Biological indicators for the identification of ionizing radiation exposure in humans

Sally A Amundson[†], Michael Bittner, Paul Meltzer, Jeffrey Trent and Albert J Fornace, Jr.

While the effects of acute high-dose irradiation are well-documented, less is known about the effects of low level chronic radiation exposure. Physical dosimetry cannot always be relied upon, so dose estimates and determination of past radiation exposure must often be based upon biological indicators. Some of the established methods used in the assessment of nuclear accidents are reviewed here, including cytogenetic analyses, mutation-based assays and electron spin resonance. As interest in research on low-level radiation exposures expands, there is an increasing need for new biomarkers that can identify exposed individuals in human populations. Developments in high-throughput gene expression profiling may enable future development of a rapid and noninvasive testing method for application to potentially exposed populations.

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[†]*Author for correspondance:*
NIH, National Cancer Institute
37 Convent Dr., Bldg. 37,
Bethesda, MD 20892, USA
Tel.: +1 301 594 7772
Fax: +1 301 480 2514
amundson@mail.nih.gov

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Since in cases of accidental or suspected radiation exposure physical dosimetry is often incomplete or absent, the need for biological indicators of exposure has long been recognized. In the case of industrial accidents, information from biodosimetry can assist in determining dose distribution as well as overall exposure, important factors for triage of affected individuals. The inclusion of biomarker information in epidemiological studies may also contribute to our understanding of the long-term risks of both acute and chronic exposures.

While the primary biological indicators of radiation exposure have been developed and applied to populations exposed to acute and often relatively large doses, such as the atom bomb survivors, the Chernobyl 'liquidators' and even radiotherapy patients, there is increasing interest in developing biomarkers for lower doses and more chronic exposures. Such exposures are generally more common, both among radiation workers and the population at large. Several cohorts of chronically irradiated people are under study, such as residents of the Techa River area in Russia and to a lesser extent the populations surrounding the Hanford facility in the US and the Sellafield nuclear plant in England. Attempts are also being made to define bioassays specific for more densely ionizing radiations, such as the α-particles produced by radon gas or the heavy ions in cosmic rays. Such exposures can be of concern everywhere from homes in high radon areas, to airline personnel, to extended space-flight, such as on the new international space station or the planned mission to Mars.

A number of characteristics determine the practicality and usefulness of a biological indicator of radiation exposure. The time required to perform the assay can be critical, especially in accident or potential military situations where dosimetry is needed as soon as possible and may need to be applied to large population. A short turnaround time is needed both to assign victims to appropriate treatment and to determine which workers have exceeded allowable dose limits and should be removed from high-exposure areas. Assays with a potential for automation are highly desirable in this context, while those requiring high levels of expertise are less useful. The time after exposure when an assay is informative requires consideration. Some assays are appropriate in a

narrow window of time from hours to days after an acute exposure, while a few biomarkers persist for years. For any marker, minimal variations in the range of normal background levels are also important, as in real world situations, individual preexposure measurements are unlikely to be available for comparison. The ideal biomarker would be specific for ionizing radiation exposure. Unfortunately, many of the available markers can be confounded by effects of age, smoking or other environmental toxins. It may, however, be possible to define a signature of the more densely ionizing high linear energy transfer (LET) radiations.

Finally, a clearly demonstrable dose-response relationship is necessary. While some *in vivo* relationships have been determined in animal models or radiotherapy patients, many biodosimetry techniques rely on calibrations to dose-response curves determined *in vitro*. When all these factors are considered, it is clear that there is no one perfect biodosimeter for all applications. Continuing technological improvements and combined approaches may provide valuable support for medical triage, epidemiology and mechanistic insight into the late effects of ionizing radiation exposure.

Current biodosimetry methods

The hematopoietic system contains some of the most radiation-sensitive and easily sampled cells in the human body. This has been exploited by many of the biodosimetry methods developed to date, including those based on somatic mutation, cytogenetics and gene expression (TABLE 1). One of the earliest and most direct methods of dose determination following radiation exposure involves charting daily counts of different cell types circulating in the peripheral blood. Total leukocyte counts decline rapidly in the first week following radiation exposures in excess of about 1 Gy and the extent and duration of the decline and subsequent recovery have been shown to correlate well with dose [1]. Total body irradiation (TBI) doses of 1 Gy and higher can also be well estimated from peripheral blood neutrophil counts. The dose estimates derived from these two methods agree closely with each other and were widely used and confirmed following the Chernobyl accident as well as other well documented accidents at research facilities in Russia [2].

