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Use of the Comet-FISH Assay to Demonstrate Repair of the *TP53* Gene Region in Two Human Bladder Carcinoma Cell Lines

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The alkaline single-cell gel electrophoresis (comet) assay can be combined with fluorescence *in situ* hybridization (FISH) methodology to investigate the localization of specific gene domains within an individual cell. The position of the fluorescent hybridization spots in the comet head or tail indicates whether the sequence of interest lies within or in the vicinity of a damaged region of DNA. In this study, we used the comet-FISH assay to examine initial DNA damage and subsequent repair in the *TP53* gene region of RT4 and RT112 bladder carcinoma cells after 5 Gy γ irradiation. In addition to standard comet parameter measurements, the number and location of TP53 hybridization spots within each comet was recorded at each repair time. The results indicate that the rate of repair of the *TP53* gene region was fastest during the first 15 min after damage in both cell lines. When compared to overall genomic repair, the repair of the *TP53* gene region was observed to be significantly faster during the first 15 min and thereafter followed a rate similar to that for the overall genome. The data indicate that the *TP53* domain in RT4 and RT112 cells is repaired rapidly after γ irradiation. Furthermore, this repair may be preferential compared to the repair of overall genomic DNA, which gives a measure of the average DNA repair response of the whole genome. We suggest that the comet-FISH assay has considerable potential in the study of gene-specific repair after DNA damage.

INTRODUCTION

The alkaline single-cell gel electrophoresis (comet) assay is a quick, simple, sensitive and relatively inexpensive way to measure DNA damage (*1–3*). The experimental potential of the comet assay can be enhanced when it is combined with fluorescence *in situ* hybridization (FISH) (*4, 5*). The comet-FISH assay uses fluorescence-labeled DNA probes that hybridize to specific gene sequences. Hence the localization of a particular DNA sequence within a given comet can be established. Despite the potential of this technique, few studies to date have employed the comet-FISH combination. The detection of UV-radiation-induced breakage in lymphocyte chromosomes (*6*) and DNA breakage in plant cells (*7*) has been demonstrated previously, while in our laboratory a preliminary study used the comet-FISH assay to examine DNA damage and repair within the *TP53* gene region (*8*). However, given the extensive application of the comet assay in biology, medicine and genetic toxicology, the comet-FISH assay will undoubtedly become more widely used as its potential in these research areas is realized.

One promising application of the comet-FISH assay is the study of gene-specific repair after DNA damage. The *TP53* gene is a particularly attractive candidate for measuring repair related to specific DNA sequences, since it is actively transcribed throughout the cell cycle (*9*) and is induced by DNA-damaging agents including UV and γ radiation (*10*). Intriguingly, some studies have shown that the *TP53* gene is preferentially repaired in comparison to overall genomic repair after UV-radiation-induced DNA damage (*11, 12*). However, although much has been learned about *TP53* gene repair in response to UV radiation, no one has reported on its repair in response to γ radiation. Indeed, only a few previous studies have investigated the repair of individual genes in response to γ radiation, and the findings have been inconsistent. For example, in normal human fibroblasts, γ-radiation-induced thymine glycol lesions have been shown to be preferentially repaired in the transcriptionally active metallothionein IIA (*MT2A*) gene in comparison to overall genomic repair (*13, 14*). By contrast, in human colon cancer cells, repair of γ-radiation-induced strand breaks within another transcriptionally active gene, dihydrofolate reductase (*DHFR*), was not significantly different from global genomic repair (*15*). However, it is worth noting that this particular study employed high levels of γ radiation and that the authors acknowledged the need for a new method that would allow the study of strand break repair at low doses of γ radiation.

The comet-FISH assay is just such a method; it is capable of detecting DNA damage and repair, even after low doses of γ radiation. In addition, it offers a more rapid and

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clinically feasible alternative to previously established methods for studying gene-specific repair (*16–19*). Therefore, in the current study, we addressed the issues outlined above by investigating the rate of DNA repair in the *TP53* gene region after exposure to 5 Gy γ radiation in cells of two bladder cancer cell lines, RT4 and RT112, using the comet-FISH assay.

