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Second cancers after radiotherapy: any evidence for radiation-induced genomic instability?

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Do second primary cancers in humans arise from radiation-induced somatic genomic instability after radiotherapy for the first malignancy? The amount of truly pertinent human information on this issue is sparse, leading to the conclusion that we cannot confirm or refute that instability induction by radiation is involved. However, the *in vitro* findings of radiation-induced genomic instability through bystander effects or increased mutation rates in cell progeny of apparently normal but irradiated cells are provocative and their transferability to human *in vivo* biology deserves further investigation. We describe possible animal and human studies to stimulate ideas, but the collaborative commitment of multiple large institutions to tumor tissue procurement and retrieval will be essential. In addition, detecting the temporal progression of genomic instability and identifying the salient genetic events as being radiation-induced will be pivotal. Execution of some of the studies suggested is not possible now, but applying next-generation methods could bring the concepts to fruition. As nearly one in 10 cancer diagnoses are second (or higher) malignancies, it is important to understand the contribution of radiotherapy to second cancer induction and pursue well-coordinated efforts to determine the role of induced genomic instability.
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Introduction

Since Roentgen's discovery of X-rays in 1895, skin reactions and carcinomas were ultimately linked to radiation exposure (historical review in Miller, 1995) and the induction of numerous human malignancies by radiation exposure is unequivocal (reviewed in Boice *et al*., 1996). Similarly, second malignancies of various organ sites are related to radiotherapy for the first primary cancer (reviewed in Tucker *et al*., 1988; Kaldor *et al*., 1992; Swerdlow *et al*., 1992; van Leeuwen *et al*, 1994; Boivin *et al*., 1995; Bhatia *et al*., 1996; Boice *et al*., 1996; Curtis *et al*., 1997; Inskip, 1999; Travis *et al*., 2000, 2002; Dores *et al*., 2002). The extent to which genomic instability, whether innate or induced, plays a role in the development of a second malignancy within or near the margins of the irradiated tissue is not known. The long-held belief that a radiation effect required the traversal of the nucleus and ionizations to occur within the cell nucleus is challenged by the observations of radiation-induced genomic instability. The ideas that (1) irradiated cells confer genomic instability to unirradiated adjacent cells (bystander effects), (2) apparently normal irradiated cells confer instability after multiple cell divisions to their progeny, (3) traversal of the cell's cytoplasm confers genomic instability, or (4) clastogenic plasma factors induced after radiation exposure perennially increase free radical production creating a 'stressed' cellular environment, have not been completely recognized in radiobiological models of radiation carcinogenesis (reviewed in Morgan, 2003b). According to one hypothesis of 'radiation-activated' phagocytic cells crossing tissue boundaries, genomic instability could be conferred to cells distant from the radiation treatment site (Wright, 1999; Lorimore and Wright, 2003).

After the initial descriptions of radiation-induced genomic instability (reviewed in Little, 2000; Morgan, 2003a, b), obvious questions arose concerning the relative contribution such a phenomenon could have for risk of first and second cancers after radiation exposure, the impact on assumptions used in modeling the radiation risk estimates, and whether the cell or the organ tissue is the proper basis for these estimates (Barcellos-Hoff and Brooks, 2001). For the purposes of this review, we will define radiation-induced somatic genomic instability as an evolving and ongoing progression of events. Our definition of radiation-induced somatic genomic instability would be in addition to the known mechanism of direct and indirect radiation damage-causing mutations and chromosomal aberrations within the cellular DNA. It is known that radiation creates alterations in the genome, but the view we present concentrates on detectable changes that appear in a progression of destabilizing events, leading ultimately to malignancy. We are concerned less about continued destabilization that certainly occurs after tumor formation, and we acknowledge detection of pre- *vs* post-tumor events is presently difficult to identify.