Electron spin resonance of dental enamel

Electron spin resonance (ESR) is a physical measurement of absorbed dose that can be applied to biological material. Although samples of fingernails and clothing have been used in ESR determinations of dose shortly after exposure, dental enamel is the most widely used material. One advantage of the dental enamel technique is that the absorbed dose can be determined many years after exposure with no decrease in accuracy. The free electrons produced in the dental enamel by radiation exposure are measured by ESR and most commonly compared to a calibration curve generated in the laboratory by irradiation of dental materials with known doses of radiation [3]. ESR is now considered sensitive enough to detect low doses of about

Table 1. Comparison of some of the major methods of biodosimetry for radiation exposure.

Assay	Ease of assay	Baseline variability	Detection limit (Gy)	Factors confounding specificity	Post–exposure duration	Types of exposures	AP
ESR	Large equipment requirement	To 3-fold	0.1	Ingested β emitters	Indefinite (years)	Acute or chronic TBI	No
Blood counts	Simple, daily counts over several weeks	Low	0.5–1	Physical stress, injury other toxic exposures	Weeks–months	Acute TBI only	No
Somatic mutation							
gpa by flow cytometry	Rapid, simple	Moderate	1–2	Age, smoking	Indefinite (years)	Acute or chronic	Yes
hprt – T-cell cloning	Simple, 2+ weeks	Moderate	1–2	Other toxic exposures	Transient (<1 year)		No
Autoradiography	Rapid, cheap, simple				Transient (<1 year)		Yes
Chromosomal							
Unstable – dicentrics	Technical expertise	Moderate	0.5	Relatively rad. specific	Several years?	Acute	No
Micronuclei	Rapid, simple	Moderate	0.1–0.3	Exposure to clastogens	1 year half-life	Partial *vs.* TBI	Yes
PCC	Rapid, cheap, simple	Moderate	0.1–0.5	Exposure to clastogens	Days–weeks	Partial *vs.* TBI	Yes
Stable – FISH	Technical expertise	Moderate	0.1–0.25	Low F-ratio specific for high LET (?)	Years	High *vs.* low LET(?)	No
Gene expression	Technical expertise	Low	0.2	Specificity	At least 72 h	Acute or chronic	Yes
Profiles	Potential to simplify			Undetermined		TBI	

AP: Automation potential.

0.1 Gy and for higher doses retrospective ESR measurements have agreed well with dose estimates made by blood counts and chromosome aberration methods [2].

Cytogenetic methods

Dicentric assay

Ionizing radiation is a strong clastogen, causing chromosome breakage and resulting in cytogenetic aberrations in exposed cells. A number of cytogenetic methods have therefore been developed as measures of radiation exposure and when applied in accident situations – such as that at Chernobyl – have generally produced dose estimates agreeing well with physical dosimetry or ESR [1,2].

The dicentric assay in particular has been one of the most widely applied techniques for radiation biodosimetry and among all available assays is still considered the most specific for ionizing radiation damage [4]. In this assay, peripheral lymphocytes are separated from the blood and stimulated to divide in culture. Then, using standard staining or hybridization with centromere-specific FISH probes, metaphase chromosome spreads are scored for the observed frequency of chromosomes that have two centromeres, the so-called dicentric chromosomes. Radiation dose is then estimated from comparison to a standardized curve obtained from human lymphocytes irradiated *in vitro*. While significant increases in dicentric frequencies have been documented following *in vitro* doses above 0.02 Gy [5], practical detection limits for *in vivo* exposures appear to be closer to 0.5 Gy [2]. Studies of radiotherapy patients have also suggested that *in vivo* yields of dicentrics may indeed be considerably lower than those predicted by *in vitro* calibration curves [6].

