Do No Harm

Normal Tissue Effects

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Radiation therapy confers enormous benefits that must be balanced against the possibilities for harm including late toxicity in normal tissues and radiation-induced second malignancies. A small percentage of patients experience severe late complications. The question is, do these late sequelae occur randomly, or are they confined to individuals who are genetically predisposed to radiosensitivity. Experiments with knockout mice and with patients demonstrate that individuals heterozygous for a number of genes appear to be radiosensitive. If radiosensitive patients were identified prospectively by genetic analysis, they could be spared the trauma of late sequelae. Several large studies have shown a statistically significant excess of radiation-induced malignancies in radiotherapy patients. Most second cancers are carcinomas, developing in the lining cells of the body often remote from the treatment site. Radiation-induced sarcomas appear only in the heavily irradiated areas. These are small in number but appear with a very high relative risk.

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It is axiomatic for the therapy of any malady that whether or not it can do good, it should at least do no harm. It is a tragedy if the patient suffers more from the treatment than from the disease that it was designed to ameliorate. In the case of radiation therapy there are two possibilities for harm that may offset the undoubted benefit: first, unacceptable late toxicity in normal tissues, and secondly, radiation-induced second malignancies. These will be discussed in turn.

UNACCEPTABLE LATE TOXICITY IN NORMAL TISSUES

Whenever doses are pushed to the limit in aggressive radiotherapy, a small percentage of patients experience severe late effects that compromise their quality of life. The question that has occupied attention for years is whether these effects are random, stochastic, governed simply by the roll of the dice, or are late sequelae confined to individuals who are genetically predisposed to radiosensitivity. This is an important distinction since if the latter were true, the sensitive individuals could be identified prospectively and spared the trauma.

Much effort has been expended on the development of predictive assays designed prospectively to identify individuals likely to be radiosensitive and at risk of developing late sequelae. Techniques include the measurement of SF_2 (surviving fraction at 2 Gy) in cultured fibroblasts (1–7), the micronucleus assay (8–11), the measurement of chromosomal aberrations (12) and the immunohistochemical expression of DNA-PKCS and Ku (p70/p80) (13). The possible usefulness of these techniques has been the subject of a number of reviews (14–17). The bottom line is that all prove to be of limited usefulness, and none has been adopted in the clinic. What is more likely to succeed in the future are the attempts to identify radiosensitive individuals prospectively at the genetic level. In microorganisms, several genes have been identified that dramatically modulate radiosensitivity (18). Not so in human cells. There is an urgent need at the present time to identify genes that result in a small but significant change in radiosensitivity.

The ATM gene

Most attention to date has focused on the ATM gene. Individuals homozygous for this mutation have the serious clinical problems of ataxia and telangiectasia, they are exquisitely radiosensitive and are prone to develop cancer, but they are few in number and are easily identifiable by their clinical symptoms (19–21). What is of greater interest is the much larger number of individuals who are heterozygous for a mutation in this gene, since they make up

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1 to 3% of the population and cannot be identified by any clinical symptoms (22). The question is whether they are radiosensitive and account for the minority of radiotherapy patients that experience late sequelae?

We recently completed a project with AT knockout mice, in which it is possible to observe two highly quantifiable endpoints: (a) radiation-induced ocular cataracts—a deterministic effect; and (b) oncogenic transformation in mouse embryo fibroblasts—a stochastic endpoint.

By mating mice heterozygous for the ATM gene, it was possible to obtain embryos from the same litter that were wild-type, heterozygous or homozygous for the ATM gene. Homozygous animals were exquisitely radiosensitive to both cataract formation and oncogenic transformation, but this was not surprising and is of little interest. The important finding is that mice heterozygous for the ATM gene developed radiation-induced cataracts earlier, and that reached a higher level of opacity than those of wild-type animals. At the same time, an intra-litter comparison between ATM heterozygous and wild-type mouse embryo fibroblasts indicates that heterozygous cells are significantly more sensitive to radiation-induced transformation than their wild-type, litter-matched counterparts (23). This is the first work to demonstrate unequivocally a significant enhancement in radiosensitivity in carriers of a heterozygous mutation—in this case ATM.