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There are a number of complicating observations to keep in mind. The human genome is intrinsically unstable. The chemical instability of the genome (Lindahl, 1993) and the error proneness of some repair systems (such as nonhomologous end joining and some repair polymerases) (Ferguson and Alt, 2001; Goodman, 2002), together contribute to mutations in the genome of somatic cells. Although many changes are benign for the affected cell, others modify the function of genes that protect the genome from mutation. Loeb has written extensively about the potential of the latter type of changes to produce a mutator phenotype that increases genomic instability and accelerates progression to cancer (reviewed in Loeb *et al*., 2003). The increased risk of cancer associated with radiation, and of second cancer after radiotherapy, may, in part, reflect the induction of such genome-destabilizing mutations. Adding to the complexity of relating radiation-induced changes to risk of second cancer is variation among people in their capacity to repair DNA damage and the association of reduced repair capacity with increased risk of cancer (Berwick and Vineis, 2000; Berwick *et al*., 2002). Variation in repair capacity in the general population is likely due to the many different combinations of inherited polymorphisms in genes that protect genome integrity (Mohrenweiser *et al*., 2003). Hence, both radiation exposure and genetics contribute to risk of second cancer. In the end, having high-quality radiation dosimetry (which can be calculated from radiotherapy records by radiation physicists expert in these methods) for tissues at risk and defining the inherited capacity of those tissues to respond will be necessary, although not likely sufficient, to assess the mechanisms relating radiation and risk of second cancer.

Although the focus of this review is on radiation-induced second primary cancers and the evidence that such cancers might have arisen from induced genomic instability, it is impossible to divorce this notion from an individual's innate degree of genomic instability. For this reason, concepts of cancer susceptibility are unavoidably interwoven. In this review, we will address briefly first primary cancers that arise after radiation exposure and how these may provide clues about the influence of genomic instability, summarize the epidemiology of radiotherapy-related second cancers, evaluate studies of second tumors arising in irradiated tissues for hallmarks of induced somatic genomic instability, and describe possible future studies.

Radiation-induced genomic instability and cancer

There is ample evidence that radiation induces changes in the genome and cancer. For example, chromosome aberrations are the standard biodosimeter for radiation exposure (Tucker *et al*., 1993; Bauchinger, 1998; Finnon *et al*., 1999). This role reflects the relative ease of detecting aberrations and well-defined dose–response relationships. The stability of aberration frequencies and the dose dependence of their frequencies in blood lymphocytes even decades after radiation exposure (Kleinerman *et al*., 1990; Kodama *et al*., 2001) indicate that induction of chromosomal instability of these cells by radiation is not a sufficiently high-frequency event *in vivo* to disrupt dose–response relationships. The relationships between dose, frequency of somatic translocation and gene mutation biodosimeters and cancer in the Japanese atomic bomb survivors may provide insight into the mechanisms that relate radiation to cancer (Mendelsohn, 1995). Mendelsohn observed that the relative risks of both leukemia and translocations in blood lymphocytes increase steeply in a curvilinear manner as a function of dose, a pattern suggesting that translocations alone might be causative of leukemia in atomic bomb survivors. Such a diagnostic relationship is well established for a number of leukemias (Chen and Sandberg, 2002). In contrast, the relative risks of solid cancers and somatic mutation in the glycophorin A gene of red blood cells both increased linearly with a shallow slope as a function of dose. Mendelsohn interpreted the latter relationships as indicating that radiation induces one of the multiple mutations needed to progress to a solid tumor. His model does not rule in or out whether the radiation-induced event involves induction of genomic instability.

Documenting that radiation induces events that lead to genomic instability, which in turn contributes to progression toward cancer, is difficult (Figure 1). Other papers in this volume and reviews cited above present the experimental evidence that genomic instability, manifested as increased frequencies of chromosome aberrations, gene mutations, or microsatellite sequence instability, can occur after radiation exposure. It may not be possible to distinguish definitively cancers associated with radiation-induced genomic instability from cancers that develop in radiation-exposed cells with a pre-existing genomic instability (Figure 1b, c). One approach, however, might be to identify signatures of genomic instability in tumor cells that are associated with specific, genetically defined genomic instability syndromes (and hence specific repair genes and pathways), and then determine whether postradiotherapy tumors have mutations with a radiation mutation spectrum in any of the genes associated with inducing that signature of instability. For example, genes in mismatch repair would be assessed in tumors displaying microsatellite instability (MIN), and genes in double-strand break repair would be evaluated in tumors with chromosome instability. A given tumor may display multiple types of genomic instability, yet radiation may have been directly responsible for the induction of only one type.

To sort out the many possible effects of radiation and genomic instability, we are confined to a few examples of human genetic disease that are associated with inherited genomic instability. Fortunately, access is steadily increasing to animal models of inherited genomic instability. These model *in vivo* systems will enable comparisons of the nature of genomic instability in cancers that form in individuals with pre-existing

genomic instability with and without radiation exposure. The rarity of people with inherited genomic instability syndromes, the short lifespan many of them have, and the infrequency of their experiencing radiotherapy for conditions other than cancer limit such examples.