As centromeres are the site of chromosome attachment to the mitotic spindle, chromosomes with two centromeres will be unable to segregate properly into daughter cells at mitosis. This means that dicentrics are unstable aberrations and the lymphocytes bearing these informative chromosomes in the peripheral blood decline over time with kinetics that are not yet fully understood. A study following 15 people exposed in a radiological accident in Goiania, Brazil, suggests that the rate of decline of the dicentric frequency may depend on the initial dose, with higher doses declining most rapidly and lower doses producing more stable dicentric frequencies [7]. Despite uncertainties in interpretation of dicentric frequencies obtained at long times after radiation exposure, this assay remains one of the most practical shortly after exposure.

Micronucleus assay

Another cytogenetic assay used for biodosimetry is the scoring of micronucleus formation. This method has several advantages over the dicentric assay in that it requires less specialized expertise, is more rapid and hence can more readily be applied to monitoring large populations. In this assay, lymphocytes are mitogenically stimulated in culture, then cytokinesis is blocked by cytochalasin-B. This results in mitosis and nuclear division without cell division. Extranuclear chromatin particles (micronuclei) are then counted in binucleated cells.

Unlike the fairly radiation-specific dicentric assay, micronuclei can be induced by a range of other clastogens, including cigarette smoking and exposure to clastogenic chemicals. While most spontaneously arising micronuclei contain centromeres, it has been found that the majority of radiation-induced micronuclei represent acentric fragments formed by chromosome breakage. The use of centromeric FISH probes to allow rapid scoring of only acentric micronuclei has enhanced specificity and lowered the dose detection limit of this assay to between 0.1–0.2 Gy [8,9].

Premature chromosome condensation

Radiation damage can also be detected in interphase cells by the premature chromosome condensation (PCC) assay. This method classically uses fusion of the test cells with mitotic cells, which transmit a signal for dissolution of the nuclear membrane and condensation of the interphase chromosomes as if in preparation for mitosis. Fragments in excess of the expected 46 chromosomes occur when breaks were present within the interphase chromosomes. More recent refinements using chemical induction of PCC and FISH probes for chromosome painting have increased the speed and accuracy of the assay [10–12]. Excess PCC fragments have been shown to increase with increasing radiation exposure [13].

Heterogeneous exposures resulting in overdispersion of cytogenetic markers

The distribution of cytogenetic damage among cells can also be used to estimate partial body exposures, an important aspect of biological dose reconstruction. Following TBI the observed aberrations would be expected to follow a Poisson distribution among the scored cells, while an overdispersion of aberrations would indicate that only a subset of the cells were in the radiation field. Two mathematical approaches for this type of analysis were initially developed with the dicentric assay and continue to provide improved dosimetry in cases of heterogeneous exposure [14–16]. The extent of overdispersion of PCC fragments has also been shown to correlate with the irradiated fraction in human lymphocyte cultures *in vitro*, as well as for rhesus monkeys irradiated *in vivo* [17,18].

