Radiation-induced chromosome aberrations in human euchromatic (17cen-p53) and heterochromatic (1cen-1q12) regions

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The constitutively heterochromatic 1q12 band and the primarily euchromatic 17cen-p53 region comprise a similar size in terms of percentage of the total human genome but have a completely distinguishable chromatin structure. The aim of this study is to unravel whether this structural difference has an impact on the formation and processing of radiation-induced chromosome aberrations. To do so, we have analysed the initial induction and the long-term persistence of radiation-induced (3 Gy γ-rays) chromosomal aberrations with breakpoints in either the 1q12 band or the 17cen-p53 region in comparison with the behaviour of the overall genome. The fusigenic potential of euchromatic and heterochromatic ends was also compared. This time course experiment was performed in a human lymphoblastoid cell line with sampling times at 1, 3, 7, 14 and 56 days after irradiation. The outcome of this study, with 68 000 metaphases studied by multicolour FISH, with centromeric (1cen and 17cen), paracentric (1q12) and locus specific (p53 gene) probes, revealed: (i) a similar radiosensitivity of all regions analysed irrespective of their chromatin configuration; (ii) a possible enhanced fusigenic potential of heterochromatic chromosome ends; (iii) a rapid decline of 1q12 translocations; and (iv) a similar long-term behaviour of translocations involving 1q12 and 17cen-p53. The implications of these findings in biomonitoring studies are discussed.

Introduction

Previous studies using banding techniques have revealed that chromosomal alterations induced in human peripheral lymphocytes by many genotoxic agents occur non-randomly through the genome. In earlier banding studies, the paracentric heterochromatic regions have been found to be chromosomal breakage hotspots following in vitro exposure to a wide variety of agents and also exhibit high frequencies of chromosomal breakage in human populations (Broth, 1977; Savage and Cao, 1985; Sabatier et al., 1989). One of these breakage prone regions is the paracentric heterochromatin of chromosome 1, band 1q12. This band is commonly involved in chromosomal aberrations occurring in a wide range of human haematological and solid malignancies (Atkin, 1985; Olah et al., 1989). Breakage in 1q12 has been proposed as a biomarker of chromosome damage in populations exposed to genotoxic agents such as pesticides (Rupa et al., 1995), benzene (Eastmond et al., 1994) or ionizing radiation (Ramírez et al., 1999). 1q12 breakage is easily detectable by multicolour fluorescence in situ hybridization (FISH) with tandem probes labelling the α satellite centromeric region of chromosome 1 and the adjacent classical satellite region (q12) (Eastmond et al., 1994).

Another relevant chromosomal region in carcinogenesis is the short arm of chromosome 17 (Menon et al., 1990) harbouring the p53 gene (band 17p13.1). The wild-type p53 gene is a tumour suppressor gene encoding a nuclear phosphoprotein that plays a crucial role in the cellular response to DNA damage. Upon low or repairable levels of DNA damage, p53 mediates the delay or arrest at checkpoints preceding cell replication (the G1/S checkpoint) (Kastan et al., 1991), and is involved in delaying damaged cells prior to mitotic chromosome condensation (the G2 and premitotic checkpoint) (Cross et al., 1995). Upon high or irreparable DNA damage, p53 promotes the cells towards apoptosis (Yopnish-Rouach et al., 1991; Bates and Voussen, 1996). Observed aberrations of p53 include genomic rearrangements (Mulligan et al., 1990), homozygous deletions (Takahaski et al., 1989) and loss of heterozygosity on the short arm of chromosome 17 (Chun Chen et al., 1991). The loss of the normal p53 gene function is known to contribute to genomic instability (Lee et al., 1994; Schwartz et al., 1997). We recently applied a novel FISH methodology to detect radiation-induced breakage in the 17cen-p53 region by simultaneously labelling the centromeric region of chromosome 17 and the p53 locus, in different colours (Ramírez et al., 2000). A similar approach was previously used to detect 17p alterations in acute myeloid leukaemia (Soenen et al., 1998) and multiple myeloma (Drach et al., 1998).

