Structural Chromosome Analysis by Whole Chromosome Painting for Assessment of Radiation-Induced Genetic Damage

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INTRODUCTION

Accurate assessment of radiation exposure is important for several purposes including: 1) Detection of occupational or environmental exposure for regulatory purposes, 2) Evaluation of the level of accidental exposure to guide medical treatment, 3) Assessment of the level of exposure in populations in order to understand the long term biomedical consequences of exposure and 4) Measurement of the radiosensitivity of tumors and normal tissues in order to optimize cancer therapy. Physical dosimetry is frequently not available or unhelpful in these situations so that biological indicators of exposure are needed.

One widely used indicator of radiation exposure is the frequency of structural chromosome aberrations in peripheral lymphocytes. Numerous studies during the past three decades have shown that the frequency of such aberrations increases with dose in a well defined manner (reviewed in 1) and thus can be used as an indication of exposure. The frequency of dicentric chromosomes is measured most often in these assays since these aberrations can be scored rapidly and accurately. This allows sufficient cells (often several hundred to a thousand) to be scored so that statistically precise estimates of the aberration frequency can be made. This is especially important when studying low exposures since the frequency of aberrations may be only a few per thousand cells. Dicentric frequency assays have proved to be most useful for assessment of dose in individuals exposed to acute, low LET radiation immediately after irradiation. Indeed, dicentric frequency analysis is the method of choice for dose estimation in such situations²–⁵). However, estimation of dose from the dicentric chromosome frequency is much more difficult when the exposure is chronic or when the analysis is carried out at long times after irradiation because dicentrics are lost during cell division³). Dosimetry based on the frequency of stable aberrations such as translocations is preferable in these cases since translocations are retained in peripheral lymphocytes more-or-less indefinitely⁶). However, estimation of translocation frequencies using conventional staining or banding analysis has been too labor
intensive and time consuming to be routinely practical. As a result, translocation frequency analysis has been carried out only in a few studies.

**CHROMOSOME STAINING USING FLUORESCENCE IN SITU HYBRIDIZATION**

Chromosome staining using fluorescence in situ hybridization has substantially changed the rate at which structural aberrations can be detected and scored. This has been achieved by using FISH to stain selected regions of one or more chromosomes so that exchanges of material between a stained chromosome and an unstained chromosome can be recognized immediately, even by relatively untrained observers. In this approach, metaphase chromosome are denatured so that their DNA becomes partially single stranded and reacted with chemically modified probe DNA that binds only to sequences to which it is homologous. Unlabeled genomic or Cot 1 DNA is included in the hybridization reaction to competitively inhibit the binding of repeated sequences that are located on many different chromosomes. Commonly used chemical probe modifications include addition of molecules such as biotin, acetylaminofluorene or digoxigenin that can subsequently detected using a fluorescent ligand that binds to the chemical modification. Alternately, fluorescent molecules may be attached directly to the DNA. Thus, FISH results in the selective staining of the region of the genome to which the probe is homologous. A second stain, usually DNA specific, is applied to make the remaining chromosomal DNA visible. The fluorescing reagents are chosen so that the region of the chromosomes targeted by the probe fluoresce one color and the rest of the DNA fluoresces a different color.

**PROBES FOR STRUCTURAL ABERRATION DETECTION**

Three types of probes, illustrated in Figure 2, have been used to stain chromosomes so that structural aberration can be recognized easily. One type of probe targets chromosomal sequences that are repeated several hundred times in a limited part of the genome. Two useful probes of this type are located near the centromere of chromosome 1 and at 1p36. FISH with these probes allows rearrangements between the region of chromosome 1 between these probes and any other chromosome to be readily detected as depicted in Figure 2a. A second type of probe is useful for detection of structural aberrations occurring anywhere along one or more chromosomes. These are termed whole chromosome probes and contain large numbers of different DNA fragments that are homologous to DNA sequences at many locations along the target chromosome. FISH with these probes leaves the entire target chromosome type stained more-or-less uniformly. Whole chromosome probes type are constructed from DNA from chromosomes of one type, typically purified using fluorescence activated sorting as illustrated in Figure 1. This DNA is immortalized (eg. by cloning into plasmids, page and cosmids) so that it can be labeled and used for hybridization. To date, DNA cloned into plasmids have been most widely used as hybridization probes because of the ease with which these libraries can be manipulated. Libraries for each human chromosome
Fig. 1. Schematic illustration showing the construction of whole chromosome probes. Each probe was produced by purifying one chromosome type using fluorescence activated sorting. The resulting DNA was then digested to completion using either Hind III or Eco R1 and cloned into the phage Charon 21A (14). Inserts from Charon 21A libraries were then subcloned into Bluescribe plasmids to increase the insert size to vector size ration and to increase the ease of library manipulation (15).

Fig. 2. Schematic illustration of the use of FISH for detection of structural aberrations. The chromosome region targeted by the probe is illustrated in black and the remainder of the chromosome is white.

type are now available in plasmid form\cite{15} and many are available commercially (Imagenetics, Naperville, III). More recently, a second set of whole chromosome probes has been constructed from sorter purified chromosomes using the polymerase chain reaction\cite{16}. In this
approach, the DNA is cut into small fragments and attached at each end to an oligonucleotide that served as a target for the PCR primer. These libraries are especially useful as hybridization probes because they are complex, properly sized for hybridization (several hundred base pairs in length on average) and easy to produce and label (labels such as biotin or digoxigenin can be incorporated during the last stages of PCR). FISH with these probes reveals aberrations as illustrated in Figure 2b\textsuperscript{17,18}.