Determining LET of exposure from relative aberration frequencies

It may also be possible to distinguish between high and low LET radiation exposures by comparing different types of chromosome aberrations. High LET radiation, such as neutrons or α-particles are more densely ionizing than X- or γ-rays. When such radiations interact with a cell, they produce multiple lesions in close proximity to each other, as ionizations occur along the particle track. The creation of more sites of damage closer together increases the probability that multiple double-strand breaks (dsb) will result within a single chromosome, whereas the dsb produced by γ-rays and most chemical clastogenic agents are more or less randomly distributed (FIGURE 1). Thus, high LET irradiation has been predicted to result in a greater number of aberrations involving a single chromosome, such as pericentric inversions and
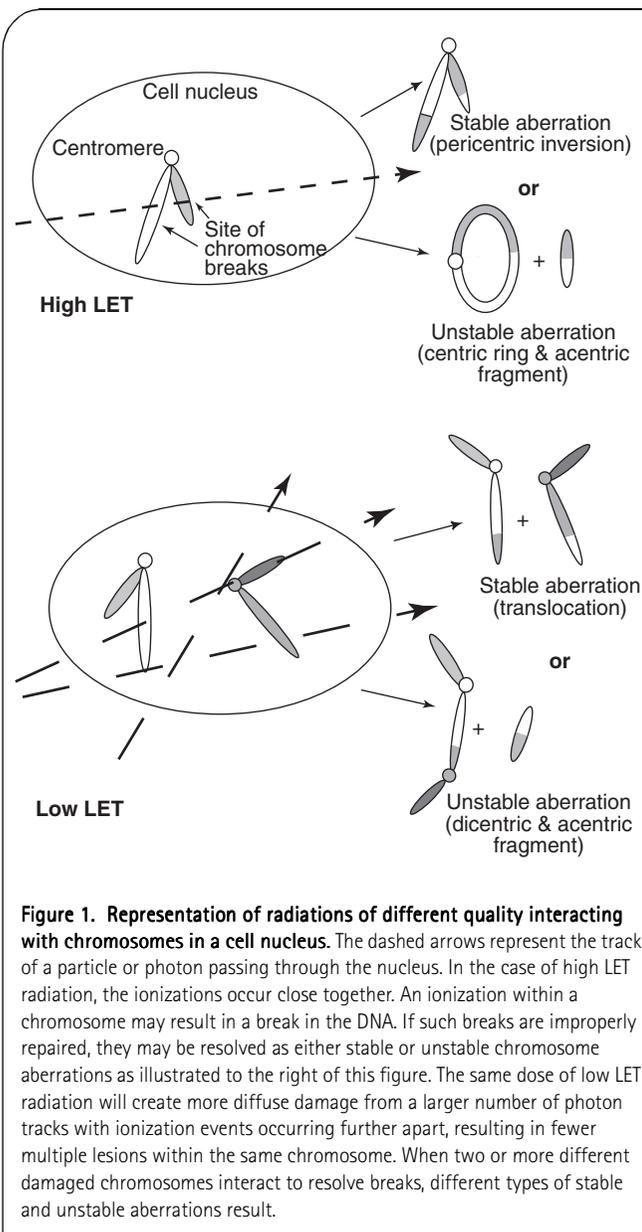
Figure 1. Representation of radiations of different quality interacting with chromosomes in a cell nucleus. The dashed arrows represent the track of a particle or photon passing through the nucleus. In the case of high LET radiation, the ionizations occur close together. An ionization within a chromosome may result in a break in the DNA. If such breaks are improperly repaired, they may be resolved as either stable or unstable chromosome aberrations as illustrated to the right of this figure. The same dose of low LET radiation will create more diffuse damage from a larger number of photon tracks with ionization events occurring further apart, resulting in fewer multiple lesions within the same chromosome. When two or more different damaged chromosomes interact to resolve breaks, different types of stable and unstable aberrations result.

ring chromosomes, while γ-rays should favor interchromosomal events, such as translocations and dicentrics [19].

Consistent with this prediction, the ratio of interchromosomal (between different chromosomes) to intrachromosomal (exchanges within a single chromosome) aberrations, termed the F value, has been found to be significantly reduced in two cell lines irradiated with α-particles as well as in human peripheral blood lymphocytes irradiated by neutrons [20,21]. Analysis of F values for the atomic bomb survivors has also indicated biological evidence supporting a major neutron component in the Hiroshima dose [22]. The usefulness of low F values as a specific marker for high LET radiation exposure remains controversial, however, not all studies have found significant differences in this ratio for high and low LET radiations [23]. Comparisons of yields of intra-arm interchanges (paracentric inversions or acentric rings) to interarm intrachanges (pericen-

tric inversions or centric rings) may eventually provide a superior marker for high LET exposures [24]. Finally, as FISH and chromosome painting techniques have allowed scoring of more complex chromosome aberrations, especially in samples exposed to high LET. Recent experimental evidence suggests that insertions may also provide a specific marker for past exposure to high LET radiation [25].

Somatic mutation

Repair of DNA damage caused by radiation in hematopoietic stem cells can result in somatic mutations in marker loci that can be monitored as biological indicators of dose. Mutations in several different loci have been exploited for detection of radiation exposure, including expression of hemoglobin (*Hb*) and glycophorin A (*gpa*) variants in erythrocytes and mutations at the HLA or hypoxanthine-guanine phosphoribosyltransferase (*hprt*) loci in T-lymphocytes. A drawback common to these somatic mutation end-points is their relative lack of specificity for radiation exposure as other environmental exposures or physiological states can also increase the observed mutant frequencies *in vivo*.

