on heterologous chromosomes as the repair substrate (Johnson and Jasin 2001; Liang et al. 1998; Richardson et al. 1998, 1999). A comparison of cell survival in NHEJ proficient and deficient human glioma cell lines revealed higher survival in the NHEJ proficient cell line at high doses (>1 Gy), but no difference at lower doses (<0.2 Gy), implying a qualitative difference in DNA repair pathway at low vs. high doses (Wykes et al. 2006), at least in this test system. Using fluorescently labeled markers of RAD51 and BRCA2 (both involved in homologous recombinational repair) and for DNA-PK (involved in NHEJ), a second study of human glioblastoma cells also observed a difference in repair pathway between high and low doses (Short et al. 2005). There appeared to be limited involvement of NHEJ following doses <0.5 Gy; however, the activity of HRR increased significantly in cells exposed to 0.1 Gy compared to controls. This activity increased more slowly between 0.1–1.0 Gy, and was saturated at

higher doses. HRR appeared to be indicated in all phases of the cell cycle, though most markedly in G_2.

Furthermore, in response to ionizing radiation, interphase cells reorganize chromosome domains to bring homologous chromosomes into close proximity (Abdel-Halim et al. 2004; Dolling et al. 1997). This movement involves bringing homologous chromosomes and specific genes closer to the center of the nucleus where transcription is known to be more active (Skalnikova et al. 2000). Pairing of homologous chromosomes is also observed after exposure to other types of DNA damaging agents, such as mitomycin C (Abdel-Halim et al. 2005), ultraviolet radiation, and hyperthermia, and appears to represent a general stress response (Abdel-Halim et al. 2006). This phenomenon does not appear to be the result of random interactions of chromosomes, but appears to reflect an active cellular process, possibly homologous DNA repair (Abdel-Halim et al. 2005, 2006; Dolling et al. 1997; Plan et al. 2005). The pairing of homologous chromosome domains in response to exposure to DNA damage or to stress in interphase is at least suggestive that HRR is operative in interphase, though other interpretations are possible. One important caveat is that the studies showing changes in chromosome locations used high dose, high dose-rate irradiation protocols (Abdel-Halim et al. 2004; Dolling et al. 1997; Skalnikova et al. 2000). It is not clear whether these results can be extrapolated to low dose, low dose-rate situations. This evidence must be weighed against the conclusions from other high dose studies of bulk DNA repair that HRR is only important in G_2 and S phase, and it suggests that interpreting the evidence on this topic should be approached cautiously.

There is yet another reason to be cautious about drawing conclusions from assays of gross DNA repair—lack of resolution. The total estimated size of the human genome is about 3,200 Mb (IHGSC 2001), of which only approximately 34 Mb consists of coding exons (IHGSC 2004). While the cellular decision between repair and apoptosis appears to be influenced by chromatin structure (Berardi et al. 2004), it is not known whether the repair pathway that operates on particular radiation-induced DNA lesions is influenced by the transcriptional status of the affected DNA. There is solid evidence that the repair kinetics of some types of DNA damage through nucleotide excision repair (NER) is influenced by transcription status (Mellon 2005), and data from gene expression profiling has demonstrated that at least some genes involved in NER may be upregulated following irradiation at high doses (5 Gy) (Tusher et al. 2001) in human lymphoblastoid cells. It has also been demonstrated that NER is important in the adaptive response (Hafer et al. 2007). It is plausible that chromatin structure at the break site may be a factor in determining which repair mechanism is employed to deal with radiation-induced DNA damage (Hassa and Hottiger 2005; Moore and Krebs 2004), but this has yet to be conclusively demonstrated. If the small subset of DSBs occurring in coding regions is preferentially repaired by either HRR or precise NHEJ (discussed in the next section), then assumptions about the repair fidelity of radiation-induced lesions based on gross repair assays that cannot differentiate between coding and noncoding regions may be misleading. At this point, this is an untested possibility, but it seems prudent to refrain from drawing conclusions about the risk associated with misrepair of radiation-induced lesions until such questions have been addressed.