Frequency of ATM heterozygotes among prostate cancer patients with severe late responses to radiotherapy

The clear and unequivocal data with AT knockout mice lend credibility to the preliminary report that we published in 1998 (24), which suggested that a disproportionate number, but by no means all, of prostate cancer patients who experience severe late effects after radiotherapy are AT heterozygotes. The approach was as follows:

A group of prostate cancer patients were selected who experienced severe late sequelae, specifically proctitis or cystitis, after high-dose external-beam conformal radiation therapy, together with a control group of patients treated in the same way but who did not have severe late effects. Blood samples were taken from these patients, genomic DNA extracted, and mutations sought in the ATM gene by direct sequencing.

Of 17 patients with late-effect, in whom most or all of the ATM gene had been examined, significant mutations were identified in 3 (18%). No significant mutations were found in the control group. The incidence of ataxia telangiectasia heterozygotes in the United States population is 1% to 2%.

All the mutations observed involve a change in the amino acid coded for and were in regions of the gene where mutations are commonly found in AT homozygotes. These preliminary results, although not conclusive and too small in number to reach statistical significance, suggest that AT heterozygotes, that is, individuals carrying a significant mutation in one copy of the ATM gene, are over-represented in prostate cancer patients who developed serious late sequelae after high-dose external-beam conformal radiation therapy.

If these, and perhaps other genetically radiosensitive individuals, could be identified prospectively, a dose de-escalation regimen could spare them a great deal of discomfort and suffering, probably (because it is likely that their tumor cells will also be radiosensitive) without loss of tumor control. As a corollary, if most of the late-effects population were prospectively identifiable, the dose to the remaining patients might potentially be escalated, with a concomitant improvement in tumor control.

Other candidate genes

Other candidate genes that may confer radiosensitivity include:

NBS—Perhaps 10 times less prevalent in the population as a whole, but perhaps as frequent at ATM in individuals from central Europe owing to a 'founder effect'.
HRAD9—Prevalence not known—human analogue of rad 9, which has such a dramatic effect in yeast.
BRCA1 & 2—Prevalent in certain populations, again probably because of a founder effect. This gene certainly confers a predisposition to breast cancer, but there is some evidence that it may also confer increased radiosensitivity.

An unpublished report from the MD Anderson Hospital in Houston, Texas focused attention on breast cancer patients who were shown, by direct sequencing, to be BRCA heterozygotes (T. Buchholz, personal communication).

Dermal fibroblasts were studied using an in vitro clonogenic survival assay. These data were compared with results obtained from a previous set of prospectively studied cancer patients who had a negligible risk for a BRCA mutation. In addition, results from a mutagen sensitivity assay performed on lymphocytes obtained from BRCA heterozygotes were compared with results from normal controls with no cancer history. Results from both assays suggested that cells containing a heterozygous mutation in BRCA1 or BRCA2 were more radiosensitive than controls. For the fibroblast studies, the mean surviving fraction at 2 Gy (SF2) for carriers was 0.279 versus 0.348 for the control set ($p = 0.007$). For the lymphocyte studies, the mean number of chromatid breaks after 1.25 Gy of radiation was 0.79 breaks per cell for the carriers versus 0.45 for the controls ($p = 0.0005$). These data must be regarded as preliminary since they rely on historical controls, but the results are consistent with a relationship between a germline mutation in BRCA1 or BRCA2 and a hypersensitivity to radiation.

RADIATION INDUCED SECOND MALIGNANCIES

There are many single-institution studies in the literature involving radiotherapy for a variety of sites which conclude that there was no increase in second malignancies, although a more accurate assessment would have been that the studies had limited statistical power to detect a relatively small increased incidence of second malignancies induced by the treatment (25).

Most radiation oncologists who see a limited number of patients with any given type of tumor do not see second malignancies as a serious problem. There are the well-known exceptions, such as the significant incidence of breast cancer in young women receiving radiotherapy for Hodgkin's lymphoma (26–28), or the spectrum of carcinomas in women irradiated with intracavitary and external-beam radiotherapy for carcinoma of the cervix (29, 30). This is the nightmare of any radiation therapist, and the ultimate disaster for the patient.