1cen-1q12 and 17cen-p53 regions comprise a similar size in terms of percentage of the total human genome (0.5%) but have a completely distinguishable nuclear architecture and chromatin structure and function. The 1q12 region is constituted by repetitive DNA packaged by underacetylated histones and frequently associated with the nuclear envelope (Miller et al., 1974; Manuelidis, 1990; van Dekken et al., 1990; Vogt, 1990; Surralles et al., 1997). In contrast, 17p is mainly euchromatic, early replicating, transcriptionally active, rich in gene density and packaged by acetylated histones (Craig and Bickmore, 1994; Jeppesen, 1997). The aim of this study is to unravel whether these structural differences have an impact on the expression and processing of radiation-induced chromosome aberrations. To do so, we have analysed the initial induction and the long-term persistence of radiation-induced (3 Gy γ-rays) chromosomal aberrations with breakpoints in either the 1cen-1q12 band or the 17cen-p53 region in comparison with the behaviour of the overall genome represented by chromosome 1, as previously reported by us in replicated slides from the same cultures (Puerto et al., 1999). The fusigenic potential of euchromatic and heterochromatic ends was also compared.

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Materials and methods

Cell culture procedures, irradiation and harvesting
The study was performed with a human wild-type lymphoblastoid cell line (wtTK6) which is karyotypically stable and is commonly used as a control cell line in in vitro chromosome instability studies (Zhang et al., 1995). The cultures were established in 89% RPMI 1640, 10% heat inactivated foetal calf serum and 1% streptomycin/penicillin (all the components of the culture medium were obtained from Gibco). At the time of irradiation, a single 10 ml flask was subdivided to form four cultures at a cell density of 2.2×10^6 cell/ml, and two of them were exposed to 3 Gy Co-γ-rays at a dose rate of 1 Gy/min, and the other two cultures were left unexposed (control cultures). The cultures were maintained at a density of 1–2×10^6 cell/ml in 5 ml volume throughout the study. The number of cells was counted five times a week by using the Trypan blue exclusion method and the number of cell divisions gone up to each fixation time was calculated. Harvesting was performed at 1, 3, 7, 14 and 56 days after irradiation by using standard cytogenetic procedures. Finally, tandem labelling of 1cen-1q12 region and 17cen-p53 FISH were performed on different slides from the same culture.

Tandem labelling FISH
Tandem labelling FISH and immunodetection were performed as described previously with minor modifications (Surralles et al., 1997; Ramirez et al., 1999). The probes used were an α-satellite, biotin labelled, DNA probe that hybridizes specifically with the centromeric region of chromosome 1 (D1Z15; ONCOR, Gaithersburg, MD) and a classical satellite, digoxigenin-labelled, DNA probe detecting the adjacent heterochromatic band 1q12 (PUC1.77; Boehringer Mannheim). 1cen was visualized in green with FITC–avidin reaction and 1q12 was coloured in red with TRITC-conjugated antibodies.

17cen-p53 FISH
17cen-p53 FISH was performed as described previously with minor modifications (Ramirez et al., 2000). The probes used were an α-satellite, biotin labelled, DNA probe that hybridizes specifically with the centromeric region of chromosome 17 (D17Z1; ONCOR) and a p53 locus specific probe, directly labelled with Cy3 (LSI p53; VYSIS, Downers Grove, IL). 17cen was visualized in green with FITC–avidin reaction and p53 locus in red directly with Cy3. The slides were counterstained with 0.01 μg/ml DAPI in antifading solution (Vectashield; Vector). All the slides were stored at 4°C until microscopy.