Caution is also warranted in extrapolating the results of studies utilizing other DNA-damaging agents, even those studies using agents that cause DSBs. There is evidence that the repair of DSBs via NHEJ is impeded by increasing complexity of the damage (Pastwa et al. 2003). For example, blunt-ended, simple DSBs induced by endonucleases appear to be readily repairable by NHEJ, while more complex DSBs induced by the radiomimetic drug bleomycin or by low-LET radiation are more challenging to repair by NHEJ. Highly complex DSBs induced by high-LET radiation or by Auger electrons may be almost completely refractory to repair by NHEJ.

Is NHEJ error-prone?

It has been conclusively demonstrated that NHEJ can result in the deletion of a few base pairs upon repair of DSBs (Phillips and Morgan 1994), and therefore NHEJ has traditionally been considered an error-prone pathway (Hoeijmakers 2001). However, there is growing evidence that this is an oversimplified view of NHEJ. In particular, while fast and slow NHEJ pathways have been observed, recent studies indicate that there is also at least one high-fidelity NHEJ subpathway (Bau et al. 2004, 2006; Wang et al. 2006), possibly mediated by microhomologous sequences near the break site (Zhong et al. 2002). Furthermore there is evidence that the fidelity of NHEJ depends on the dose-rate (Rothkamm et al. 2001). Repair via NHEJ after low dose-rates (3.96 mGy min^{-1}) resulted in no detectable genomic rearrangements, while chromosomal rearrangements were observed after high dose rate exposures (23 Gy min^{-1}). However, it should be noted that this study involved very high total doses (80 Gy), and the types of detectable misrepair were limited to rearrangements involving fragments >100 kbp.

APOPTOSIS

Does error-prone DNA repair result in an increase in risk?

One of the lines of reasoning offered in support of the LNT theory is that if (1) most cells exist in G_0/G_1 in vivo, and if (2) radiation-induced DNA damage occurring in G_0/G_1 is repaired via NHEJ, and (3) NHEJ is error-prone, then (4) even the smallest dose can result in misrepair, and (5) there is therefore some risk associated with even the smallest dose. This argument relies on the implicit assumption that an increase in risk *necessarily follows* from the possibility, no matter how remote, of error-prone repair of radiation-induced DNA damage. Even if doubts about the first three premises of this argument are set aside, it is not inevitable that an increase in risk results from error-prone repair.

While it may be the case that some radiation-induced DNA damage, perhaps damage clusters, present a greater challenge to the cell's DNA repair systems, this does not necessarily equate to an increase in carcinogenic risk. Repair is but one option for the cell in dealing with DNA damage. Some tissues where a large fraction of the cells are terminally differentiated appear to be relatively radioresistant and maintain normal function even after high radiation doses. In these noncycling cells, it is not clear that there is any increase in risk even if the DNA damage goes unrepaired.

In the case of DNA damage that is difficult to repair, apoptosis may be the primary cellular response, with the choice being dictated by excessive amounts of single-stranded DNA generated by repeated unsuccessful attempts at DSB repair (Bree et al. 2004).

Even before cells attempt to repair DSBs, it appears that apoptosis is initiated as a protective mechanism. Evidence in human glioblastoma and normal fibroblast cell lines suggests that at very low dose-rates, on the order of 2–9.4 cGy h^{-1}, the cell's damage detection pathway mediated by ATM is not activated, and this leads to apoptosis (Collis et al. 2004). Since dead cells do not present a carcinogenic risk, this would seem to represent a dose-rate threshold for radiocarcinogenesis. This failure to activate the cell's damage detection mechanism was not observed at a high dose rate (45 Gy h^{-1}), and this represents yet another qualitative difference in response to low and high dose rate exposures in the cell lines and conditions used in this study. The generality of this observation has yet to be determined.

