Chromosome Painting in Biological Dosimetry: Assessment of the Ability to Score Stable Chromosome Aberrations Using Different Pairs of Paint Probes

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We exposed human peripheral lymphocytes in vitro to 0.3 and 1 Gy of $^{60}$Co-gamma rays to evaluate whether the ability and sensitivity to detect chromosomal aberrations by chromosome painting is independent or not to the specific paint probes. To detect structural aberrations (translocations), we painted chromosome spreads simultaneously with two whole-chromosome libraries for chromosomes 1, 2, 3, 4, 5, 6, 7, 11, 13, 16, and 18. To compare the rate of chromosome translocations detected by the different pairs of chromosomes, data were normalized according to the fraction of genome painted and evaluated by unconditional logistic regression. Our results show that any combination of paint probes can be used to score induced chromosomal aberrations. We observed that the amounts of translocations are dose dependent and quite homogeneous within each dose of radiation, independently of chromosomes painted. However, the use of small chromosome probes is not recommended because of the high number of cells to be analyzed due to the small amount of genome painted and because it is more difficult to detect translocations in small chromosomes. — Environ Health Perspect 104(Suppl 3):475–477 (1996)

Key words: chromosome painting, dosimetry, radiation, translocations, stable chromosome aberrations, mutagenesis, induced chromosome damage, fluorescence in situ hybridization

Introduction

Since the original description of the procedure of chromosome painting by fluorescence in situ hybridization (FISH), it was thought that the procedure could be used to score induced chromosomal aberrations, for example, in radiation dosimetry. FISH is an easy technique and it can detect chromosome translocations by allowing visualization of color changes, which is preferable to the time-consuming G-banded pattern analysis (1–4). Chromosome painting is based upon using different stains for specific chromosomes, with labeled DNA probes used for whole chromosomes. It allows the detection of color changes in the painted chromosomes, which reveals chromosome exchanges with unpainted chromosomes. Furthermore, in low doses of radiation where the rate of dicentrics can be detected, the use of FISH techniques to score chromosome exchanges (more frequent than dicentrics) can be applied as a biological dosimeter in ionizing radiation exposition.

Chromosome painting is now being widely used in many areas of cytogenetics (such as cancer cytogenetics) to define chromosome markers (5), to detect specific translocations, or to detect spontaneous aberrations in instability syndromes (6) or in normal populations (7).

FISH is also being used more frequently in environmental mutagenesis because it can detect stable chromosome aberrations, in contrast to unstable aberrations such as dicentrics (7–8). There are many different possibilities with different DNA probes. The ideal use of this technique would include chromosome paint probes of many different colors. But today a limited number of fluorochromes—colors—are available. Most often, the procedure uses between two and five paint probes with two to three colors, including the counterstain. This limitation leads to a great variability in procedures (different combinations of chromosome probes) and introduces difficulties in the comparison of similar experiments.

In order to evaluate the ability and sensitivity of chromosome painting to detect chromosomal aberrations and whether it is dependent on the specific paint probes used, we designed the following experiment: chromosome spreads from lymphocytes from a normal donor were exposed in vitro to gamma rays. They were then hybridized with a sequence of different pairs of biotinylated whole chromosomes to detect structural chromosome aberrations—mainly translocations. The objective of this study is to compare the rate of these anomalies scored by the different paint procedures.

Methods

Human peripheral whole blood from a single healthy 28-year-old male donor was irradiated in vitro with gamma rays from $^{60}$Co at doses of 0.3 and 1 Gy. This peripheral blood was cultured for 48 hr according to the standard techniques to obtain chromosomes. RPMI culture medium was supplemented with 20% fetal calf serum, and phytohemagglutinin was used as a stimulator. During the final 2 hr of the cultures, Colcemid was used to inhibit metaphase. After harvesting, all the chromosome spreads were stored at −20°C until they were hybridized with chromosome libraries.

For chromosomal in situ suppression hybridization (chromosome painting), whole biotinylated chromosome libraries for chromosomes 1, 2, 3, 4, 5, 6, 7, 11, 13, 16, and 18 were used in sequences of two (1,2; 2,3; 4,6; 5,7; 13,16; 11,18) and...
detected with fluorescein-labeled avidin as follows. Fifteen microliters of biotinylated total library DNA for the two chromosomes chosen was denatured for 10 min at 65°C and then incubated at 37°C for 30 min. Chromosomes were denatured on slides for 2 min in 70% formamide/2× standard saline citrate (SSC) at 65°C and then quickly quenched in ice-cold 70% (v/v) ethanol and dehydrated in serial ethanol washes (80%, 90%, 100%). The probe was pipetted on the slides and incubated overnight at 42°C. Signal detection was performed by incubation in fluorescein-labeled avidin, followed by one amplification with biotinylated goat-antiamvidin and fluorescein-labeled avidin. After several washes, metaphases were counterstained with propidium iodide (PI).

For each hybridization, we analyzed all the readable metaphases on the slides. Metaphases were scored using a microscope equipped with the appropriate filters to detect FITC (fluorescein isothiocyanate) and PI. The criteria to score metaphases were as follows: hybridization for both chromosomes was successful; it was possible to clearly distinguish painted chromosomes from counterstained chromosomes, and the metaphase appeared to be intact (without counting chromosomes). In cases where chromosome anomalies such as translocations, dicentrics, deletions, or rings were indicated by painted chromosomes, photographs were taken.