The nature of the postradiation cancers of Fanconi anemia (FA) patients provides one illustration of how people with innate genomic instability may provide models to investigate genomic instability, radiation, and cancer progression. FA is an inherited genomic instability syndrome with a number of serious clinical consequences; bone marrow failure as a young adult is the primary cause of death (Wright, 1999 and references therein). Bone marrow transplantation has been used to rescue many FA patients from bone marrow failure, and total body irradiation has been the primary conditioning regime used prior to bone marrow transplant (Alter and Young, 1998). A comparison of the cancers that occur in nontransplanted and transplanted FA patients could provide insight into the contributions of the innate genomic instability of FA and radiation-induced events. Intriguingly, the suite of post bone marrow transplantation malignancies in FA patients is distinctive; all post-transplant cancers were solid tumors, whereas myelodysplastic syndrome (MDS) and leukemia dominated in nontransplanted FA patients who have no radiation exposures (Deeg *et al*., 1996 and reviews cited therein). It could be instructive to analyse both sets of cancers for genomic instability, to determine whether radiation treatment has induced signatures distinct from those attributable solely to the inherited instability of FA. A similar pattern of solid tumors appearing after bone marrow transplantation was associated with increasing radiation dose in a study of many patients with diverse diseases prompting the procedure (Curtis *et al*., 1997). However, in those cases and for those with FA, one must be aware that immunosuppression or extended lifespan associated with the procedure may be responsible for, or contributing to, the change in tumor spectrum.

Animal models provide invaluable experimental systems for relating inherited predisposition to genomic instability, radiation exposure, and cancer. To illustrate the potential of these systems, we present an example in which an observed interstrain variation in radiation-induced cancer has been traced to a polymorphism in a specific gene, and also point to the wealth of opportunities that transgenic technologies provide for creating animal models with defined alterations in genes associated with genomic instability. Recently, a functionally significant polymorphism in the Prkdc gene (which encodes the DNA-dependent protein kinase catalytic subunit) was found to be the genetic basis of the higher

