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COMMENTARY

Chromosomal "Fingerprints" of Prior Exposure to Densely Ionizing Radiation

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A biomarker that would distinguish radiation-induced biological damage from damage produced by other agents has long been a goal in radiation biology. We suggest that densely ionizing radiations such as α particles from radon daughters, or fission neutrons, leave a distinctive chromosomal marker that may be detected and measured long after radiation exposure. Specifically, they produce an anomalously low ratio (*F*) of interchromosomal to intrachromosomal, interarm exchange-type chromosome aberrations, in comparison with either X rays or chemical carcinogens. For densely ionizing radiations and for other agents, experimental values of this *F* ratio, determined both *in vitro* and *in vivo*, are quantitatively consistent with theoretical expectations based on considerations of chromosomal geometry and radiation track structure. The use of fluorescence *in situ* hybridization to measure *F* values in stable chromosomal aberrations, together with recent developments in techniques for harvesting viable human cells, makes the application of this biological marker quite feasible. For example, the use of this marker could greatly facilitate epidemiological studies of radon-exposed cohorts.

INTRODUCTION

Various groups of people, such as inhabitants of areas with high levels of radon, uranium miners, nuclear energy research workers, well loggers, airline flight personnel and survivors of the Hiroshima A-bomb, have potentially been exposed to significant doses of densely ionizing radiation, such as α particles or neutrons. Consequently, determination of whether individuals, particularly those who later developed cancer, did in fact receive significant densely ionizing radiation doses is an important societal and legal issue. Thus there has been considerable interest in finding a detectable biological "fingerprint" preferentially produced by densely ionizing radiation. Indeed, a recent expert report on laboratory-based methods to arrive at risk estimates for radiation-induced cancer in humans (*1*) suggested that identification of a radiation "signature" was a key research need.

Several observations have been made suggesting biological lesions that may be preferentially or uniquely produced by densely ionizing radiation: For example, Vahakangas *et al.* (*2*) studied 19 lung tumors from uranium miners and reported p53 point mutations in 7 (37%). These point mutations were unusual in that (a) they were clustered around codons 146–161 and 195–208 and (b) none were G:C→T:A transversions, which are the most common inversions associated with tobacco-related lung cancer. This observation was widely considered to represent a "fingerprint" of radon-induced biological damage. Subsequently, however, Taylor *et al.* (*3*) examined a larger sample of 52 lung tumors from uranium miners. They found 29/52 (56%) contained p53 point mutations, and, of these, 16 had G→T transversions at codon 249. This observation was again suggestive of a radon-related biomarker. In contrast, however, Vahakangas *et al.* (*2*) had found zero G→T transversions, and also zero mutations at codon 249. At present, we have no mechanistic understanding of either of these differing observations.

Another suggested marker of high-LET radiation damage is the induction of sister chromatid exchanges (SCEs), which were suggested (*4*) to be inducible by high-LET but not low-LET radiation. Again this is not a mechanistically driven hypothesis, and in fact other investigators (*5*) have reported significant induction of SCEs by low-LET radiation.

In contrast to these reports, we discuss here a potential biomarker of prior exposure to densely ionizing radiation which is based on a preferential effect that is expected mechanistically and is observed experimentally. The pro-

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posed biomarker relates to yields of inter- vs intrachromosomal exchange-type aberrations. The primary mechanism for the production of exchange-type chromosomal aberrations is the pairwise interaction of two double-strand breaks (DSBs),[1] in which ends from different breaks meet and join in an illegitimate recombination (*6*). The likelihood that two DSBs become sufficiently close to undergo such an exchange depends on their relative proximity, which in turn depends on whether the DSBs were in the same or different chromosomes. Consequently, comparing yields of intrachromosomal aberrations (from two DSBs on the same chromosome) with interchromosomal aberrations (from two DSBs on different chromosomes) can be seen as a probe of radiation track structure; it is this observation which forms the basis for the proposed marker of the passage of a densely ionizing radiation track.

In this Commentary, experimental data regarding the magnitude of this effect are reviewed, and it is concluded that published data are in agreement with mechanistically based expectations. Finally, the potential implications of a detectable and stable biomarker for densely ionizing radiation are discussed.

THE NATURE OF THE FINGERPRINT

After DSBs are produced by ionizing radiation, restitution occurs in competition with illegitimate recombination between DSBs, the latter process producing exchange-type chromosomal aberrations; these can be *interchromosomal* if the DSBs are on different chromosomes, or *intrachromosomal, interarm* if the DSBs are on different arms of the same chromosome. Figure 1 shows schematically the production of these aberrations.