GPA variants

The *gpa* assay in erythrocytes has been widely used for biodosimetry. Although mature human red blood cells do not have nuclei, mutations occurring in progenitor cells in the bone marrow can be monitored by the measurement of phenotypic variants among circulating erythrocytes. Two alleles of *gpa* encode the cell surface proteins that determine the M and N blood group antigens. In M/N constitutional heterozygotes, variant red blood cells expressing only one allele can be quantified rapidly by flow cytometric techniques [26]. The obvious drawback to this method is that it can only be applied to heterozygotes, approximately 50% of the population. The mutant progenitor cells appear to persist for years in the bone marrow, however a significant dose-response relationship has been found for *gpa* variants even years after high-dose acute exposures in atomic bomb survivors and victims of the Chernobyl accident and in lower dose-rate exposures in patients treated with Iodine-131 [27–29]. No correlation with dose was found among workers at the Sellafield nuclear plant, perhaps due to a relatively high apparent threshold dose (about 1–2 Gy) for detection of significant *gpa* variants [30].

Hprt and mutant spectra

Functional inactivation of the *hprt* gene has probably been the most extensively used of the T-cell biodosimetry assays. In contrast to the erythrocyte assays, the T-cell assays monitor mutations occurring directly in the circulating peripheral cells. The *hprt* gene codes for a salvage pathway enzyme that allows the phosphoribosylation of hypoxanthine and guanine as precursors for DNA synthesis. It can also utilize purine analogs, such as 6-thioguanine, which can then incorporate into DNA and kill the cells. Mutant cells that have lost this enzyme can grow in concentrations of 6-thioguanine that are toxic to wild type

cells, thus allowing mutant selection. Furthermore, the location of the *hprt* gene on the human X-chromosome means it is functionally hemizygous, allowing detection of the loss of a single allele. An assay using T-cell cloning and *hprt* mutant fraction determination has been used to show a strong relationship between dose and induced mutations in atomic bomb survivors and patients receiving high doses of radiation therapy [31,32]. An increase in *hprt* mutant fractions may also be detectable following lower dose exposures but these results seem more variable depending on the time of sampling [33].

An additional benefit of the T-cell cloning assay is the potential for molecular analysis of mutants arising *in vivo* and the subsequent determination of induced and spontaneous mutational spectra to enhance the specificity of the assay. While spontaneous *hprt* mutants show a wide variety of point mutations and deletions [34,35], an increase in gross structural changes has been seen in individuals undergoing radiation therapy [36]. The observed mutations also show an increase in size and frequency of deletions correlating with dose. *In vitro* exposure of human T-cells to radon resulted in an increase in small partial deletions with a low frequency of total gene deletions [36], suggesting a possible LET-dependent component in the *hprt* mutant spectrum. Although molecular mutant spectra have been derived largely from *in vitro* results, there does appear to be general agreement between these studies and the existing data on *in vivo* exposures [36].

One of the drawbacks of the cloning assays is the length of time required before results are obtained. A short-term autoradiography or immunofluorescence assay can also be used to more rapidly quantitate *in vivo hprt* mutations [37]. This assay is based on the fact that only cells that have lost expression of *hprt* will incorporate tritiated thymidine or bromodeoxyuridine in the presence of 6-thioguanine in short-term phytohemagglutinin-stimulated cultures. The labeled cells can then be quantified using either standard or automated image analysis. Results obtained from individuals suspected of radiation exposure in two different accidents suggest this assay may be useful as an indicator of exposure [38]. As with the *gpa* assay, a relatively high threshold of 1–2 Gy for detection limits the usefulness of the *hprt* assay in low-dose exposure situations.

Molecular profiling by gene expression

Recent technological advances may allow an additional exploitation of the molecular responses of cells to ionizing radiation. Exposure of cells to DNA-damaging agents elicits a highly complex molecular response, much of which is mediated through changes in gene expression. A transcriptional response to genotoxic stress, estimated to involve 1% or more of the genome, was initially identified in yeast and similar complex transcriptional responses were soon confirmed in mammalian cells [39–42]. The stress response pathways responding to different environmental and physiological stresses have many overlapping components, including growth factors, cytokines, oncogenes and genes involved in cell cycle, apoptosis, signaling pathways and DNA repair. The recent development of functional genomic approaches to simultaneously quantify expression of thousands of genes in a single experiment may allow the determination of expression signatures indicative of exposure to ionizing radiation or other environmental toxins. Although presently still in the speculative realm, this approach would be highly attractive as it would be amenable to rapid, even automated, noninvasive analysis and may additionally have the potential to discern competing effects from incidents involving different quality radiations or mixed chemical and physical exposure components.