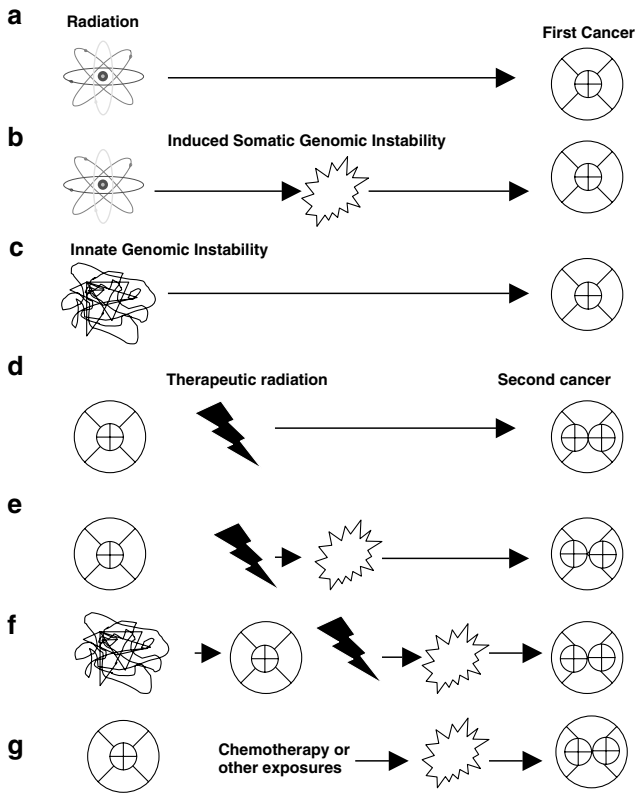

Figure 1 Radiation and genomic instability as inducers of first and second cancers. These models are not meant to be comprehensive, but to provide testable hypotheses to study events leading to second cancers. The experimental challenges are to distinguish between the alternatives. (**a**) Radiation induces DNA damage that initiates cellular events leading to a first cancer. Although the exact sequence or the nature of events in radiation carcinogenesis is unknown, this model represents the long-held notion that traversal of the cell's nucleus is required for radiation-induced cancer. (**b**) Radiation traverses but does not obviously damage the parent cell (the cell survives) or the parent cell neighbors a hit cell but is otherwise undamaged. After many cell divisions, the parent cell's progeny exhibit an 'acquired' or induced somatic genomic instability within a cellular subpopulation. Through additional stochastic events and cell divisions, a radiation-induced first cancer occurs. (**c**) Germline or innate genomic instability is responsible for cancer occurrence. Although the person may or may not have been exposed to radiation, this exposure was irrelevant or was an insignificant contributor on a background of generalized genomic instability. The germline or innate genomic instability spans a continuum between a person with a rare genetic syndrome and an apparently normal individual with a suite of polymorphic variants conferring increased cancer susceptibility. (**d**) The first cancer was treated with radiotherapy and the treatment induced the second cancer. This model is analogous to (**a**), but illustrates the need to distinguish radiation-induced second cancers from second cancers arising in models e–g. (**e**) The first cancer was treated with radiotherapy and the treatment induced somatic genomic instability. After many cell divisions, the second cancer occurs. This model illustrates the need to distinguish between an intervening induction of genomic instability rather than an inducing effect of radiation treatment (model d). (**f**) Germline or innate genomic instability is responsible for first cancer occurrence, for which therapeutic radiation is given. After many cell divisions, radiation induces a somatic genomic instability that, with additional stochastic events and ensuing cell divisions, leads to a second cancer. This model might represent (at one extreme) a person with bilateral retinoblastoma, who is treated with radiation and after many years develops a sarcoma within the irradiated field. Distinguishing between germline or innate genomic instability and radiation-induced somatic genomic instability is essential. (**g**) The first cancer is treated by CT or the person is exposed to additional carcinogens (e.g. continues smoking) and these exposures induce somatic genomic instability; additional stochastic events lead to second cancer occurrence. This model illustrates the need to distinguish between the induction of somatic genomic instability that is related to carcinogens other than radiation

risk of radiation-induced mammary cancer in BALB/c mice (Yu *et al*., 2001). The studies leading to this discovery elegantly illustrate the synergy possible by applying genetics, *in vivo* and *in vitro* radiation exposures, the ability to study tissues at different times after exposure, assays for cytogenetic instability, and molecular biology (reviewed in Ullrich and Ponnaiya, 1998). A large number of mouse strains with targeted mutations of genes that affect repair of DNA damage are already available (Friedberg and Meira, 2003). Most are knockouts although a few conditional mutants have been reported. One clear advantage of working with mice as a model system is that mutations can be bred into different strain backgrounds and mice with mutations in multiple genes involved in genomic instability can be produced. One example illustrates this point. Radiation-induced mammary cancer was over fivefold higher and lymphomas were twofold lower in mice heterozygous for a p53 gene deleted for exons 2–6 on a BALB/c background (known from the work of Yu *et al*. (2001) to be deficient in DNA-dependent protein kinase catalytic subunit) than in mice with the same p53 genotype on a DBA/2 background (Backlund *et al*., 2001). Comparison of the spectrum of genomic instability of tumors in these cases could be informative.

Second cancer risk after radiotherapy treatment

Several studies have investigated second cancer risk following radiotherapy treatment for a first primary cancer. Table 1 summarizes the second cancer sites associated with radiotherapy for a first malignancy. These studies clearly indicate increased risk of subsequent cancers with increasing dose to the surrounding tissues, including the bone marrow (reviewed in Boice *et al*., 1987, 1996; Curtis *et al*., 1997; Inskip, 1999; Travis *et al*., 2000; Gilbert *et al*., 2003, citations not intended to be exhaustive), and include information that not all tissues uniformly exhibit increased risk. In addition, among follow-up studies with sufficient numbers and radiation dosimetry, the dose–response curve for leukemia after irradiation for cervical cancer indicates a curvilinear pattern in that risk increases up to 4 Gy and then decreases with doses above 4 Gy (Boice *et al*., 1987). Occurring around 5 Gy, 'flattening' of second thyroid risk is suggested among childhood cancer survivors, but this observation is based on small numbers and the referent groups included patients with nonzero thyroid doses (Tucker *et al*., 1991; De Vathaire *et al*., 1999). The pattern of increasing risk at lower doses that begins to 'plateau' or decrease at high doses is consistent with nonlinearity (or a suspected high-dose