Microscopic analysis and scoring
Microscopic analysis was performed on an Olympus BX-50 microscope equipped with a triple band pass filter to simultaneously visualize DAPI, FITC and TRICT (Chroma). 2000 metaphase cells were analysed for each culture and chromosomal region, making a total of 4000 cells per time point and treatment for each region. All types of CA involving the painted chromosomes were scored. These include dicentrics, ring, excess chromosome fragments, insertions, translocations and complex aberrations. Complex exchanges were scored by transferring them into aberration base types. Depending on the FISH pattern, translocations were classified as reciprocal and incomplete/terminal ‘one way’ translocations. Dicentrics were classified as complete reciprocal dicentrics and incomplete dicentrics. Likewise, bicolour acentrics fragments were also scored as incomplete dicentrics. Excess fragments means an acentric element with non-associated aberrations. Chromosome aberrations involving 1cen-1q12 and 17cen-p53 region in metaphase were classified following the same criteria when possible. The minimum number of breaks required to generate the observed pattern of chromosome aberrations was also calculated. The total number of chromosomal ends (rejoined and unrejoined) generated by the radiation exposure was also calculated for each chromosomal region to measure the fusigenic potential of chromosome ends formed within euchromatin and heterochromatin. Two chromosome ends are generated after a breakage event. When one of these two ends is joined with another chromosome end present in the cell, then it is counted as a rejoined end. When a chromosome end is left unrejoined, then it is counted as an unrejoined end. However, there are a few cases where the fragment was not present in the metaphase spread and, therefore, only one chromosome end was considered when calculating the rejoining of chromosome ends. In the case of 17cen-p53 FISH, the pattern observed in five translocations did not allow us to classify them as reciprocal or terminal so these aberrations were not considered when the ratio of rejoined and unrejoined events was calculated.

Statistical analysis
The χ²-test was used to evaluate the differences between replicates and between control and irradiated cultures in the frequency of damage detected in the 1q12 band and the 17cen-p53 region. The χ²-test was also performed to compare initial breakage and different chromosomal aberrations in 1q12, 17cen-p53 region and chromosome 1. The expected frequency was estimated as a function of the percentage of the DNA content which is 0.45, 0.57 and 8.10% for 1cen-1q12, 17cen-p53 and chromosome 1, respectively (Human Genome Database). 1q12 is polymorphic in size but the differences between individuals are not large enough to quantitatively affect the results.

The obtained data were mathematically transformed in order to compare the different regions with respect to the persistence of the initially induced chromosome damage. The initial number of breaks or translocations detected, i.e. 1 day after irradiation, was expressed in percentage terms as 100%. The kinetics of disappearance of translocations fitted an exponentially declining model. A logarithmic transformation of the percentage of translocations and breaks was therefore performed, which resulted in a considerable straightening of the response curve. The slopes of the straight lines obtained per region were compared by statistical regression analysis to determine whether translocations or breaks involving the different regions showed similar elimination kinetics over time.

Results
To determine the impact of chromatin structure on the induction and persistence of chromosomal aberrations, the frequency of chromosomal aberrations was determined in the band 1q12 and in the region 17cen-p53 in untreated and irradiated cultures at 1, 3, 7, 14 and 56 days after 3 Gy γ-rays exposure. Table I indicates the number of translocations, dicentrics, fragments and minimum number of breaks required to generate all observed CA in untreated and irradiated cultures for each region studied. The same data are also indicated for chromosome 1 (Puerto et al., 1999). Because of the stable low level of chromosome aberrations observed in the non-irradiated cultures, the level of spontaneous chromosome breakage in 17cen-
Chromosome breakage in euchromatin and heterochromatin

Table I. Chromosomal aberrations detected in the band 1q12 by tandem labelling, in the 17cen-p53 region by FISH using chromosome 17 centromere specific and p53 gene specific probes, and in the whole chromosome 1 by chromosome painting, in 4000 metaphase wtTK6 cells in untreated and irradiated (3 Gy γ-rays) cultures at 1, 3, 7, 14 and 56 days after treatment

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\( t \), translocations; \( dic \), dicentrics; \( frag \), fragments.

\( ^a \)Data on chromosome 1 was obtained in a previous study in replicated slides (Puerto et al., 1999).

\( ^b \)In the case of the irradiated samples harvested 1 day after treatment, 3126 instead of 4000 metaphase cells were analysed by 1cen-1q12 FISH and the results were extrapolated to 4000 cells to allow statistical comparison between target chromosome regions.

\( ^c \)Total breaks is not the simple addition of \( t \), dic and frag as rings and insertions were also considered and in some complex aberrations, a single break can generate two aberrations.