If it is simply assumed (contrary evidence aside) that there is some frequency of misrepair even after low dose, low dose-rate radiation exposure, does this lead to an increase in cancer risk? The implicit assumption is that the types of abnormalities produced by such misrepair (e.g., chromosome aberrations, especially translocations) equate to increased cancer risk. There are varying opinions about this assumption. Some specific translocations are associated with specific cancers (Mitelman et al. 2007). There is also some suggestive evidence from studies of cancer-prone mice that chromosome aberrations appearing as early as the first post-irradiation mitosis are related to cancer (Bouffler et al. 1995, 1996, 1997); however, generalization of these results to normal mice, let alone to humans, should be approached with the appropriate degree of caution. A correlation between the frequency of chromosome aberrations in human peripheral blood lymphocytes and cancer risk has been observed in some large studies (Boffetta et al. 2006; Bonassi et al. 2008; Hagmar et al. 2004; Norppa et al. 2006), though the connection between these aberrations and exposure to carcinogens is tenuous. This is especially true since this assay depends on stimulation of normally nondividing lymphocytes to enter the cell cycle. This stimulation is accomplished by the use of mitogens such as phytohemagglutinin (PHA). Unfortunately, another effect of PHA is the suppression of apoptosis (Belloni et al. 2008b; Carloni et al. 2001), and apoptosis is one of the primary protective mechanisms cells use to deal with DNA damage. It has been definitively demonstrated that in the absence of mitogenic stimulation, cells bearing unstable aberrations are preferentially eliminated via apoptosis (Bassi et al. 2003; Belloni et al. 2008a), though it is unknown whether this also applies to cells bearing ostensibly stable reciprocal translocations. Accordingly, it has been definitively demonstrated that the frequency of unstable aberrations, and to a lesser but still significant extent, the frequency of translocations, are not constant but decline with time from the levels initially observed following radiation exposure (Matsumoto et al. 1998; Tucker et al. 1997, 2004, 2005), though the frequency of reciprocal translocations may plateau above pre-irradiation levels following the initial decline. The initial decline may be due at least in part to the co-occurrence of reciprocal translocations with unstable forms of chromosome damage (Tucker 2008). Recent studies of radiologic technologists have observed increased frequencies of chromosome aberrations in this cohort occupationally exposed to radiation (Bhatti et al. 2007, 2008; Sigurdson et al. 2008a). These studies suggest that chromosome aberrations, particularly reciprocal translocations, hold promise as a biomarker of both exposure and cancer risk, but numerous concerns about potential confounding due to factors such as age, cigarette smoking, and phenotypic differences remain (Sigurdson et al. 2008b; Tucker 2008).

It has also been argued that the levels of chromosome aberrations in irradiated cells at a particular point in time is

more properly thought of as a biomarker of dose rather than as a biomarker of risk due to the factors discussed above, and because factors such as species, strain, tissue of interest, and genetic background confound the relationship between aberration frequency and cancer risk (Brooks 1999, 2001; Brooks et al. 2003). It seems reasonably clear that specific aberrations are related to, and probably contribute to causing specific cancers, at least in some cases. It is also reasonably clear that moderate to high dose and dose-rate exposure to radiation increases the frequency of chromosome aberrations as well as cancer risk, but it is less clear that low dose, low dose-rate exposures have the same effect. Some mouse studies (Bouffler et al. 1995, 1996, 1997) suggest a direct link between radiation exposure and the development of the types of aberrations associated with murine cancers, but it is not clear that there is a linear relationship between dose, the frequency of these aberrations, and the eventual increase in cancer risk. Considering the size of mammalian genomes, it is not clear how radiation, which is presumed to distribute damage randomly across the genome, could directly produce the specific translocations associated with specific cancers with any regularity. It seems inevitable that while radiation damage may be distributed randomly, the probability of misrepair and concomitant production of potentially carcinogenic aberrations is not random. There is some evidence from studies of human papillary thyroid carcinoma that the specific translocation involved in this disease involves two genes that are in close proximity in interphase cells. It has been hypothesized that this makes it more likely that a single radiation track could induce the requisite DSBs that lead to the characteristic translocation, and eventually to papillary thyroid carcinoma (Nikiforova et al. 2000). The generality of this observation to other translocations and other types of cancer is uncertain.

It should be noted that the studies mentioned here are not necessarily in conflict, but rather this may be another issue of resolution. It is quite plausible that radiation causes a subset of potentially carcinogenic chromosome aberrations, as well as a spectrum of aberrations with little carcinogenic potential. Assessing the frequency of bulk chromosome aberrations across the genome generated soon after radiation exposure may have little value in predicting cancer risk.