A number of high-throughput gene expression measurement methods are currently available, including serial analysis of gene expression (SAGE), oligonucleotide arrays and cDNA arrays [43,44]. In our own laboratory, we have used cDNA microarrays constructed by printing PCR amplified cDNA sequences onto glass followed by hybridization to 2-color fluorescent labeled probes [101]. The measurements made by this technique agreed with those obtained by more conventional single probe quantitative hybridization analysis of genes induced by γ-irradiation in a human tumor cell line [45].

As gene expression profiles of radiation exposure are developed, it will remain crucial to validate the findings by independent means, such as single probe quantitative hybridization or real-time PCR. While the majority of published studies of gene induction by ionizing radiation have used large, sometimes supra-lethal doses to ensure easily measurable effects, extrapolation of the results of such studies to doses relevant to human exposures is not entirely satisfactory. Changes in mRNA levels have been documented at doses of less than 1 Gy and we have recently shown five genes with a linear relationship between induction and γ-ray dose in the range of 0.02–0.5 Gy *in vitro*, indicating that such molecular responses may provide potential indicators of exposure in physiologically relevant ranges [46].

As an initial step towards a gene expression assay with biomonitoring applicability, we have recently used cDNA microarray analysis to identify a set of genes with linear dose-dependent elevation in human peripheral blood lymphocytes (PBL) as long as 72 h after *ex vivo* irradiation with between 0.2 and 2 Gy γ-rays (FIGURE 2) [47]. Of the genes examined, there was only slight variation in expression levels in untreated PBL from different individuals, supporting the possibility of establishing ranges of expression correlating with normal and exposed populations. Elevated gene expression appears to be maintained for at least a day in several organs following TBI of mice with doses from 0.2–2 Gy and in the PBL of human patients undergoing TBI prior to bone marrow transplantation. Although these experiments are still in preliminary stages and require more extensive studies for validation, the possibility of using gene expression changes to monitor for radiation exposure is extremely enticing.

Expert opinion

There is general consensus in the field that there is as yet no perfect biological indicator of radiation exposure. An approach of using combined dosimetry from the most appropriate methods in a given situation has been advocated
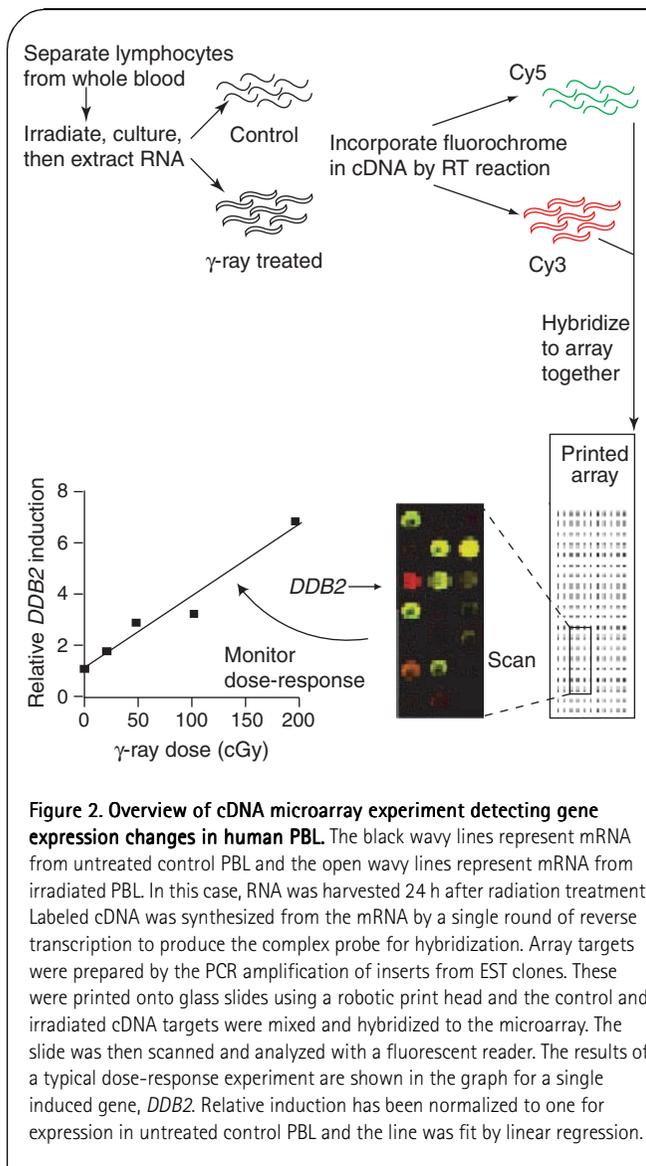
Figure 2. Overview of cDNA microarray experiment detecting gene expression changes in human PBL. The black wavy lines represent mRNA from untreated control PBL and the open wavy lines represent mRNA from irradiated PBL. In this case, RNA was harvested 24 h after radiation treatment. Labeled cDNA was synthesized from the mRNA by a single round of reverse transcription to produce the complex probe for hybridization. Array targets were prepared by the PCR amplification of inserts from EST clones. These were printed onto glass slides using a robotic print head and the control and irradiated cDNA targets were mixed and hybridized to the microarray. The slide was then scanned and analyzed with a fluorescent reader. The results of a typical dose-response experiment are shown in the graph for a single induced gene, *DDB2*. Relative induction has been normalized to one for expression in untreated control PBL and the line was fit by linear regression.

