Rapid Metaphase and Interphase Detection of Radiation-Induced Chromosome Aberrations in Human Lymphocytes by Chromosomal Suppression In Situ Hybridization

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Chromosomal in situ suppression (CISS)-hybridization of biotinylated phage DNA-library inserts from sorted human chromosomes was used to decorate chromosomes 1 and 7 specifically from pter to qter and to detect structural aberrations of these chromosomes in irradiated human peripheral lymphocytes. In addition, probe pUC1.77 was used to mark the 1q12 subregion in normal and aberrant chromosomes 1. Low LET radiation (60Co-γ-rays; 1.17 and 1.33 MeV) of lymphocyte cultures was performed with various doses (D = 0, 2, 4, 8 Gy) 5 h after stimulation with phytohaemagglutinin. Irradiated cells were cultivated for an additional 67 h before Colcemid arrested metaphase spreads were obtained. Aberrations of the specifically stained chromosomes, such as deletions, dicentrics, and rings, were readily scored after in situ hybridization with either the 1q12 specific probe or DNA-library inserts. By the latter approach, translocations of the specifically stained chromosomes could also be reliably assessed. A linear increase of the percentage of specifically stained aberrant chromosomes was observed when plotted as a function of the square of the dose D. A particular advantage of this new approach is provided by the possibility to delineate numerical and structural chromosome aberrations directly in interphase nuclei. These results indicate that cytogenetic monitoring of ionizing radiation may be considerably facilitated by CISS-hybridization.

Key terms: Biological dosimetry, ionizing radiation, fluorescence in situ hybridization

Chromosome aberration analysis in human lymphocyte metaphase spreads has been established as a reliable tool for biological dosimetry (22,41). Analyses performed with conventional cytogenetic techniques, however, are tedious and depend on skilled personnel (3,35). Another severe limitation of conventional cytogenetic analyses in biological dosimetry relates to the fact that it can only be performed in mitotic cells, i.e., in a small fraction of the whole irradiated cell population. Radiation induced chromosome damage could be much more reliably assessed if the whole cell population were amenable to analysis. For evaluation of interphase nuclei the technique of premature chromosome condensation (PCC) has been successfully used (4,5,37), but cytogenetic analyses of prematurely condensed chromosomes may be too laborious for practical use in biological dosimetry. The micronucleus test has provided a simpler method for scoring damage to chromosomal material in cells at interphase which have undergone one cell division after irradiation (42,43). However, the micronucleus test is indicative largely for cells with non-stable chromosome aberrations, which are rapidly eliminated during subsequent cell cycles (6,16,27). For the assessment of long term biological effects of radiation damage the evaluation of stable chromosome aberrations, such as reciprocal translocations, appears to be of particular practical importance. Non-lethal chromosome exchanges have been implicated in the multistep process of malignant cell transformation (31). Recently, X-ray induced translocations have been correlated with transformation to anchorage-independent growth of human diploid fibroblasts...
techniques may provide the potential to overcome most
human-hamster hybrid cells. These translocations
have used fluorescence in situ hybridization to estab-
lishe γradiation damage. Pinkel et al. (38)
rectly in human cell types (32,33,39). Chemically mod-
ifed DNA inserts from libraries established from
sorted human chromosomes (9,17,48) were used as a
complex probe in this approach. It has been shown that
numerical changes, deletions, and rearrangements of
the visualized chromosomes can be readily detected by
CISS-hybridization both in mitotic and interphase tu-
mor cells (14). In addition, subregional chromosome
specific probes were used for more detailed analysis of
chromosome aberrations (14,15,33). In this study we
present a first application of this new approach to the
assessment of chromosomal damage induced by irradia-
tion with 60Cobalt-γ-rays in peripheral human lym-
phocytes and discuss its future potential for biological
dosimetry.

MATERIALS AND METHODS

Cell Material

Human lymphocytes from a healthy, male donor
(46,XY) were cultivated in vitro by using standard
 techniques (46). Five hours after stimulation with phy-
tohaemagglutinin (PHA) cultures were irradiated at
room temperature with 0, 2, 4, and 8 Gy of 60Co-
gamma-rays (1.17 and 1.33 MeV). After additional 67 h
of cultivation, Colcemid arrested metaphase spreads
were obtained after hypotonic treatment (0.075 M KCl)
and fixation with methanol/acetic acid (3:1, vv). For
color of non-targeted chromo-
somes. It results from the superposition of green fluo-
rescence (as derived from the detection of biotinylated
sequences with fluorescein isothiocyanate [FITC] con-
jugated avidine) and red fluorescence (as derived from
counterstaining of the whole chromosome complement
cell nuclei were counterstained with propidium io-
dide (PI) and viewed with a Zeiss photo microscope

equipped with epifluorescence. Pictures were taken
with Agfachrome 1000 ASA films.