If DSBs were produced at random in a human cellular nucleus, and all the DSBs were equally likely to interact with one another, the ratio (F) of interchromosomal to intrachromosomal, interarm aberrations would be approximately 86, based on the patterns of chromosome arm lengths in humans (*7*), close to the value of 90 ($4n - 2$) that would hold if all 92 chromosome arms were of equal length. However, it has long been known (*8–10*) that pairs of DSBs on different chromosomes that are distant from each other within the nucleus are less likely to interact. If, as is observed experimentally (*11, 12*), individual chromosomes are localized in domains that are smaller than the cell nucleus, the increased interaction probability of nearby DSBs will result in a bias toward intrachromosomal aberrations, and thus a decrease in the F value. In fact, decreased F values, interpreted as evidence for chromosomal localization and a limited DSB interaction range, are observed after exposure to X and γ rays (*7–10*).

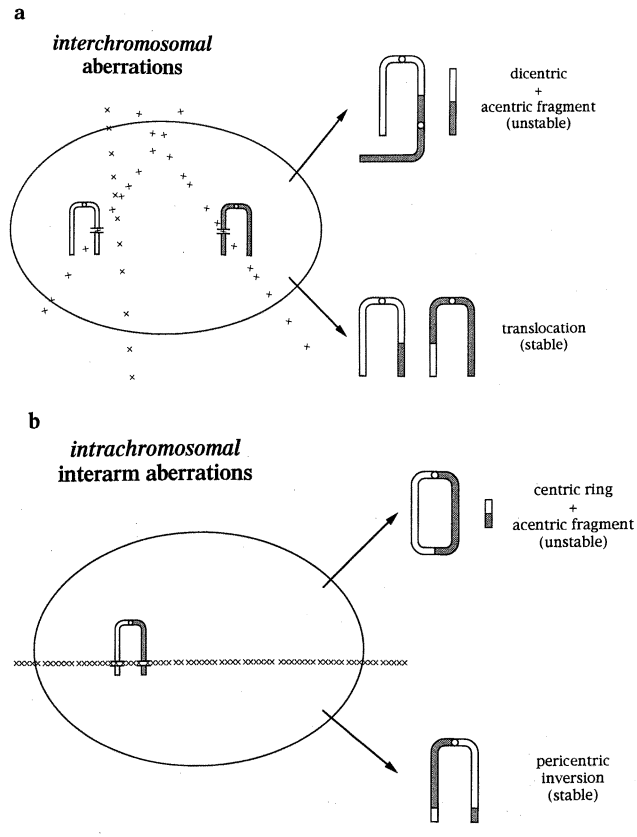

FIG. 1. Panel a: Schematic of interchromosomal aberrations resulting, in the case shown here, from two independent sparsely ionizing radiation tracks; each cross represents an ionization cluster of sufficient localization and multiplicity to potentially produce a DSB. This aberration could also be produced by two DSBs from a single radiation track. Panel b: Intrachromosomal, interarm aberrations resulting, in the case shown here, from a single densely ionizing radiation track.

Densely ionizing radiations, however, exhibit a unique property that should reduce the F value even further. These radiations produce spatially inhomogeneous energy depositions, and thus DSBs, that are much closer together than those produced by sparsely ionizing radiations such as X or γ rays or by chemical clastogens. Consequently, it would be expected that yields of intrachromosomal aberrations would be increased further relative to interchromosomal aberrations; the resulting smaller F value would then be a "fingerprint" of densely ionizing radiation.

The proposed chromosomal fingerprint is the ratio, F, of interchromosomal to intrachromosomal, interarm aberrations, either for stable aberrations (translocations to pericentric inversions) or for unstable aberrations (dicentrics to centric rings). Unstable aberrations are often lethal to cells

[1]We refer here to the elementary lesions that interact pairwise to produce exchange-type aberrations as DNA double-strand breaks. However, this identification is not essential for the arguments presented here.

in division, but stable aberrations are typically nonlethal, and can often be measured in irradiated cells and their progeny many years after exposure (e.g. *13–15*). It is thus the measured F value in stable aberrations which has the potential to be a practical biomarker of past exposure to densely ionizing radiation.

EXPERIMENTAL DATA

There are many data in the literature on yields of inter- and intrachromosomal aberrations. Often F values are not well determined, because of the smaller number of intrachromosomal aberrations produced. What adequate data are available support the notion that densely ionizing radiation produces characteristically low F values. We quote data for sparsely and densely ionizing radiations, for irradiation *in vivo* and *in vitro*, and for stable and unstable aberrations.