![Fig. 1. Genomic frequencies of minimum number of breaks calculated from the observed frequencies involving 1q12, 17cen-p53 and the overall chromosome 1 after 3 Gy γ-rays and corrected for the size of each region in a human lymphoblastoid cell line.](image-url)

As shown in Table I, the great majority of chromosome aberrations involving 1q12 and 17cen-p53 were translocations. The statistical analysis revealed that the frequency of translocations involving breakpoints in both regions was higher than expected on the basis of the DNA content. No statistically significant differences in dicentric or fragments were found between chromosome 1 and the euchromatic 17cen-p53 region. However, we observed a deficiency of dicentrics involving 1q12 when compared with the overall chromosome 1 \( (P < 0.029) \). The frequency of fragments with breakpoints in 1q12 was also lower than in the overall chromosome 1 but only at the limit of statistical significance \( (P = 0.053) \).

To quantify the fusigenic potential of radiation-induced chromosome ends involving different chromosomal regions, the proportion of rejoined and unrejoined chromosome ends was also considered and in some complex aberrations, a single break can generate two aberrations.

![Table II. Fusigenic potential of radiation-induced chromosome ends involving different chromosomal regions](image-url)

Some 1. We therefore conclude that all regions analysed had a similar radiosensitivity irrespective of their chromatin structure.
by constitutive heterochromatin were rejoined. This finding suggests that chromosome ends generated by breakage at constitutively heterochromatic DNA are more fusigenic than ends generated by breaks elsewhere in the genome, represented by the overall chromosome 1 and the 17cen-p53 region.

To compare the persistence of CAs involving the various regions, the initial frequency of CAs was converted to 100%, so that the remaining fraction of CAs at different times after irradiation could be directly compared in percentage terms (Puerto et al., 1999). Figure 2 shows the decline of translocations involving chromosome 1, 1q12 band and 17cen-p53 region observed at 1, 3, 7, 14 and 56 days after irradiation. No differences were found in the overall declining ratio of translocations involving 1q12 band and 17cen-p53 region ($t = 1.288; P > 0.05$). However, statistically significant differences were found between the declining kinetic of translocations involving chromosome 1 and 1q12 band ($t = 4.117; P < 0.05$) but not between chromosome 1 and 17cen-p53 ($t = 1.984; P > 0.05$). This intermediate behaviour of 17p translocations is represented in Figure 2. This apparent differential behaviour of 1cen-1q12 and 17cen-p53 when compared with chromosome 1 would be attributable to the rapid decline of 1q12 translocations 3 days after exposure, whereas chromosome 1 and 17cen-p53 translocations remained relatively stable at early sampling times. The fact that both 1cen-1q12 and 17cen-p53 translocations declined to baseline levels between 7 and 14 days after irradiation explains why the long term kinetics of 1cen-1q12 and 17cen-p53 translocations was similar. The same results were found when comparing the decline of the minimum number of breaks detected in chromosome 1, 1q12 band and 17cen-p53 region.

Discussion

There are a number of studies analysing the interchromosomal distribution of radiation-induced chromosome aberrations in banded chromosomes (reviewed by Johnson et al., 1999). The assignment of breakpoints in constitutively heterochromatin regions by banding has four major limitations in human cells:

(i) mapping exchange breakpoints to the light bands, either G or R, could be a pattern-recognition artefact, which forces their placement in light staining bands (Savage, 1977); (ii) breakpoints close to the border between a dark and a light band are difficult to assign; (iii) the number of metaphases analysed and, consequently, of breakpoints localized is usually low because of the intrinsic complexity and time-consuming nature of the analysis of banded chromosomes; and (iv) unlike hamster cells, the human karyotype does not contain large blocks of heterochromatin, making the target of study relatively small. These limitations are circumvented by the use of the molecular cytogenetic techniques proposed in the present investigation which allowed us to study radiation-induced chromosome breakage specifically involving euchromatic (17cen-p53) and constitutively heterochromatic (1cen-1q12) regions in as many as 68 000 metaphases. Interestingly enough, similar frequencies of breaks were detected in both regions 1 day after irradiation. Moreover, a proportional distribution of radiation-induced chromosome breaks was observed when the results were compared with those obtained in chromosome 1, as a representative of the overall human genome, in a previous study in replicated slides that involved the analysis of over 120 000 metaphases by multicolour painting (Puerto et al., 1999). Therefore our study provided quantitative evidence that the heterochromatic band 1q12 is as radiosensitive as the genome overall, at least in our experimental conditions. This result is apparently in disagreement with previous banding and FISH studies reporting that regions with repetitive DNA, such as centromeric and telomeric chromatin and heterochromatic regions, seem to be more vulnerable to chromosome damage (Johnson et al., 1999; Xiao and Natarajan, 1999). However, we must keep in mind that the present study was carried out on a continuously growing lymphoblastoid cell line, whereas the majority of the previous studies were performed with the above mentioned limitations of the banding techniques and on G0 lymphocytes, where the interphase nuclear organization and the genetic activity is different. On the other hand, our data are in good agreement with our previous studies comparing the facultatively heterochromatic inactive X-chromosome with its active counterpart, where a similar number of radiation-induced breaks were observed in both chromosomes after 1 or 2 Gy of X-rays (Surrallés and Natarajan, 1998).