Table 1 Summary of radiotherapy-related second (multiple) cancers for selected types of first primary cancer[a]

Type of first primary cancer	*Type/site of second cancers associated with radiotherapy*	*Comments*
Childhood cancers[b]		
Retinoblastoma	Bone and connective/soft tissue, brain (?)	Strong genetic component to susceptibility
Wilm's tumor	Bone and connective/soft tissue, thyroid, leukemia, liver (?)	Radiotherapy used less often now than previously
Neuroblastoma	Bone and connective/soft tissue, thyroid	Possible shared etiologic factors between thyroid cancer and neuroblastoma (?)
Ewing's sarcoma	Bone, leukemia	
ALL[c]	Brain and nervous system	Prophylactic craniospinal radiotherapy used less often today
Meduloblastoma	Brain and nervous system, skin	High skin cancer risk among patients with Gorlin's syndrome (rare)
Hodgkin's lymphoma (in adults)	Breast, lung, bone and connective/soft tissue, thyroid, leukemia (?), esophagus, stomach, bladder	High relative risks for breast, thyroid, and bone caner associated with irradiation at a young age. Leukemia risk much greater for alkylating agents
Non-Hodgkin's lymphoma	Leukemia, bladder, thyroid, kidney (?)	Thyroid risk associated with young age at irradiation
Testicular	Stomach, bladder, leukemia, bone and connective/soft tissue, pancreas (?)	
Ovarian	Leukemia, bladder, connective and soft tissue, rectum (?), pancreas (?)	Leukemia risk much greater for alkylating agents
Breast	Leukemia, contralateral breast, lung, thyroid (?), bone and connective/soft tissue, esophagus (?)	Possible interaction with alkylating agents for leukemia; little or no radiation-induced cancer of contralateral breast following exposure past age 45 years
Uterine	Leukemia, bladder, stomach, kidney (?), rectum, vagina, ovary, bone and connective/soft tissue, thyroid (?), breast (?)	Low risk of leukemia despite high dose to bone marrow; protective effect against breast cancer among women with ovaries

[a]Inskip (1999 and references therein), reproduced with the permission of Lippincott Williams and Wilkens; updated with Dores *et al*. (2002), Travis *et al*. (2000, 2002), and Zablotska and Neugut (2003). [b]Based on follow-up through early adulthood. [c]ALL: acute lymphoblastic leukemia

Table 2 Type of abnormalities found in radiation-associated second tumors by organ site or tissue, with comparison to sporadic or *de novo* tumors

Author, year	*Tumor or organ evaluated*	*First cancer or irradiated site*	*N (tumors)*	*p53 mutations*	*Microsatellite instability*	*Other analyses*	*Comments*
Mertens, 2000	Postirradiation sarcomas	Breast cancer (4), Ewing sarcoma (2), others varied	10	Not done	Not done	Karyotyping with loss of 3p21–3pter in 8/10	Loss of material on 3p significantly more likely in radiation-related sarcomas than expected[a]. 5/10 patients had CT in addition to XRT
Tarkkanen, 2001	Postirradiation sarcomas	Variable	27	Not done	Not done	CGH gain and loss patterns differed *vs* sporadic[b]	8/26 patients had CT in addition to XRT
Lefevre, 2001	Osteosarcoma, MPNST, leiomyosarcoma	RB	7	Increased deletions;[a] unique types of mutations[a]	Stable	High chromosomal instability; loss of normal RB allele	Minisatellites stable
Gafanovich, 1999	Osteosarcomas (4), MDS, AML, GBM, B-cell lymphoma, thyroid	Variable	9	High frequency[a]	High (9/9)[a]		Suceptibility (mutator phenotype) *vs* induced instability? 6/9 patients had CT in addition to XRT
Chauveinc, 1999	Sarcomas (5), meningioma, rectum cancer, malignant schwannoma	Breast cancer (4), others varied	8	Not done	Not done	Karyotyping and description of chromosomal abnormalities.	Descriptive rather than comparison study with aim to determine progression and events in radiation-associated carcinogenesis
Ben-Yehuda, 1996	Therapy-related leukemia and MDS	Variable: hematologic (49%) and solid tumors (51%)	56	Increased compared to sporadic AML/MDS[a]	High (94%; 15/16)	Increased karyotypic abnormalities[c]	Patients young at first cancer, +FH, multiple cancers. Suceptibility (RER+) *vs* induced instability? Half treated with XRT or XRT+CT; only 7% were XRT alone
Horiike, 1999	Therapy-related leukemia and MDS	Hematologic (57%), solid tumors (43%)	21	Similar to *de novo*[a]	Similar to *de novo* MDS[a]	RER+ phenotype in 2/10; hMSH2 Gln419Lys mutation in both	Could not establish radiation related *vs* chemotherapy related contributions *per se*; two patients had evidence of likely germline susceptibility (RER+)
Brat, 1999	Postirradiation astrocytomas	Cranial tumors	9	Similar to sporadic[a]	Not done	No PTEN mutations	Mutational spectra similar to sporadic tumors[a], but high grade at diagnosis and unusual anatomic cranial locations. 5/9 patients had CT and XRT