Although it is the F value in stable aberrations that has the potential to act as a practical biomarker of past exposure to high-LET radiation, it is to be expected, on the basis of the equalities between stable and unstable aberrations (e.g. *13*, *14*, *16*), that F values for unstable aberrations follow the same pattern as those for stable aberrations. Thus, in investigating the validity of the proposed biomarker, it is reasonable to use data for unstable aberrations to augment those for stable aberrations. Experimental evidence supporting this suggestion is discussed below.

In vivo, there are many measurements of F values after accidental or radiotherapeutic exposures to sparsely ionizing radiations such as X or γ rays. For inhabitants of the Chernobyl region, a ratio of 37 ± 19 was measured (*17*); for individuals exposed to γ rays in the Goiania radiation accident, the measured ratio was 20 ± 3 (*18*). For radiotherapy patients exposed to X or γ rays, where data are adequate for analysis, the mean F value is 18 ± 9 (*19–21*); these data were derived from both stable and unstable aberrations. For densely ionizing radiation, the most extensive *in vivo* data are for individuals injected with the contrast agent Thorotrast, which emits α particles: the largest study yields $F = 5.0 \pm 0.3$ (*22*). Measurements for workers exposed to plutonium (emitting α particles) yielded $F = 5.6 \pm 3.0$ for stable aberrations and $F = 4.5 \pm 2.0$ for unstable aberrations (*23*). Finally, measurements (*24*, *25*) for workers who were accidentally exposed to radiation consisting of ~88% (by dose equivalent) densely ionizing neutrons yielded $F = 5.7 \pm 3.5$ for stable aberrations and 5.0 ± 2.4 for unstable aberrations.

Two of the *in vivo* studies cited here (*23*, *25*) give F values measured in stable and unstable aberrations several years after exposure. In both cases, the measured F values from stable and unstable aberrations were very similar, suggesting that these ratios are stable over long time scales.

In vitro, several very large-scale studies have been conducted. The largest data set for low-LET radiation (*26*) yielded $F = 16.7 \pm 0.9$, in agreement with the value of 16 ± 5 obtained from a literature survey (*7*). By contrast, analysis of human lymphocytes exposed to densely ionizing neutrons (*27*) gave a significantly smaller value of $F = 5.6 \pm 0.5$.

There are other studies in the literature, but many suffer from inadequate statistics or incomplete definitions of the different exchange-type aberrations. As far as we know, no data sets with adequate statistics and adequate definitions of the measured aberrations are inconsistent with the pattern discussed above, indicating significantly smaller F values for densely ionizing radiation.

Although chemical carcinogens often produce damage at specific sites within the genome, such damage is unlikely to be located preferentially on pairs of sites on opposite arms of one chromosome (*28*). Chemical carcinogens therefore would not be expected to produce anomalously low F values, although few adequate data sets are available. An *in vitro* value of 14.6 ± 0.8 was obtained for aberrations induced by restriction enzymes which, on the scale of interest here, produce DSBs randomly within chromatin (*29*). A value of 30 ± 5 for bleomycin-induced damage *in vitro* has been reported (*30*), though this value is not unexpected in that bleomycin is a mimetic of sparsely ionizing radiation.

In summary, F values *in vitro* are consistent with those measured after irradiation *in vivo* and both show significantly smaller values for densely ionizing radiations than for X or γ rays. These data suggest that an F value of around 6 is characteristic of densely ionizing radiation, in contrast to values of ~15 or above for X or γ rays and for other clastogens.

MECHANISTIC BACKGROUND

In this section, we argue that the observed F values summarized in the previous section (~15 for sparsely ionizing radiation, and ~6 for densely ionizing radiation) are quantitatively consistent with what we know about chromosomal localization and about DSB interaction probabilities. Should this be the case, it would provide a strong mechanistic underpinning for the existence of the densely ionizing chromosomal "fingerprint" discussed here.

Any model for calculating F values requires information in three areas: (1) radiation track structure, to describe the initial spatial locations of ionization clusters which could produce DSBs; (2) the probability that two DSBs initially formed a given distance apart will ultimately produce an exchange aberration; and (3) chromosome geometry.