cytogenetic methods of dose determination are likely to become more precise and increasingly informative in the time immediately following exposure. It should be noted, however, that some of these more complex analyses may require a high degree of expertise and so may be more useful for research purposes than in the field.

Close monitoring of blood counts will also remain important in tracking the course of disease. It is only through continued comparisons of cytogenetic measurements with dose estimates derived from direct clinical observation and any available physical dosimetry that calibration of dose-response curves obtained from *in vitro* irradiations can be refined to more accurately reflect the *in vivo* response. Measurements from ESR or mutation in lymphocytes may also assist in refining the estimates of absorbed dose that can be made by more immediately applicable techniques.

It is important to continue these refinements of dose estimates made by biological indicators because these measurements are not only of use to prevent exceeding occupational dose limits, but also for assignment to acute medical care. Retrospective dosimetry can be important in epidemiological studies and ultimate prediction of long-term risks of radiation exposure. Without accurate dosimetry information, estimates of cancer and other risks cannot be made and exposure limits for radiation protection cannot be set effectively.

Accurate means to distinguish between high and low LET components of exposure are also sorely needed. The relative biological effectiveness (RBE) of the same amount of energy absorbed as different qualities of radiation is still a matter of much debate and the assignment of different RBE values can greatly alter the prediction of long-term risks for a specific exposure. Techniques comparing the yields of various specific complex types of chromosome aberrations are currently the best available hope in this regard. While these techniques require complex FISH probes and expert analysis, if they can be proven *in vivo* they will provide a powerful tool for biological monitoring.

There are as yet no truly satisfactory biological indicators for chronic low-dose and low-dose-rate radiation exposures. This is of concern as the majority of exposures – both occupational and among the population at large – are of this low-dose chronic type. Since epidemiological doses are less certain and even *in vitro* effects can be slight and difficult to study, the risks of these exposures are not clearly understood. Recent scientific initiatives have stimulated basic research in this area and as mechanisms of chronic radiation action and biological response become better understood, it is likely that biomarkers will be refined and developed to assist in the monitoring of potentially exposed populations. Aside from the slightly increased risks of cancer which may be associated with low-dose chronic radiation exposure, the psychological impact on populations fearing potential exposures also needs to be considered. A screening method able to detect significant exposure or the lack of it is likely to have a positive effect on public health in this regard.

and is likely to be the best current option [48]. Overall, biological indicators of exposure are currently most informative in situations of acute uniform exposure to the entire body in relatively high doses. In such situations, measurements of chromosome aberrations can be made within the first few days after exposure and can assist in determining the best course of treatment for radiation casualties. Among these methods, scoring of dicentric chromosomes remains the most broadly applied, i.e., while micronuclei and excess PCC fragments may also be manageable in large populations with suspected exposure [49]. Improvements in automation of these techniques will add to their attractiveness for triage and other field applications.