However, whenever a large, well-controlled study is possible, the whole spectrum of second malignancies is clearly attributable to radiation therapy. We recently completed the largest ever study of second malignancies in patients treated for prostate cancer, using the SEER data (31). Data regarding the rate of incidence from the Surveillance, Epidemiology, and End Results Program cancer registry (1973–1993) were used directly to compare second malignancy risks in 51 584 men with prostate carcinoma who received radiotherapy (3549 of whom developed second malignancies) with 70 539 men who had undergone surgery without radiotherapy (5055 of whom developed second malignancies). Data were stratified by latency periods, age at diagnosis and site of the second malignancy. Directly comparing the risks in the radiotherapy group with those in the surgery group largely precludes problems associated with the underreporting of second malignancies.

Radiotherapy for prostate carcinoma was associated with a small, statistically significant increase in the risk of solid tumors (6%; $p = 0.02$) relative to treatment with surgery. Among patients who survived for ≥ 5 years, the increased relative risk reached 15%, and was 34% for patients surviving ≥ 10 years.

It is concluded that radiotherapy for prostate carcinoma is associated with a statistically significant, although fairly small, enhancement in the risk of second solid tumors, particularly for long-term survivors. The pattern of excess second malignancies among men treated with radiotherapy was consistent with radiobiologic principles in terms of site, dose, and latency. In absolute terms, the estimated risk of developing a radiation-associated second malignancy was 1 in 290 for all prostate carcinoma patients treated with radiotherapy, increasing to 1 in 70 for those who survived 10 years or more. Improvements in radiotherapeutic techniques, along with diagnosis at younger ages and earlier stages, are resulting in longer survival times for patients with prostate carcinoma. Because of the long latency period for radiation-induced tumors, this may result in radiation-related second malignancy risk becoming a more significant issue.

A closer look at this study of prostate cancer patients reveals some interesting biological insights. On analyzing the solid tumors site by site, we found significant radiation-associated increases in bladder carcinoma, rectal carcinoma, and lung carcinoma, as well as sarcoma in or near the treatment field. As with the overall data, there is a strong indication of an increasing radiation-associated risk with increasing survival time for the individual sites. It is interesting to note that no significant increase in the rates of leukemia was noticed.

Although the majority of radiation-associated malignancies clearly are carcinomas, as in the Japanese A-bomb survivors, the largest increase in relative risk is for in-field sarcomas, a category of malignancy not observed in excess in the A-bomb survivors. In this, as in the majority of other studies, radiation-induced sarcomas occur only in heavily irradiated sites, close to the treatment volume. These observations most likely reflect a different mechanism for the induction of sarcomas compared with carcinomas. Carcinomas arise in tissues where, even in the adult, cells are turning over and/or are under hormonal control. By contrast the target cells for sarcoma typically are dormant cells and large doses are needed to produce sufficient tissue damage to stimulate cellular proliferation. The sarcoma data in this study appear to follow this pattern, with significant radiation-associated risks being observed for sites in and close to the treatment volume but not for more distant sites, which received lower doses.

The most probable reason that so few sarcomas were observed in the prostate patients is that most survived for such a short time after radiation therapy. A comparison with animal data is enlightening. A study conducted at the National Institute of Health in the US involved the irradiation of beagle dogs with large single doses in order to determine the tolerance of various organs, in preparation for a program of Intraoperative Radiation Therapy (IORT) (32). An unexpected observation was that 25% of the dogs that received 25 Gy or more developed an in-field sarcoma with a latency of 3.6 years. This was an incidental observation, and not the purpose of the study. Two decades ago, Herman Suit studied the incidence of radiation-induced sarcoma in defined flora and specific pathogen-free mice, which had a life expectancy of 900 to 1000 days (33). He showed that 50% of the animals developed a sarcoma within 480 days after a dose of 6.5 to 7.5 Gy, while after 800 days, 85% of the animals developed a sarcoma. In comparing the animal data with the human experience, the latency periods must be thought of relative to the life span of the animals, i.e., the animals were observed for a much longer period post-irradiation relative to their life than was the case with the radiotherapy patients.

The 'cautionary tale' for the radiation therapist is that

as radiotherapy is used for younger patients, with a higher probability for cure, and with a longer post-treatment life span, radiation-induced second tumors will assume an ever-increasing importance. Radiation-induced second malignancies may represent the price of success, but it could become a high price, and must be considered in the complex equation of competing relative risks.

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