We use a simple two-parameter model designed to analyze the measured F values discussed above. In this model, detailed in the Appendix, the yield, Y, of exchanges in nuclei of diameter d is quantified in terms of these three types of information:

$$Y \propto \int_0^d \frac{t(x)g(x)s(x)}{x^2} dx, \quad (1)$$

where $t(x)$ describes the radiation track structure, $g(x)$ is the DSB interaction probability, and $s(x)$ describes the chromosome geometry. The proportionality constants in this and subsequent equations will be irrelevant for our purposes, since we are interested in estimating yield ratios. We briefly discuss the three terms in Eq. (1):

The first term in Eq. (1), describing the radiation track structure, is termed (*31*) the proximity function, $t(x)$. It can be interpreted (see Appendix) by considering a cluster of ionizations of sufficient localization and multiplicity to produce a DSB in a chromosome. Then $t(x)dx$ is proportional to the probability of another such cluster at a separation between x and $x + dx$. In general, $t(x)$ is estimated by Monte Carlo track-structure simulation (*32*, *33*). However, at the large separations of interest here (>50 nm), the "LET approximation," in which energy loss occurs in straight lines with no radial extension, is valid (*31*):

$$t(x) \propto 2L + 4\pi x^2 \rho D, \quad (2)$$

where L is the stopping power (dE/dx) and D is the dose. At low doses of densely ionizing radiation, the first term, referring to pairs of energy-deposition clusters in a single radiation track, will dominate, while for sparsely ionizing radiation, the second term, referring to pairs in independent tracks, will dominate. Consequently, in the limit of both very low and very high LET, the F value [which would have $t(x)$ in both the numerator and the denominator] will be independent of dose.

The second quantity in Eq. (1) describes the probability, $g(x)$, that two DSBs induced with separation x will eventually undergo an exchange. A step-function form has often been employed (*31*) and has been shown (*34*) to constitute a reasonable approximation:

$$g(x) = constant, x \le d_i; \quad g(x) = 0, x > d_i, \quad (3)$$

where d_i defines an effective range for DSB interactions and is our first relevant adjustable parameter. The normalization constant in Eq. (3) is again irrelevant for our purposes. Equation (3) is sometimes described as the "site" approximation (*35*). Earlier work has suggested that d_i is on the order of 1 μm (*34*).

The third function in Eq. (1), $s(x)$, describes the geometry of the chromatin in a cell nucleus. As detailed in the Appendix, $s(x)$ is proportional to the probability that any two points in the chromatin are separated by a distance x. Here we use a simple model for chromosomal geometry whose main features are the following: (1) all 46 chromosomes are considered identical [since corrections for actual arm length patterns are small (*7*)]; (2) each chromosome consists of a cloud of points which randomly occupy a sphere of radius d_c, where d_c is our second (and last) adjustable parameter; and (3) different chromosomes intertwine and overlap freely. As discussed in the Appendix, given these assumptions, we can write $s(x)$ as follows:

$$s(x) = 46 \times {}^1/_2 \times s_1(x) + 46 \times 45\, s_2(x), \quad (4)$$

where $s_1(x)$ and $s_2(x)$ are derived in the Appendix (Eq. A9). The term $s_1(x)/2$ refers to pairs of points on opposite arms of one chromosome, while $s_2(x)$ refers to points on two different chromosomes. We ignore a term for points on the same arm of one chromosome, because we do not consider the corresponding aberrations in the proposed assay. From Eqs. (1–4), F values can be calculated as a function of d_c/d and d_i/d.

At relevant doses of sparsely ionizing radiation, aberrations are primarily from interactions of DSBs produced by independent tracks. Thus the proximity function, $t(x)$, is dominated by the second term in Eq. (2). Combining Eqs. (1–4) and integrating gives the F value at low LET:

$$F(low\ LET) = 90\,(8u^3 - 9u^4 + 2u^6)/g(w), \quad (5)$$

$$g(w) = (8w^3 - 9w^4 + 2w^6), w \le 1; \quad g(w) = 1, w > 1, \quad (6)$$

where $u = d_i/d$ and $w = d_i/d_c$. While the model parameters are d_i and d_c, the model is scalable in the sense that the predicted results are invariant to changes in d_i and d_c, as long as the ratios, d_c/d and d_i/d, where d is the nuclear diameter, remain unchanged. We plot in Fig. 2a the predictions of Eq. (5) as a function of d_c/d and d_i/d.

In Fig. 2a, if $d_c = d$, i.e., if the domain of each chromosome is as large as the whole nucleus, the F value is the value, 90, obtained by assuming that all DSBs are produced randomly throughout the genome, and all DSB pairs are equally likely to interact. Likewise, if the interaction cutoff is as large as the whole nucleus, i.e. $d_i = d$, a value of 90 is obtained. However, if both the interaction distance d_i and the chromosome localization diameter d_c are less than the nuclear diameter d, proximity effects come into play because DSBs on the same chromosome have an increased probability to interact, and the F value decreases.