An additional benefit of these analyses is their usefulness in reconstructing partial exposures, a critical concern in many accident situations where physical dosimetry is generally inadequate. As the use of FISH probes in conjunction with the classical chromosome aberration techniques is further refined,

One of the major areas offering hope for potential new biological indicators of radiation exposure is expression profiling. Many changes in gene expression have been documented following radiation exposure both *in vivo* and *in vitro*. With the advent of high-throughput screening and information-intensive analysis, expression profiles correlating with specific types of exposure may be defined. Although it remains to be seen what the limits of *in vivo* detection would be with such methods, they could provide a very powerful tool for biomonitoring.

Many approaches are developing to sift large data sets for informative patterns. Clustering programs developed for the analysis of expression array data look for correlations between the most similar patterns, allowing molecular definition and classifying expression profiles [50,102]. Similar mathematical analysis of cancer cell expression profiles has identified previously unclassified subtypes of lymphoma and melanoma [51,52]. Several organizations including Phase I and the Health and Environmental Sciences Institute of ILSI (International Life Sciences Institutes) are actively involved in defining the gene response signatures resulting from chemical exposures as informative for general toxicology and environmental monitoring. The application of similar techniques to radiation exposure may eventually enable exposure profiling based on expression signatures unique to acute or chronic, high or low LET radiation or absorbed dose. Once defined, such profiles could be assessed accurately using real-time PCR or other rapid and cost-effective methods amenable to high-throughput and automated analysis.

Five-year view

The next 5 years are likely to see rapid advances in the arena of expression profiling. Mathematical models and computer algorithms are being developed and refined for exploitation of expression databases tailored to specific questions and the question of *in vivo* response to ionizing radiation is sure to be included. Developments in the nascent field of proteomics – the quantitation of protein expression profiles – may also impact on biodosimetry. As our ability to monitor protein changes in a cell catches up with our current ability for transcription profiling, proteomics may ultimately prove more informative. While changes in transcription are common mediators of cellular response to radiation, protein changes may be more relevant, longer lasting or more indicative of long-term cellular damage correlating with biological impact and risk.

As molecular biological tools are now available to address mechanistic issues of cellular response to low doses and chronic irradiation states, an explosion of basic research is likely to inform our application of all available biological markers. However, the continued refinement of extrapolation between *in vitro* experiments and *in vivo* effects will perhaps remain the most crucial factor in the success or failure of any biological indicator of radiation exposure.

Key issues

- A number of factors determine the usefulness of potential radiation biomarkers:
  - Time after exposure in which they are informative
  - Simplicity and rapidity of assay, automation potential
  - Applicability to large populations (considerations of invasiveness)
  - Good dose-dependence *in vivo* as well as *in vitro*
  - Minimal variation in the normal range of unirradiated individuals
  - Radiation specificity or few confounding factors

- While no single method meets all these criteria in every situation, cytogenetic assays, such as PCC, dicentrics and chromosome aberrations scored with the aid of FISH probes are currently among the most informative and widely used methods for acute exposure situations. Further refinements of the assays and validation of results *in vivo* are still needed.

- None of the currently available biological indicators of exposure is very satisfactory for low-dose, low-dose-rate chronic radiation exposure. As the effects of such chronic exposures become better understood, it will be increasingly important to find means of monitoring populations for these exposures.

- A specific radiation signature remains the 'Holy Grail' of molecular radiation biology. While no absolute specificity for radiation has yet been found, some types of mutations and chromosome aberrations (i.e., dicentrics) are strongly associated with radiation exposure, especially high LET. More research is still needed in this area.

- Expression profiling by such methods as microarray analysis is one newly developing technology that offers great promise for biomonitoring, both in terms of exposure assessment and for predicting the specific risks of exposure. More research is required, however, to prove the validity of profiling *in vivo* and its predictive power.

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