Shoshan, 2000	Radiation-induced meningioma	Patient's scalp irradiated for tinea capitis (ringworm)	7 RIM compared to 8 sporadic meningioma	Not done	Not done	No NF2 mutations; low LOH 22q, high LOH 1p	Nonrandom allelic loss found in 4/7 (57%) RIM *vs* ~30% expected[a]
De Benedetti, 1996	Lung	Hodgkin's lymphoma	11	Increased[a] G: C to A: T transitions at non-CpG sites	Not done		May have been unable to distinguish a smoking from a radiation effect. 6/11 patients had CT in addition to XRT
Behrens, 2000	Breast and lung cancer	Hodgkin's lymphoma	19 lung 19 breast; 57 lung, 20 breast	Similar in second cancers *vs* sporadic	Increased in second cancers *vs* sporadic	Minisatellite results similar in second cancers *vs* sporadic	Comparisons made between the 38 second cancers after HL and the 77 sporadic cancers. 23/38 second cancer patients had CT in addition to XRT

[a]Mutations compared to expectation based on literature review. [b]Postirradiation tumors compared to previous work in sporadic tumors by the same authors. [c]Cannot assess if pattern unusual for postirradiation tumors compared to appropriate control tumors

cell killing effect); however, linear relationships also describe second cancer risk, even in instances where the field is irradiated in excess of 5 Gy. For example, in a follow-up study of lung cancer after Hodgkin's lymphoma, the risk with radiation dose to the tumor (in cases) and to corresponding lung tissue (in controls) was consistent with linearity (risk increases as the radiation dose increases), even though the majority of lung doses were above 30 Gy (Gilbert *et al*., 2003). Many of these studies represent collaborative efforts for which extremely large numbers of patients are required to describe risk adequately, highlighting the difficulty in obtaining such information. For example, in a multicenter international study of solid tumors after radiation therapy (nested within a cohort of approximately 150 000 cervical cancer patients), the risk patterns by dose lacked the numbers for definitive characterization of risk (linear *vs* curvilinear) (Boice *et al*., 1988). Unfortunately, it is impossible to ascribe the shape of a dose–response curve or any proportion of second cancers arising within a cohort of cancer survivors to late effects of radiation-induced genomic instability, although the observational risk estimates must include the effect, if it exists.

Tumor tissue analysis of second primary cancers

Second primary human tumors arising in an irradiated field for a first cancer are intuitively attractive tissues for attempting to discern a signature for radiation causation or, in this context, induced somatic genomic instability. On the other hand, tumors are notoriously complicated, displaying a wide variety of aberrant conditions such as karyotypic abnormalities, proliferative signaling, p53 mutations, gene amplification, loss of heterozygosity, multinucleation, gene expression changes, micro- and minisatellite instability, etc. The investigator typically detects these in a cross-sectional study design, rather than longitudinally. It may be difficult to isolate definitively early tissue or tumor changes within second tumors, as these can be high grade and late stage at presentation (Brat *et al*., 1999). Nevertheless, we selected reports of second tumor analyses that arose within or near a previously irradiated field and reviewed these for MIN, complex karyotypic changes, or other unique features such as single base mutations *vs* larger gene deletions/rearrangements that might be indicative of radiation-induced somatic genomic instability. We recognize the inherent limitations of such a survey, not the least of which is that all the tumor studies were not necessarily designed to assess radiation-induced genomic instability *per se*.