In Situ Hybridization With Probe pUC1.77

Probe pUC1.77 was a generous gift from Dr. Howard
Cooke. It represents a 1.77 kb EcoRI fragment of hu-
man satellite II/III DNA subcloned in pUC9 (7). Plas-
mid DNA preparation, purification, nick translation
(Nicktranslation System, Bethesda Research Labora-
tories, cat. no. 8160SB; Biotin-n-dUTP, Sigma, cat. no.
B7645), and fluorescence in situ hybridization were
performed as described (15,19).

RESULTS

Delineation of Structural Chromosome
Aberrations in Metaphase Spreads of Irradiated
Lymphocytes by CISS-Hybridization

Figure 1 shows results of CISS-hybridization of chro-
mosome 7 (Fig. 1a–c) and chromosome 1 (Fig. 1d–f,h–l)
in metaphase spreads of PHA-stimulated human lym-
phocytes using biotinylated inserts of the respective
libraries from sorted chromosomes. Figure 1a shows a
metaphase spread from an unirradiated control
(46,XY), Both chromosomes 7 are completely and spe-
cifically decorated from pter to qter with biotinylated
chromosome 7 sequences. Their yellow color clearly
contrasts with the red color of non-targeted chromo-
somes. It results from the superposition of green fluo-
rescence (as derived from the detection of biotinylated
sequences with fluorescein isothiocyanate [FITC] con-
jugated avidine) and red fluorescence (as derived from
counterstaining of the whole chromosome complement
with propidium iodide). Figure 1b,c shows several re-
arrangements (monocentric and dicentric transloca-
tions) of chromosome 7 material in metaphase spreads
obtained after γ-irradiation of lymphocyte cultures.
Figure 1d–f,h–l provides examples of γ-radiation-
induced structural aberrations of chromosome 1, in-
cluding simple translocations, insertions, dicentrics,
rings, and fragments. At the time when metaphase
spreads were collected many cells were in a tetraploid
state showing identical twin dicentrics and other rear-
rangements (e.g., Fig. 1b,d–f). These duplicated aber-
ations confirm the reliability with which even small
translocations can be detected by this approach (Fig.
1b,d). CISS-hybridization provided a powerful tool with
which to detect the involvement of specific chromo-
somes in all types of aberrations (with the exception of
inversions). Specific staining of chromosomal band
1q12 also allowed the detection of dicentrics (Fig. 1g),
as well as deleted chromosomes 1 or rings containing
this band. In contrast to the CISS-hybridization ap-
proach, however, simple translocations of chromosome
1 material with breakpoints outside the specifically la-
beled region could not be scored.

Table 1 summarizes the results of our quantitative
FIG. 1.
of the aberrant chromosomes as a function of the square of the dose D was noted. These quadratic dose-effect relationships were observed with regard to the percentages of breaks, deletions, and fragments, of monocentrics with translocations, as well as of dicentrics (Fig. 2; compare Table 1). For all doses tested the increase in the yield of all types of aberrant chromosomes (Fig. 2, curve 1) was significant (99% confidence level) over the next lower dose.

### Table 1

Detection of Radiation-Induced Chromosome Aberrations by In Situ Hybridization in Metaphase

<table>
<thead>
<tr>
<th>Specific delineation</th>
<th>Dose (Gy)</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Monocentrics with translocations and inserts&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Deletions and fragments</th>
<th>Dicentrics&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Rings</th>
<th>Total aberrant chromosomes</th>
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<tr>
<td>Complete</td>
<td>0</td>
<td>250</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>#1</td>
<td>2</td>
<td>250</td>
<td>2</td>
<td>2</td>
<td>6.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.2</td>
<td>1.6</td>
</tr>
<tr>
<td>#7</td>
<td>8</td>
<td>350</td>
<td>38.9&lt;sup&gt;e&lt;/sup&gt;</td>
<td>24.3&lt;sup&gt;e&lt;/sup&gt;</td>
<td>16&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.8</td>
<td>80&lt;sup*e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1q12</td>
<td>0</td>
<td>400</td>
<td>n.d.</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>250</td>
<td>3.2</td>
<td>2.4</td>
<td>0.8</td>
<td>0</td>
<td>6.4&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>250</td>
<td>14.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>4.8</td>
<td>1.2</td>
<td>1.2</td>
<td>22&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>350</td>
<td>35.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>9.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.4</td>
<td>60.3&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>250</td>
<td>n.d.</td>
<td>0.8</td>
<td>0.4</td>
<td>0</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>250</td>
<td>6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2</td>
<td>0</td>
<td>5.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>N, number of chromosomes examined containing hybridized material.