At moderate doses of densely ionizing radiation, most aberrations will be produced by interactions between DSBs from one track. In this case, the proximity function will be dominated by the first term in Eq. (2), and we obtain the F value at high LET:

$$F(high\ LET) = 90\,(u/w)^2\,(24u - 18u^2 + 3u^4)/h(w), \quad (7)$$

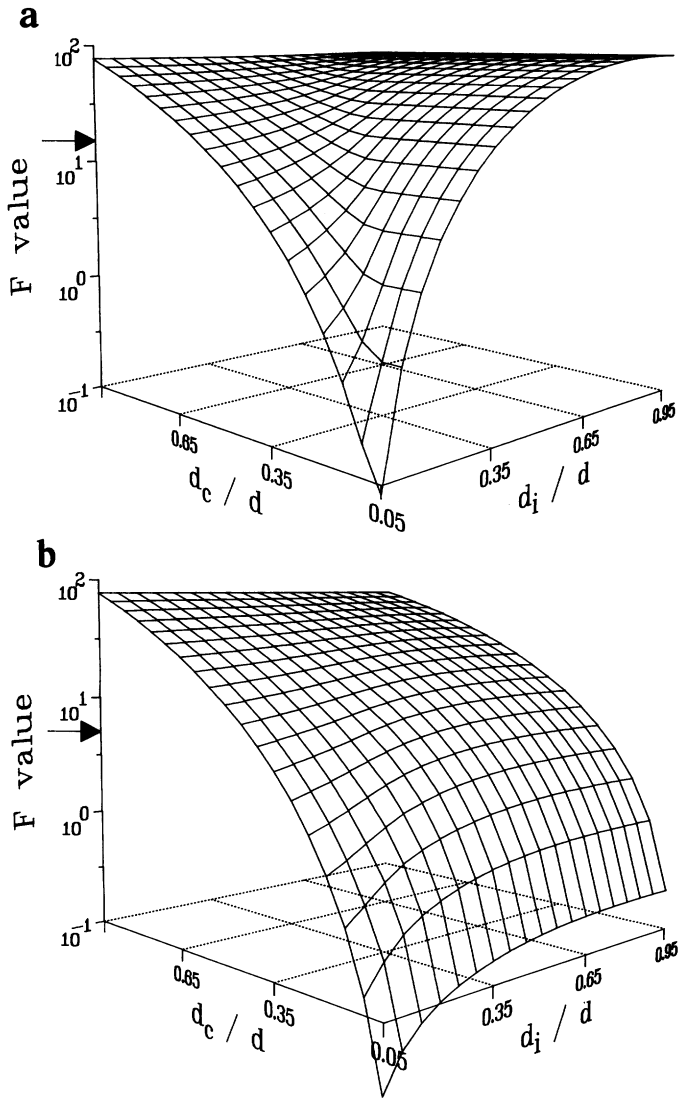

FIG. 2. Panel a: Predicted F values for sparsely ionizing (low-LET) radiation. Here, d is the nuclear diameter, d_i is the maximum DSB interaction distance, and d_c is the diameter of the chromosomal domains. The arrow gives an indication of the consensus sparsely ionizing experimental F value. Panel b: Predicted F values for densely ionizing (high-LET) radiation. The arrow gives an indication of the consensus experimental F value.

$$h(w) = 24w - 18w^2 + 3w^4, w \le 1; \quad h(w) = 9, w > 1, \quad (8)$$

where u and w are as in Eq. (5). The results for densely ionizing radiation are shown in Fig. 2b. As with sparsely ionizing radiation, if $d_c = d$, the limiting value of 90 is reached. In contrast, for densely ionizing radiation, assuming $d_i = d$ and $d_c < d$ results in an F value less than 90. The reason is that densely ionizing radiation produces DSBs close to each other, and whose interaction probabilities are roughly proportional to the square of their number; thus, even with no spatial limitations on interactions ($d_i = d$), a localized chromosome will sometimes contain multiple DSBs, with a resulting quadratic yield of intrachromosomal aberrations that more than compensates for cases where the chromosome is missed entirely.

Assuming, on the basis of the data quoted above, that $F(low\ LET) \sim 15$ and $F(high\ LET) \sim 6$, Eqs. (5, 7) can be solved numerically for d_c and d_i. The result is

$$d_c/d \approx 0.32 \pm 0.05, \quad d_i/d \approx 0.32 \pm 0.05, \quad (9)$$

where the uncertainty limits are based on corresponding estimated uncertainty limits for the F values at low and high LET. For human lymphocytes, where $d \sim 6$ μm, then

$$d_c \approx 2.0 \pm 0.3 \text{ μm}, \quad d_i \approx 2.0 \pm 0.3 \text{ μm}. \quad (10)$$

These estimates are consistent with those derived from different types of data (*12*, *34*).