Several studies were performed on sarcomas that developed within tissue irradiated for a previous malignancy and were diagnosed many years after treatment (Table 2). Most of the sarcomas occurred after varying types of first tumors, except for one study among patients treated for retinoblastoma (Lefevre *et al*., 2001). Among the second tumor studies that

included sarcomas, one found evidence of MIN (Gafanovich *et al*., 1999) while another did not (Lefevre *et al*., 2001). In the study by Gafanovich *et al*. (1999), all the tumors showed evidence of MIN. It was unclear if the instability was radiation induced or the second tumors occurred in a highly selected group of individuals with innate susceptibility since five tumors and corresponding normal tissues out of seven evaluated showed MIN. Among patients with therapy-related leukemia or MDS, a high proportion with MIN was found (among the subset analysed), but again the group was characterized by unique features often associated with genetic predisposition, such as early age of onset of the first cancer, multiple primary cancers, and a history of cancer within the family (Ben-Yehuda *et al*., 1996). In a subsequent study of therapy-related leukemia and MDS, a high proportion with MIN was not found, but the two unrelated patients with MIN remarkably revealed the same mutation in the mismatch repair gene hMSH2 (Horiike *et al*., 1999). Studies of astrocytomas and meningiomas after cranial irradiation describe several unusual characteristics (Brat *et al*., 1999; Shoshan *et al*., 2000), although the prevalence of MIN was similar in postirradiation compared to sporadic astrocytomas (Brat *et al*., 1999). MIN was increased in second lung or breast cancers after Hodgkin's lymphoma compared to sporadic tumors, although the presence of p53 mutations was similar (Behrens *et al*., 2000). This finding contrasts with an earlier observation among lung cancer tumors (again post-Hodgkin's lymphoma) in which unique p53 mutations were reported (De Benedetti *et al*., 1996).

The small number of studies investigating second tumors after radiation provides few unifying threads of commonality. Certainly, findings of MIN within these tumors are consistent with genomic instability, but based on these data it is impossible to place MIN as a tumor-specific, progressively destabilizing, driving event. In fact, MIN could have predated the tumor as a characteristic of the individual in whom the second tumor developed. Very few studies included patients treated only with radiation (Shoshan *et al*., 2000; Lefevre *et al*., 2001) and it is virtually impossible to divorce radiation-related effects from chemotherapy (CT)-related effects (see Table 2, comments column). Karyotypic patterns unique to radiation-associated second tumors compared to *de novo* tumors (Ben-Yehuda *et al*., 1996; Mertens *et al*., 2000; Shoshan *et al*., 2000; Lefevre *et al*., 2001; Tarkkanen *et al*., 2001) suggest that events in radiation-induced carcinogenesis do differ from spontaneous cancers. Unfortunately, more specific details related to the hypothesized order of these events are difficult to evaluate, even when taking advantage of karyotypic clues (Chauveinc *et al*., 1999).

Conclusions

The published studies to date do not provide sufficient evidence to determine whether the phenomenon of radiation-induced genomic instability contributes to secondary malignancies in humans after radiotherapy. This potential mechanism for radiation-induced cancer in humans remains speculative (Wright, 1999; Barcellos-Hoff and Brooks, 2001) and there are no sources, other than indirect inferences, that support or refute that such an instability induction occurs *in vivo*. However, the *in vitro* findings of radiation-induced genomic instability are provocative, and their transferability to human *in vivo* biology deserves additional attention. We describe the experimental and epidemiological resources needed to succeed in addressing the unanswered questions about human radiation-induced genomic instability in the next section.

Future studies/recommendations for research/speculations

Given that genomic instability is a hallmark of cancer in general, finding genomic instability in a tumor that occurs after radiotherapy is not sufficient to prove that radiation was causative. More convincing would be identification of mutations in the second tumor in genes that can lead to the specific types of genomic instability that are documented in that tumor, and determination that the mutations have a spectrum consistent with radiation. It is necessary to determine that the mutations do not appear in normal tissues that were not in the radiation field; however, they might be present in normal tissue adjacent to the tumor of an individual. The mutations ought not be present in the first, radiation-independent tumor. The tools to perform such analyses are being developed now. Highly sensitive methods are needed to screen small tissue samples for all types of genomic instability, in order to classify a tumor with respect to the pathways and hence genes that are candidates for the radiation-induced somatic mutations that started the progression of instability. One can envision a suite of assays that apply next-generation methods related to comparative genomic hybridization and gene expression arrays (Gray and Collins, 2000) to detect chromosomal alterations and related expression phenotypes and the detection of microsatellite mutations. As knowledge of the genes responsible for DNA repair and different types of genomic instability becomes ever more complete (Ronen and Glickman, 2001; Wood *et al*., 2001), it will be relatively easy to define the sets of genes to screen for mutations in each individual. Oligonucleotide arrays could be used to search exhaustively for mutations in each gene (as recently carried out for ATM mutations in lymphomas, Fang *et al*., 2003). Finding somatic mutations in candidate genes associated with genomic instability is the first step. Then it is necessary to compare the spectra of mutations to determine if there is a mutation signature in the tumors after radiotherapy that distinguishes (some of) them from the mutation signatures in unexposed tissues and tumors from subjects with no radiotherapy.