<sup>b</sup>n.d., not determined.

<sup>c</sup>Containing a few tricentrics observed at 8 Gy.

<sup>d</sup>The increase over the next lower dose value is significant on the 99% confidence level.

<sup>e</sup>The increase over the value at 0 Gy is significant on the 99% confidence level.

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**Delineation of Chromosome Aberrations in Interphase Nuclei of Irradiated Human Lymphocytes by CISS-Hybridization**

Figure 3a–e shows examples of interphase nuclei with normal and abnormal staining patterns of chromosome 1 domains after CISS-hybridization with the respective library inserts. Many nuclei from irradiated samples showed a grossly disturbed staining pattern including extra domains of various sizes probably resulting from irradiation-induced mitotic non-disjunction and translocation events (Fig. 3b,c). Other nuclei showed both an increased size and increased numbers of hybridized chromosome domains and were considered as polyloid (Fig. 3d). Similar interphase staining patterns were obtained after CISS-hybridization with chromosome 7 library inserts (not shown). The potential of interphase cytogenetics as compared to the evaluation of metaphase spreads in detecting cells with extra copies and translocations of specific chromosomes is further demonstrated by Figure 3e and f. Figure 3f shows a metaphase spread from a testicular human germ cell tumor cultivated in vitro (20). Besides two apparently normal chromosomes 1 (as revealed by additional banding studies, results not shown), a third chromosome 1 was deleted and three translocations of...
chromosome 1 material were detected. The number and size of interphase domains seen in nuclei with appropriate domain separation (Fig. 3e) were in complete agreement with the analysis of the corresponding metaphase spreads (20). Similar results were obtained for other tumor lines (14).

Figure 4 presents data for the quantitative evaluation of nuclei in irradiated lymphocyte cultures. For this, classification of nuclei was performed according to the following criteria: Nuclei with one or two distinct domains were counted as normal (Fig. 3a), while nuclei showing no hybridization signals were excluded from further consideration. Nuclei with more than two signals as exemplified in Figure 3b–d were considered abnormal.

In agreement with the evaluation of metaphase spreads, the interphase dose-response curves suggest a quadratic dependence on dose. About 5% of control nuclei also exhibited staining patterns which were arbitrarily classified as abnormal by these criteria. This contrasts with the evaluation of metaphase spreads where no structural chromosome aberrations were seen in non-irradiated cells. The reason for this discrepancy is presently unclear but is likely to reflect technical shortcomings rather than indicating actual chromosomal aberrations in a subset of non-irradiated interphase cells which could not be evaluated at metaphase (see Discussion).

**DISCUSSION**

Staining of entire individual human chromosomes or parts thereof by using fluorescence in situ hybridization of probes with various complexity has provided a new and simple means for the rapid detection of numerical and structural aberrations in both metaphase and interphase cells. Here we have applied CISS-hybridization to the detection of chromosome aberrations in $^{60}$Co-γ-irradiated human lymphocytes by using libraries from sorted chromosomes 1 and 7 as complex probes. In addition, probe pUC1.77 (7) was used to delineate the lq12 band in normal and aberrant chromosomes 1. In agreement with other investigations (35), a linear increase of dicentrics was observed with the square of the dose D within the dose range used. Our data demonstrate the usefulness of CISS-hybridization as a method for the rapid assessment of a broad spectrum of chromosome aberrations in metaphase spreads of irradiated cells. This technique allows the rapid scoring of both unstable and stable chromosome aberrations after radiation exposure. Specific chromosomes can be easily screened for their participation in aberration events even in metaphase spreads of poor quality (14). The easiness with which stable translocations can be detected (14,20; A. Jauch and T. Cremer, unpublished data) should make it possible to screen for radiation-induced damage even in cells which have undergone many successive mitoses after a radiation event or to screen for cumulative effects of repeated radiation exposures. Aberration scoring of specific chromosome bands by CISS-hybridization with appropriate chromosome band specific DNA-probes or probe sets (33) should provide a possibility for the rapid testing of site-specific chromosomal rearrangements in irradiated human diploid cells. Such specific rearrangements may be involved in radiation-induced transformation events (28–30). For the immediate purposes of biological dosimetry, however, limitation of aberration detection to single chromosomes is disadvantageous in cases where the total yield of aberrations induced in an irradiated cell population is small. The advance of multi in situ hybridization and multicolor detection protocols (36) in combination with fluorescence digital image microscopy (2) is likely to overcome this limitation soon (D.C. Ward, personal communication).