DISCUSSION

The F values for densely ionizing radiation appear to be around 6, significantly lower than those observed for any other clastogens including X rays, γ rays or chemical carcinogens. The observation of anomalously low F values for densely ionizing radiations has both mechanistic and pragmatic implications.

Mechanistically, F values provide significant constraints on (a) the spatial localization of individual chromosomes within the nucleus and (b) the range of interaction of DSBs with each other to form exchange-type aberrations. The data suggest that the individual chromosomes are constrained to a mean diameter about one-third that of the nucleus, and that DSB interaction probabilities are small at distances larger than about 2 μm. These estimates are consistent with those derived from entirely different types of data (*12*, *34*). This consistency provides the mechanistic underpinning for the suggestion that F values could be a useful biomarker of exposure to densely ionizing radiation.

The fact that the proposed biomarker refers to ratios of yields results in it possessing considerable robustness against possible confounding effects. For example, such effects as cell turnover and clonogenic or interphase death, while affecting aberration yields, are unlikely to affect ratios of yields. Of course, the use of F values from stable aberrations, possibly measured long after exposure, presupposes that there is no differential loss over time of translocations relative to pericentric inversions. No data are available about the relative rates of loss of these two types of aberrations, though the structural similarities between them suggest that significant differential loss would be unlikely.

An example of the application of this biomarker relates to the question of the exposure of A-bomb survivors at

Hiroshima to densely ionizing neutrons. The 1986 reanalysis of the A-bomb dosimetry (*36*) had suggested that the neutron component at Hiroshima was negligible, a conclusion which affected estimates of the risk for low-LET radiation significantly, as well as effectively removing the only available source of estimates of risk for humans exposed to fission neutrons. However, recent measurements (*37*, *38*) have suggested that neutrons may still be significant and might even dominate the equivalent dose at relevant locations. For Hiroshima A-bomb survivors, an F value of 6.2 ± 0.7 has been measured (*39*). This may be interpreted as evidence that a significant proportion of the equivalent dose to which the survivors were exposed was from neutrons, in accordance with recent measurements, but in contrast to the dose reassessment calculations.

The most likely application of this potential "fingerprint" is in the field of radon. In the past decade, the relationship between low exposures to radon daughters and lung cancer risk has been seen to be of major importance. Detection in exposed populations of molecular markers and adducts associated with particular carcinogens and with particular cancers is now used to study chemical carcinogenesis risks (*40*). This approach, termed "molecular epidemiology," has become an accepted tool for assessing chemical risks. The same approach could be considered in relation to radon-induced lung cancer. Currently, epidemiological studies to determine radon risks at low exposures are limited by the large "background" lung cancer rates produced by carcinogens other than radon.

For epidemiological radon studies of this kind, nontumorous bronchial epithelial cells (basal cells and their progeny) would be obtained from bronchoscopy (using the bronchial brush technique, ref. *41*), the cells disaggregated, stimulated to mitosis and then assayed for F values for stable aberrations. In a recent report describing a technique for harvesting viable cells from the bronchial epithelium, Kelsen *et al.* (*41*) reported a 36 ± 4% viable cell yield and subsequent primary-culture plating efficiencies of 50 to 60%.

Although measurements of F values can be undertaken using G banding, the large numbers required to reduce the confidence interval on this ratio will generally require the use of fluorescence *in situ* hybridization (FISH) techniques. Translocations can be measured using standard techniques described by Lucas *et al.* (*14*), involving a cocktail of composite DNA probes specific to several large chromosomes. For example, if chromosomes 1, 2 and 4 are labeled, this covers about 23% of the genome. Theoretical considerations, confirmed by experiment (*14*), indicate that this scheme will sample about 35% of all translocations. Pericentric inversions can be identified efficiently using a pancentromeric probe in one color, and another color labeling two loci of one arm of several large chromosomes, these two loci respectively being located close to the telomere and close to the centromere. For example, if the centromeric probe was red and the other probes blue, the sequence blue *blue-red* would be characteristic of a normal chromosome, and blue *red-blue* characteristic of a pericentric inversion. Appropriate probes can be developed using the techniques described by Meltzer *et al.* (*42*, *43*). Again, if chromosomes 1, 2 and 4 were labeled as above, about 33% of pericentric inversions in the genome would be detectable.