In Figure 1, we illustrate the conceptual and crucial elements associated with radiation-induced somatic genomic instability as an event in cancer progression that must be distinguished from the alternative models. Our depiction of various scenarios downplays the complex processes; however, these are important points. (1) In the absence of radiation-induced somatic genomic instability, radiation exposure alone induces cancer, although the relative contributions of each are unknown. (2) Germline or innate genomic instability probably varies among individuals, and the relation of radiation to cancer risk could be modified by the innate genomic instability such that radiation exposure ranges from irrelevant to synergistic (i.e. strong radiation–genetic interaction). (3) Therapeutic radiation given for a first cancer may or may not induce a second cancer, but the ability to distinguish the mutational spectra between a second cancer with or without an induced somatic genomic instability and to be able to attribute the induced genomic instability to radiation (and not CT) treatment are essential.

Assuming that such radiation mutational spectra can be identified, a potentially ideal study setting would be within a cohort of childhood cancer survivors where follow-up of patients into adulthood is ongoing (Robison *et al*., 2002) or, alternatively, where a national system of cancer registries linked to individuals is available (such as many European countries). Treatment records would be obtained for radiation therapy so that accurate dosimetry to organ sites could be calculated and CT doses could be included in statistical analyses. Second, cancers ascertained would then be referred for tissue studies. Collaborative coordinated biologic sample procurement support would be required, such that snap-frozen tissues from both first and subsequent malignancies (and normal unirradiated material if available) would be stored for later retrieval. A large cohort or population base is essential for sufficient numbers of cases to arise over time for testing. Sample size requirements for a human study would depend on the prevalence of the genomic instability among tumors likely to have a radiation-related cause compared to likely nonradiation caused tumors. (Clearly, misclassification of the second tumor as radiation related will complicate matters, but we will presume that such issues can be minimized by appropriate selection criteria.) In rare but non-negligible instances, some cancer survivors develop two or more subsequent malignancies. It would be possible to identify these patients and select tumor tissue that developed within an irradiated field and a tumor that did not for comparison. This would control, to some extent, for the individual genetic background on which each tumor arose.

Determining whether second cancers that occur after radiotherapy result from radiation-induced genomic instability presents multiple challenges. Criteria and experimental methods that enable one to distinguish between radiation-induced and radiation-independent cancers are needed. Another critical component is designing epidemiologically sound studies and establishing repositories of the requisite biological materials and information. We outlined the conceptual elements of each, with the intent of stimulating research in all the disciplines required. Given the challenges and time required to develop resources for studies of human cancers, studies in mouse models may be most fruitful in the near future. Based on data from the Surveillance, Epidemiology and End Results program, 225 154 of 2.25 million cancers diagnosed between 1973 and 2000 in the United States were second (including third, fourth, etc.) cancers, or approximately 10% (R Curtis, personal communication, 2003). As treatment and survival of those with first cancers are expected to continue to improve, these numbers indicate the importance of understanding the contribution of radiotherapy to subsequent cancer risk and the potential for well-coordinated efforts to execute studies such as that we have described.

Abbreviations used in Table 2

AML, acute myeloblastic leukemia; CGH, comparative genomic hybridization; CT, chemotherapy; +FH, positive family history of cancer; GBM, glioblastoma multiforme; HL, Hodgkin's lymphoma; LOH, loss of heterozygosity; MDS, myelodysplastic syndrome; MPNST, malignant peripheral nerve sheath tumor; NF2, neurofibromatosis type 2; RB, retinoblastoma; RER+, replication error or mutator phenotype; RIM, radiation-induced meningioma; XRT, radiotherapy.

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