As expected, very few chromatid-type aberrations were detected although the cell culture is asynchronous. This is explained by the fact that the first harvesting time was 24 h after irradiation. This means that most of the metaphase cells analysed were actually exposed either during G0 or were in their second mitosis after treatment. This is supported by our flow cytometry data indicating that most of the lymphoblastoid cells in culture are indeed in G1 (data not shown).

The design of the experiment reported here does not allow us to state that the initial number of radiation-induced DSB was equal in both regions since DNA repair did occur during the period between irradiation and harvesting. In human cells, we reported that radiation-induced DSBs are not equally processed in the active and inactive X chromosome (Surrallés and Natarajan, 1998). However, in a recent study in hamster cells using arm-specific painting probes in prematurely condensed chromosomes, we concluded that the heterochromatic state had little effect on the processing of the initially induced breaks (Puerto et al., 2000). Therefore it would be interesting to study initially induced breaks in 1q12 and their processing by applying tandem labelling 1cen-1q12 FISH to PCCs at different sampling times after irradiation.

On the other hand, the great majority of chromosome aberrations involving 1q12 and 17cen-p53 breaks observed in metaphases were translocations and very few fragments arising from breaks in heterochromatin were observed. This observation suggests the interesting possibility that chromosome ends...
formed by highly repetitive heterochromatin are very ‘sticky’ and have an enhanced fusigenic potential. The fact that the proportion of unrejoined ends involving 1q12 is very low (15.2%) compared with those involving breaks in the rest of the genome represented by the overall chromosome 1 and the 17cen-p53 regions (45–7%), further support this hypothesis. Considering that the band 1q12 is formed by highly repetitive classical satellite DNA, this finding might be explained by an hypothetical enhanced repair of DSB in highly repetitive DNA or by an excess toxicity of unrejoined 1q12 breaks. Further experiments are needed to uncover the mechanisms explaining our observations.

Translocations are thought to be stable and, therefore, have been proposed as good biomarkers of chromosome damage in retrospective studies (Lucas et al., 1992a,b). The persistence of the genetic alterations is particularly relevant for cancer clonal development and for human retrospective biomonitoring studies. The role of chromatin structure in the persistence of the formed CA has been also addressed in the present investigation. The initial rapid decline of translocations is due to the presence of multiple aberrant cells including cells with complex aberrations as extensively discussed in our previous paper (Puerto et al., 1999). The key question is why cell selection is differently expressed depending on the chromosome target studied. Thus, 1q12 translocations were extremely unstable in culture with a 60.8% decline one cell cycle after induction. Translocations involving 17cen-p53 region initially fall at the same rate as those for chromosome 1, but they do not flatten out at the same time (Figure 2). The persistence of chromosomal alterations affecting the 1cen-q12 region in AZH-1 cells (derivative of TK6 cells) exposed to diepoxybutane and mitomycin C have been studied for a period of up to 20 days (Murg et al., 1999). In their study, the authors observed that only 6% of the damage persisted over time in metaphase cells. The rapid loss of translocations involving 1q12 could have implications when these methodologies are used as biomarkers of chromosome damage in biomonitoring studies. Thus, the observed rapid decline of 1q12 translocations could explain why we detected an increase of 17cen-p53 breakage but not of 1q12 breakage in buccal cells from thyroid disease patients therapeutically exposed to radioactive iodine (Ramírez et al., 1999, 2000).

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