To estimate how many cells would need to be examined, suppose we examine N cells, and measure X interchromosomal aberrations and Y intrachromosomal, interarm aberrations. Assuming the detection efficiency is the same for both X and Y, the estimated F ratio is simply X/Y. It can be shown that the variance of this estimate is approximately given by

$$(1/NY^3)\,[XY\,(N - X) + X^2\,(N - Y)]. \qquad (11)$$

As an example, suppose we are interested in subjects who actually had a cumulative exposure to radon progeny of ~50 WLM (working level months: for comparison, the average lifetime domestic cumulative exposure in the U.S. is ~15 WLM). Based on an estimated bronchio-epithelial dose/WLM (*44*), and a measured yield of dicentrics per unit α-particle dose (*45*), we might expect a yield of ~0.24 translocations per cell and, assuming an F value of 6, 0.04 pericentric inversions per cell. Given a FISH detection efficiency of 33% (see above), examination of, say, 3000 cells would yield ~240 translocations and ~40 pericentric inversions, and an estimated F value of 6 ± 1; this estimate would easily distinguish it from F values of ≥15. Of course, smaller exposures would require measurements of larger numbers of cells and vice versa.

In practice, the individuals under study might well have been exposed to a mixture of clastogens, such as α particles + tobacco (e.g. uranium miners) or neutrons + X rays (e.g. survivors at Hiroshima). In such cases an intermediate F value might be anticipated, and experimental calibrations of F value vs proportion of damage induced by high-LET radiation would be important.

In conclusion, we have suggested the existence of a potentially useful biomarker for prior exposure to high-LET radiations. The supporting theoretical and experimental data are quite convincing, though further experiments with human cells other than peripheral blood lymphocytes would be desirable. For individuals or cohorts exposed to radon, measurement of the F value could provide a local history of α-particle exposure in the lung and, in individuals with lung cancer, in the vicinity of the tumor. As with all epidemiological studies, such results could not demonstrate a causal relationship between radon exposure and lung cancer. They do, however, have the potential to demonstrate and quantify much stronger associations than are currently

possible between low-dose exposure to radon daughters and induction of lung cancer.

APPENDIX

Here, we discuss the track-structure proximity function, $t(x)$, the DSB interaction function $g(x)$ and the chromosomal proximity function $s(x)$. Finally, we derive Eq. (1), which combines these functions to predict aberration yields, and thus F values.

Radiation Track Structure

Let $\tau(\mathbf{r})$ be the density of ionization clusters of sufficient multiplicity and localization to create a DSB if a chromosome is hit (*33*). Formally, we take τ to be a random function. In that we are interested in interactions between spatially separated DSBs, the quantity needed in our calculation is the cluster-density autocorrelation function,

$$\Gamma(\mathbf{r},\mathbf{r}') = \langle \tau(\mathbf{r})\tau(\mathbf{r}') \rangle, \tag{A1}$$

where $\mathbf{r}$, $\mathbf{r}'$ are points within the nucleus and $\langle\ \rangle$ denotes an average. Assuming that the radiation is, on average, spatially homogeneous throughout the region of interest implies

$$\Gamma(\mathbf{r},\mathbf{r}') = \Gamma(\mathbf{r} - \mathbf{r}'). \tag{A2}$$

DSB Interaction Probabilities

Let $g(x)$ denote the probability that two DSBs initially formed a distance x apart will ultimately interact in a chromosome exchange event. It has been shown (*31*, *34*) that an appropriate approximation to $g(x)$ may be the simple cutoff form given in Eq. (3), where the cutoff interaction radius, d_i, is an adjustable parameter (of order of magnitude 1 μm).

We also assume that $g(x)$ is independent of whether the DSBs are on different chromosomes or on different arms of the same chromosome; i.e., any bias toward higher interaction probabilities between DSBs on one chromosome is assumed to be due solely to their initial proximity, with no additional bias caused by the restricted motion of DSBs.

Chromosomal Geometry

Let $\sigma(\mathbf{r})$ be the density of chromatin within a representative cell nucleus centered at the origin. Then

$$\sigma = \sum_{j=1}^{46} \sigma_j(\mathbf{r}), \tag{A3}$$

where σ_j refers to chromosome number j and σ is zero outside the cell nucleus. For convenience we normalize σ using the chromosome number:

$$\sigma(\mathbf{r}) = 0 \text{ if } |\mathbf{r}| > d; \quad \int d^3\mathrm{r}\ \ \sigma(\mathbf{r}) = 46. \tag{A4}$$

Here, and in subsequent equations, integrals with unspecified limits go over all of three-dimensional space and have cutoffs supplied automatically by integrands such as σ.