Beyond the factors discussed above, it is still not necessarily true that some probability of producing a potentially carcinogenic aberration by low dose and/or dose-rate radiation exposure necessarily equates to an increase in cancer risk. Accepting for the sake of discussion that carcinogenic risk is directly proportional to the number of potentially carcinogenic chromosomal aberrations occurring in cycling cells, then the total number of potentially carcinogenic aberrations can be expressed by the following equation:

$$A_{t1} = A_S\,(1 - P) + (DSB_S + DSB_R)(1 - C - P), \quad (1)$$

where:

A_{t1} = the total number of potentially carcinogenic aberrations present in cycling cells at some time after irradiation, and after all cellular processing is completed;
A_s = the total number of potentially carcinogenic aberrations present before irradiation (i.e., the spontaneous background load of aberrations);
DSB_S = the number of background, or spontaneous unrepaired double-strand breaks present before irradiation;
DSB_R = the number of radiation-induced double-strand breaks;
C = the probability of correct repair of double-strand breaks; and
P = the probability of removal of aberrant cells through radiation-induced apoptosis.

In this example, it is assumed that DSBs are the lesions that lead to chromosome aberrations, which in turn increase cancer risk. However, the argument would also hold for other types of radiation-induced DNA damage. The number of potentially carcinogenic aberrations at some point in time after radiation exposure and completion of the subsequent repair/misrepair/apoptosis will be a balance between removal of cells containing potentially carcinogenic aberrations (both radiation-induced *and* spontaneous, or background, aberrations) through radiation-induced apoptosis and the creation of newly aberrant cells through misrepair of radiation-induced DNA damage.

Assuming risk is correlated with the number of potentially carcinogenic aberrations, there are three possible effects of radiation on carcinogenic risk. If:

$A_S < A_{t1}$, then radiation increases the risk;
$A_S = A_{t1,}$ then the risk is unchanged by radiation;
$A_S > A_{t1}$, then radiation decreases the risk.

Now consider, for example, the situation if radiation does not affect risk. By definition:

$A_S = A_{t1}$;
$A_S = A_S\,(1 - P) + (DSB_S + DSB_R)\,(1 - C - P)$;
$A_S - A_S + PA_S = (DSB_S + DSB_R)\,(1 - C - P)$; and
$PA_S = (DSB_S + DSB_R)\,(1 - C - P)$.

The term on the left is the frequency that a cell containing a background carcinogenic aberration is removed by apoptosis. The term on the right is the

probability that a DSB will be misrepaired and result in a potentially carcinogenic aberration. In order for the risk to remain unchanged, these two terms must exactly balance. If the probability of misrepair is greater than the probability that a background carcinogenic aberration is removed by radiation-induced apoptosis, and if misrepair results in a potentially carcinogenic aberration, then the risk increases. Conversely, if the probability of misrepair resulting in a potentially carcinogenic aberration is less than the probability that a background carcinogenic aberration is removed by radiation-induced apoptosis, then the risk decreases. Of course, a decrease in the level of carcinogenic aberrations below the spontaneous level is only possible if the spontaneous level is greater than zero (Calabrese and Baldwin 2001; Sykes and Day 2007), as has been demonstrated for transformation (Azzam et al. 1996; Redpath et al. 2003; Redpath 2004). The conclusion that the possibility of misrepair *necessarily* implies an increase in cancer risk requires that the probability of removal of a background carcinogenic aberration by radiation-induced apoptosis is zero. There is strong evidence suggesting that this is not the case, neither in transformed cells (Azzam et al. 1996; Ko et al. 2004; Redpath and Antoniono 1998; Redpath et al. 2001) nor in co-cultures of irradiated normal cells and unirradiated transformed cells, where low doses have been shown to selectively induce apoptosis in transformed cells (Portess et al. 2007). As discussed by the authors of this latter study, radiogenic selective induction of apoptosis in transformed cells by normal cells may represent a mechanism for the protective effect of low-dose radiation, though the generalization of this conclusion to all physiologically relevant conditions requires further study (Bauer 2007). Interestingly, this response was nonlinear, as it appeared to saturate at 25 mGy. If these results are relevant to ultimate carcinogenicity, then they directly contradict the second assumption of the microdosimetric argument—that the ways in which cells or cell systems modify the biological response to radiation exposure are linear across all doses.