The third type of probe that is useful for structural chromosome analysis is used to stain the chromosome centromeres to allow unambiguous discrimination between stable aberrations in which the derivatives have one centromere each and unstable chromosomes where the derivative chromosomes have no centromeres or more than one. Probes to tandemly repeated sequences found at the chromosome centromeres are used for this purpose\textsuperscript{8}. Hybridization procedures have been developed for these probes so that they can be used in conjunction with the whole chromosome probes\textsuperscript{19}. Hybridization of the whole chromosome probes is typically detected with a green fluorescing dye, hybridization of the centromeric probe is detected using a blue fluorescing dye and chromosomal DNA is detected using a red fluorescing dye. Thus, structural aberrations can be detected rapidly by analysis of the red-green hybridization pattern and aberrant chromosome can be classified by analysis of the blue centromeric pattern.

**STRUCTURAL ABERRATION ANALYSIS AND DOSIMETRY**

The results of a FISH analysis of structural aberrations using repeat sequence probes flanking chromosome 1p are shown in Figure 3\textsuperscript{11}. This figure shows the increase in translocation frequency with increasing dose for peripheral lymphocytes irradiated in vitro with \textsuperscript{60}C\(\gamma\)-irradiation. The aberration frequency increases with dose in the expected linear-quadratic manner. The power of this approach comes from the speed of analysis. With it, aberrations involving 1p can be scored essentially as rapidly as they can be detected. Thus, thousands of cells can be scored for dosimetry. This is particularly advantageous for studies of large populations (eg. in Hiroshima or Chernobyl) or for analysis of the effects of low level radiation. The utility of this approach for low dose analysis is illustrated in Figure 3 which shows clearly that the effects of \(<0.2\) Gy \(\gamma\)-irradiation can be detected by scoring sufficient cells (over 20,000 in this study). The disadvantage of this approach is that the target size is small. Chromosome 1p comprises only about 4\% of the genome so that only about 8\% of all structural aberrations are detected. In addition, it is sometimes difficult to distinguish between dicentrics and translocations since the distinctness of the centromere may be obscured by the hybridization process. This may complicate analyses of exposed individuals a few years after exposure when dicentrics may still be present but at an unknown level.

The results of a FISH analysis of translocation frequency with whole chromosome probes also is illustrated in Figure 3\textsuperscript{17}. This figure shows the translocation frequencies measured for peripheral blood lymphocytes irradiated in vitro. The efficiency of the FISH measurements was increased by using whole chromosome probes for chromosomes 1, 3 and 4. These chromosome comprise 20\% of the genome and allow detection of 34\% of all structural aberrations\textsuperscript{17}. Figure
Fig. 3. Translocation frequency dose response curves measured for human lymphocytes irradiated in vitro (11, 17). Translocations were scored after fluorescence in situ hybridization with probes flanking 1p (solid squares; 11) or whole chromosome probes for chromosomes 1, 3 and 4 (solid diamonds; 17). Data for the lower dose portion of the 1p experiment also are shown after multiplication by 10.

3 shows the increased efficiency for translocation detection using probes for chromosomes 1, 3 and 4 compared to that using repeat sequence probes flanking chromosome 1p. In general, the efficiency of aberration detection increases until 50% of the genome is covered by whole chromosome probes. Translocations appear to be randomly distributed throughout the genome so the exact chromosomal targets are not important (17). Analysis of a few large chromosome is easier than analysis of many smaller chromosomes so that FISH with probes covering chromosomes 1 through 4 seems nearly optimal.

STABLE VS UNSTABLE ABERRATIONS

The advent of FISH with either whole chromosome probes or repeat sequence probes makes it possible to score stable or unstable aberrations with approximately equal speed and ease
in order to assess exposure to ionizing radiation. Thus, it now is important to consider the circumstances where one or the other of the endpoints may be more appropriate.

**Unstable aberrations (dicentrics):** The frequencies of stable and unstable aberrations immediately after exposure are generally thought to be approximately equal. Thus, either would seem to be a suitable dosimeter immediately after exposure. However, the background frequency of dicentrics in unexposed individuals is approximately $10^{-3}$/cells while the background frequency of translocations is approximately ten-fold higher\textsuperscript{1,17}. Since detection of low dose exposure is limited by the frequency of aberration prior to exposure, analysis of very low dose exposure seems best accomplished by analysis of the dicentric chromosome frequency as long as the analysis is conducted immediately (i.e. within a few months) of exposure. Thus, dicentric frequency analysis may be particularly useful in monitoring occupation exposure at short times after exposure.

**Stable aberrations analysis:** Translocation frequency analysis seems to be appropriate for assessment of chronic or acute exposure when analysis must be accomplished years after exposure. In such cases, the frequency of dicentric chromosomes will be reduced by an unknown amount by the proliferation of the host lymphocyte population so that estimation of dose from the frequency of dicentric chromosomes is unreliable. Translocations, on the other hand, seems to be retained more-or-less indefinitely\textsuperscript{6}. Thus, assessment of dose is likely to be more accurate based on the frequency of translocations. Of course, the dynamics of the hemopoietic system (e.g. clonal proliferation of a subpopulation of lymphocytes) also may obscure the relationship between translocation frequency and dose. The extent to which this occurs remains to be established.

**CONCLUSION**

Measurement of the frequency of structural aberrations such as dicentrics and translocations can now be accomplished rapidly and accurately using FISH with repeat probes flanking 1p or that target one or more entire chromosomes. The aberration signatures are sufficiently distinct that the aberrations can be scored almost as rapidly as metaphase chromosomes can be found. In fact, metaphase spread finding is now the rate limiting step in aberrations analysis; a limitation which can be removed by application of automated metaphase finding. Dicentric chromosome frequency analysis seems to be the method of choice for dosimetry immediately after exposure and translocation frequency analysis seems to be the method of choice for analysis of chronic exposure or at long times after exposure.

**REFERENCES**