Assuming that the chromatin distribution in a cell nucleus is, on average, spatially isotropic, we can define a chromosomal proximity function, $s(x)$, which depends only on scalar distance, as follows:

$$s(x) = 4\pi x^2 \quad \int d^3\mathbf{r}'\ \langle \sigma(\mathbf{r}' + \mathbf{x})\sigma(\mathbf{r}') \rangle. \tag{A5}$$

Here the factor $4\pi x^2$ gives a conventional normalization which, from Eq. (A4), is

$$\int_0^d s(x)dx = (46)^2, \quad s = 0 \text{ if } x > d. \tag{A6}$$

To compute $s(x)$, and to distinguish between intra- and interchromosomal exchanges, we need a geometric model for chromatin geometry. We consider only interphase chromosomes on length scales of 50 nm or more, on time scales of seconds or more and with DNA locations separated by 10^4 base pairs or more. We take all the chromosomes to have the same average geometry, so that for any i, $j = 1, \ldots, 46$ with $i \neq j$:

$$\langle \sigma_i(\mathbf{r})\sigma_i(\mathbf{r}) \rangle = \langle \sigma_1(\mathbf{r})\sigma_1(\mathbf{r}) \rangle, \ \langle \sigma_i(\mathbf{r})\sigma_j(\mathbf{r}) \rangle = \langle \sigma_1(\mathbf{r})\sigma_2(\mathbf{r}) \rangle. \tag{A7}$$

We also take each centromere to be in the center of its chromosome. The corrections needed for differing chromosomal arm lengths lead to only minor corrections (*7*).

We assume each chromosome is dispersed randomly within a chromosome localization sphere of radius $d_c \leq d$, and different chromosomes are independent of each other. The assumption of independence means different chromosomes can overlap freely. We shall assume that point pairs on different chromosomes are random pairs within the cell nucleus. For $d_c << d$ this amounts to an additional assumption that each chromosome is centered at random within the nucleus. Inserting Eqs. (A3) and (A7) into Eq. (A5), the chromosome proximity function can be written:

$$\begin{aligned} s(x) &= 4\pi x^2 \int d^3r' \langle \sigma(\mathbf{r}' + \mathbf{x})\sigma(\mathbf{r}') \rangle = \\ &\quad 4\pi x^2 \int d^3r' \left\langle \sum_{i=1}^{46} \sigma_i(\mathbf{r}' + \mathbf{x}) \sum_{j=1}^{46} \sigma_j(\mathbf{r}') \right\rangle \\ &= 4\pi x^2 \int d^3r' \Big[46 \langle \sigma_i(\mathbf{r}' + \mathbf{x})\sigma_1(\mathbf{r}') \rangle \\ &\quad + 46 \times 45 \langle \sigma_1(\mathbf{r}' + \mathbf{x})\sigma_2(\mathbf{r}') \rangle \Big] \\ &= 46 \times {}^1\!/_2 \times s_1(x) + 46 \times {}^1\!/_2\, s_1(x) + 46 \times 45\, s_2(x), \end{aligned} \tag{A8}$$

where

$$s_1(x) = p\left(\frac{x}{d_c}\right)\Big/ d_c, \quad s_2(x) = p\left(\frac{x}{d}\right)\Big/ d. \tag{A9}$$

Here $s_1(x)$, which refers to pairs of points within one chromosome, comprises equal contributions from interarm and intra-arm terms. The function $s_2(x)$ refers to points on two different chromosomes. $p(y)dy$ gives the probability that any two points chosen at random in a unit sphere are separated by a distance between y and $y + dy$ (*31*):

$$p(y) = 24y^2 - 36y^3 + 12y^5, \; y \le 1; \; p(y) = 0, \; y > 1. \tag{A10}$$

Equation (4), used in the numerical estimates of F values, is derived from Eq. (A8).

Aberration Yields

The number of exchanges that have occurred in a particular cell is proportional to

$$\int d^3r \int d^3r' \sigma(\mathbf{r})\tau(\mathbf{r})\sigma(\mathbf{r}')\tau(\mathbf{r}')g(|\mathbf{r} - \mathbf{r}'|). \tag{A11}$$

By averaging over cells, and assuming that σ and τ are uncorrelated, the average yield, Y, of exchanges, can be calculated:

$$Y \propto \int d^3x \tau(\mathbf{x}) \; g(x) \int d^3r' \langle \sigma(\mathbf{x} - \mathbf{r}')\sigma(\mathbf{r}') \rangle. \tag{A12}$$

Using Eqs. (A2, A5, A11) now gives

$$Y \propto \int_0^d \frac{t(x)g(x)s(x)}{x^2} dx, \text{ where } t(x) \propto \frac{x^2}{D} \int d\Omega \tau(\mathbf{x}), \tag{A13}$$

and $d\Omega$ is the element of solid angle. Equation (A13), first derived using a different argument in ref. (*46*), is our Eq. (1). The present derivation facilitates decomposition of the yield, Y, of exchanges into contributions which come from DSB pairs or the same or different chromosomes.

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