The result is that it is not necessary for repair to be error-free (though it may very well be, especially at low dose-rates) for risk to be unchanged or to decrease. All that is required for the risk not to increase is that removal of spontaneously aberrant cells through apoptosis be sufficient to equal or outweigh the creation of new aberrant cells through misrepair.

TISSUE-LEVEL RESPONSES

Is carcinogenesis directly proportional to energy deposited in target cells?

There is a large and growing body of evidence (only some of which can be reviewed here) that biological responses to low doses of ionizing radiation are not solely, or even primarily, dependent on DNA damage in the target cell in a manner proportional to dose (Morgan 2003a and b; Mothersill and Seymour 2006). Rather, biological defenses against low doses appear to be dominated by epigenetic factors including hyper-radiosensitivity (Joiner et al. 1996; Skov 1999; Zeng et al. 2006), bystander effects (Brooks 2004; Mothersill and Seymour 2004b; Prise et al. 2003; Snyder 2004), adaptive responses (Day et al. 2007a, b, c; Mitchel et al. 1997; Mothersill and Seymour 2004a), induced genomic instability (Morgan 2003a) [though the significance of this phenomenon in normal cells (Dugan and Bedford 2003) and in vivo (Morgan 2003b) is uncertain], and interactions between the target cell and the extra-cellular matrix (Barcellos-Hoff et al. 2005; Barcellos-Hoff 2005; Barcellos-Hoff and Costes 2006). Rather than each cell functioning independently, multi-cellular organisms mount coordinated tissue-level responses to radiation-induced injury (Barcellos-Hoff and Brooks 2001; Feinendegen et al. 2007; Trosko 1998). None of these phenomena occur in a linearly proportional relationship with dose in target cells. For example, bystander effects (Mothersill and Seymour 2004b) and adaptive responses (Mitchel et al. 2004) saturate at low doses and dose-rates (Shadley and Wiencke 1989).

The nonlinear modulation of biological responses to low doses of ionizing radiation occurring at the tissue level represents a decoupling of initial energy deposition in target cells (which appears to occur linearly with dose), and ultimate effects occurring at the organism level. Given these intervening nonlinear phenomena, it is becoming increasingly difficult to argue that carcinogenesis is linearly proportional to dose based on the microdosimetric argument.

CONCLUSION

The recent radiobiological research reviewed here has observed that LNT theory is insufficient to account for the different processes that operate at high doses compared to low doses. While initial energy deposition and the resulting prompt damage to biological molecules appear to be linearly proportional to dose, the biological responses at the gene, cell and tissue levels are not linear. Cells respond to low dose, low dose-rate exposures either through repair of radiation induced damage, or if the damage is irreparable, through cell suicide (apoptosis). Studies of gene expression profiles, as well as direct observations of biological endpoints with possible implications for risk of cancer, indicate a qualitative difference in the biological response to low dose, low dose-rate irradiation compared to the response observed at high

doses and dose-rates. This directly conflicts with the assumption of the LNT theory that biological responses are qualitatively independent of dose.

Perhaps the most significant question regarding the evidence discussed in this paper is the relevance of the various endpoints to carcinogenesis (UNSCEAR 2008). There is always a question about generalizing the results of in vitro experiments to in vivo effects. It is critical that resources and effort be directed to determining the relevance of in vitro endpoints to carcinogenesis.

This paper did not explicitly examine radiation effects at higher levels of biological organization (e.g., whole organisms or populations). Studies of whole organisms and populations of organisms have the advantage of directly observing effects of interest (e.g., cancer incidence). The advantage of radiobiological studies at lower levels of the biological hierarchy are that exquisite control of potential external confounders can often be achieved, and this facilitates the selection of one alternative hypothesis (e.g., a LNT dose-response) to the exclusion of competing alternative hypotheses such as those suggested by the radiobiological research reviewed here. It is difficult to reconcile the observations of nonlinear dose-responses at lower levels of biological organization with linearity at higher levels of biological organization. This suggests that observations consistent with linearity in organism- or population-level studies, especially at low doses and dose-rates, as well as the relevance of radiobiological endpoints to carcinogenesis, should be examined critically.

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