To compare the rate of chromosome exchanges detected by different pairs of chromosome DNA libraries, data were normalized using a formula from Lucas et al. (1), which considers the amount of genome painted in each case. This formula is based on the calculation of the fraction of all chromosome exchanges detected by hybridization (Fb) by using the equation Fb = 2f1(1−f1)Fb where f1 is the proportion of the genome painted (Table 1). Fb was determined from the amount of the genome painted and was compared to the fraction observed by G-banding (Fg), which permits data correction by harmonizing the percentage of aberrations scored according to the paint probes used. Final data were evaluated by unconditional logistic regression.

### Table 1. Genome proportion of each individual chromosome.

| Chromosome | % Genome |
|------------|----------|
| 1          | 8.4      |
| 2          | 8.0      |
| 3          | 6.8      |
| 4          | 6.3      |
| 5          | 6.5      |
| 6          | 5.9      |
| 7          | 5.4      |
| 8          | 4.9      |
| 9          | 4.8      |
| 10         | 4.6      |
| 11         | 4.6      |
| 12         | 3.7      |
| 13         | 3.6      |
| 14         | 3.5      |
| 15         | 3.4      |
| 16         | 3.3      |
| 17         | 2.9      |
| 18         | 2.7      |
| 19         | 2.6      |
| 20         | 1.9      |
| 21         | 2.0      |
| X          | 5.1      |
| Y          | 2.2      |

### Table 2. Aberrations scored by chromosome painting.

| Donor | Paint probes | Cells scored | Chromosome aberrations | % | Cells scored | Chromosome aberrations | % | Cells scored | Chromosome aberrations | % |
|-------|--------------|--------------|------------------------|---|--------------|------------------------|---|--------------|------------------------|---|
| Ang487| 1.2          | 258          | 2                      | 0.77| 236        | 4                      | 1.69| 201        | 7                      | 3.48 |
| Ang487| 2.3          | 198          | 0                      | 0   | 282        | 3                      | 1.10| 282        | 9                      | 3.19 |
| Ang487| 4.6          | 318          | 1                      | 0.31| 232        | 2                      | 0.86| 218        | 9                      | 4.13 |
| Ang487| 5.7          | 280          | 2                      | 0.70| 201        | 2                      | 0.59| 374        | 13                     | 3.48 |
| Ang487| 13.16        | 212          | 1                      | 0.47| 156        | 1                      | 0.64| 194        | 3                      | 1.55 |
| Ang487| 11.18        | 251          | 0                      | 0   | 459        | 2                      | 0.44| 145        | 3                      | 2.76 |

### Table 3. Aberrations scored in cell equivalents.

| Paint probes | % Genome | Equivalent fraction | Cell equivalents | Aberrations | % | Cell equivalents | Aberrations | % | Cell equivalents | Aberrations | % |
|--------------|----------|---------------------|------------------|-------------|---|------------------|-------------|---|------------------|-------------|---|
| 1,2          | 16.4     | 27.50               | 71               | 2           | 2.80| 65               | 4           | 6.15| 55               | 7           | 12.7 |
| 2.8          | 14.8     | 25.20               | 50               | 0           | 0   | 57               | 3           | 4.22| 71               | 9           | 12.7 |
| 4.6          | 12.2     | 21.40               | 68               | 1           | 1.40| 50               | 2           | 4.00| 47               | 9           | 19.1 |
| 5.7          | 11.9     | 20.10               | 56               | 2           | 3.50| 40               | 2           | 5.00| 75               | 13          | 17.3 |
| 13.16        | 7.1      | 13.19               | 28               | 1           | 3.57| 21               | 1           | 4.76| 26               | 3           | 11.5 |
| 11.18        | 7.5      | 13.87               | 35               | 0           | 0   | 64               | 2           | 3.13| 20               | 3           | 15.0 |

Results

The results of induced chromosome aberration detection after irradiation are shown in Table 2 where, for each dose of irradiation and each pair of paint probes used, the number of scored metaphases (145–459) and the number of aberrations are given.

Table 3 presents the proportion of the genome painted, the fraction of all chromosome exchanges detected by hybridization for each pair of paints used, the corrected number of metaphases (number of cells equivalent) scored, and the percentage of aberrations found. These results show that the percentage of chromosome aberrations, mainly translocations, are dose dependent and quite homogeneous within each dose of radiation, which is independent of the chromosomes painted (see results plotted in Figure 1). Using unconditional logistic regression, we found no significant statistical differences between the different pairs of probes.

Discussion

In an in vitro study using ionizing radiation exposure with 137Cs gamma rays, Tucker et al. (9) compared translocation detection between G-banded chromosomes and painting of chromosome 4 and chromosomes 1,
Our results show that any combination of paint probes can be used to score induced chromosomal aberrations because, after data correction that harmonizes the percentage of aberrations scored according to the paint probes used, we observed that the amounts of translocations are dose dependent and quite homogeneous within each dose of radiation, independent of the chromosomes painted.

Both experiments suggest that when scoring induced chromosome aberrations, if the results are corrected according to the fraction of the genome painted, it is possible to compare different FISH procedures of detection. This is important because the advantage of using chromosome painting is the feasibility and ease of scoring aberrations and also includes the possibility to automatize. But both aberration scores, dicentrics and translocations, are not comparable because dicentrics are unstable (acute and recently damaged) and translocations are stable aberrations (they can be found in acute exposure to any environmental mutagenic agent as well as in acute delayed exposure or in chronic accumulated exposure).

Nevertheless, Knehr et al. (10), in a comparison of the ratio of translocations to dicentrics, found an excess of symmetrical translocations in some of the combinations of paint probes used, suggesting a need of appropriate weighting factors to the formula of the equivalent fraction.

Although they are effective, small chromosome probes are not recommended because, due to the small amount of the genome painted, a high number of cells must be analyzed and because it is more difficult to detect or visualize translocations in small chromosomes.

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