The possibility to detect numerical and structural aberrations directly in interphase nuclei opens a new avenue for biological dosimetry. Diagnostic interphase cytogenetics is still in its infancy (13,38,39,44) but has
FIG. 3. Visualization of normal and aberrant chromosome 1 domains in human interphase nuclei. a: Nucleus from a non-irradiated lymphocyte culture after CISS-hybridization of two chromosome 1 domains. The nucleus was counterstained with PI. b-d: Lymphocyte nuclei with aberrant chromosome 1 pattern after irradiation with $^{60}$Co-$\gamma$-rays (8 Gy). b: Nucleus shows three large chromosome 1 domains. c: Two nuclei with small extra domains (arrowheads) suggesting rearranged chromosomes. d: Large nucleus with at least four domains indicating polyploidization. e-f: Nucleus (e) and corresponding metaphase (f) from the testicular germ cell tumor line Germa 2 after CISS-hybridization. Two normal chromosomes 1 are shown as two large domains in the nucleus. The arrows point to a smaller nuclear domain (e) and a metaphase chromosome (f) indicating a deleted chromosome 1. In addition, three translocations (arrowheads) are visualized.
cient of correlation: $r$

From the experimental data for D2 with aberrant chromosome 7 pattern (see Fig. 3b-d) divided by the regression curve 500 nuclei were evaluated. The bars indicate the number of nuclei with aberrant chromosome 1 plus number of nuclei square (D$'^2$) of the dose D, given in Gy$^2$. 

Ordinate: percentage ($Y = \frac{1}{n}$ number of nuclei with aberrant chromosome 1 plus number of nuclei with aberrant chromosome 7 pattern (see Fig. 3b-d) divided by the total number of evaluated nuclei, × 100. For each dose value, n = 500 nuclei were evaluated. The bars indicate the 99% confidence ranges. From the experimental data for D$'^2$ = 0, 4, 16, 64 Gy$^2$, a linear regression curve was calculated ($Y = 7.67 + 0.72 D'^2$; linear coefficient of correlation: $r = 0.9936$).

The interphase cytogenetics approach can be applied to paraffin embedded tissue sections (21). Thus it should become possible in the future to test solid tissue specimens (e.g., derived by biopsies after partial body exposures) or cells from body fluids for radiation-induced chromosome damage without the necessity of prior in vitro cultivation. A combination of PCC (see Introduction) and CISS-hybridization techniques should provide an ideal tool for the detailed investigation of chromosome damage and repair in interphase nuclei.

Digital image analysis of the specifically stained metaphase and interphase chromosomes (10,11,19,25) may be applied with appropriate threshold setting to outline automatically the decorated chromatini (including translocations) even in cases of non-optimal hybridization with considerable background on non-targeted chromosomes (15). After conventional staining procedures of chromosomes, the 2D-image analysis procedures used for the automatic detection of chromosome aberrations (40) require the segmenting of all individual chromosomes of a metaphase spread. In CISS-hybridization experiments, problems due to chromosomal overlap are greatly reduced, since the specifically decorated chromosome can be easily distinguished from overlapping non-targeted chromosomes (14). Since the chromosome of origin is known for all specifically decorated chromosome fragments, these specifically decorated normal chromosomes may provide parameters to discriminate against free or translocated fragments thereof simply by means of their decreased size and/or total fluorescence intensity. In case of translocation chromosomes, the difference in fluorescence between the yellow-green (PI + FITC) fluorescence of the specifically decorated part and the red fluorescence of the PI-counterstained, non-targeted part adds another parameter. This difference may easily be exploited in the automated image analysis by using appropriate CCD-cameras (M. Kraft and C. Cremer, unpublished data). Slit-scan flow cytometry (10,11,23,24) following fluorescence in situ hybridization of individual metaphase chromosomes in suspension (12,18) may be considered as another promising approach to fast automatic scoring of chromosome aberrations (10,25). Finally, it should be noted that the approach described should also be suited for studies of chromosome damaging chemical agents including the synergistic effects between DNA-repair inhibitors and irradiation damage (1,8,47).

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LITERATURE CITED