MATERIALS AND METHODS

Cell Lines and Cell Culture

RT4 and RT112 cells were obtained from the American Tissue Culture Collection (Rockville, MD). RT4 cells were cultured in McCoy's 5A medium, supplemented with 10% fetal bovine serum (FBS) and containing 1% penicillin-streptomycin. RT112 cells were cultured in minimum essential medium, supplemented with 10% fetal calf serum and containing 1% penicillin-streptomycin. The RT4 cell line is wild-type for the *TP53* gene sequence (*20*), whereas the RT112 cell line contains a point mutation at codon 248 resulting in an Arg-Gly amino acid change (*21*).

Preparation of Metaphase Spreads

Metaphase spreads were prepared to examine metaphase chromosome numbers in RT4 and RT112 cells and to correlate with subsequent TP53 FISH hybridization spot numbers. Exponentially growing cells were harvested using 0.4% trypsin:EDTA and arrested in metaphase by addition of 200 μl of 0.1% Colchicine (Sigma) for 2 h at 37°C. The cells were then centrifuged at 1100*g* for 3 min, and 4 ml of 0.075 *M* KCl (prewarmed to 37°C) was added to the pellet. The suspension was incubated at 37°C for 5 min, and then an equal amount of chilled, freshly prepared fixative (methanol:glacial acetic acid; 3:1) was added to prevent cell clumping. The cells were centrifuged at 1100*g* for 3 min and resuspended in 5 ml of chilled fixative. This was repeated twice before the pellet was finally resuspended in 500 μl of chilled fixative. Metaphase spreads were prepared on clean glass slides that had been polished with ethanol before being immersed and maintained in ice-cold distilled water. The slides were held at 45° to the horizontal and the fixed cells were dropped onto them using a sterile Pasteur pipette. The slides were allowed to air-dry before being stained with filtered 10% Giemsa in Sorenson's buffer for 10 min. The slides were rinsed in distilled water, air-dried and mounted in DePeX. For each cell line, two stock cultures were tested. Fifty cells were selected randomly on each slide and the number of chromosomes per cell was scored blind under oil immersion (100× objective).

Alkaline Comet Assay

The alkaline comet assay was performed according to a standard protocol (*5*) with some modifications as outlined below to allow γ irradiation and subsequent repair of cells to be carried out on slides rather than in Eppendorf tubes. Cells were harvested and washed twice in 10 ml Ca^{2+} and Mg^{2+}-free phosphate-buffered saline (PBS). Cell viability was assessed using the trypan blue exclusion method. In all experiments, cell viability was >99%. One-milliliter aliquots of the cell suspensions in PBS, at a concentration of 2×10^5 cells/ml, were pipetted into Eppendorf tubes and centrifuged at 2400 rpm for 5 min at 4°C. Meanwhile, Dakin fully frosted microscope slides were each covered with 100 μl of 0.6% normal-melting-point agarose (prepared in PBS) at 50°C. A 22 × 22-mm cover slip was placed on top, and the slide was kept on ice until the agarose had solidified. Each pellet of cells was resuspended in 80 μl of low-melting-point agarose at 37°C. After the cover slip was removed gently, the cell suspension was quickly pipetted onto the first agarose layer, the cover slip was replaced on top, and the slide was left on ice to solidify the agarose. In the case of cells in which repair was to be studied, 1.2% low-melting-point agarose was mixed in a 1:1 ratio with repair medium (RPMI 1640 supplemented with 20% FBS), and 80 μl of this mixture was used to resuspend the cells as described above.

After the cover slips were removed, the slides were irradiated with a range of γ-radiation doses from 0 to 10 Gy at a rate of 2 cGy/s using a ^{137}Cs source. They were then quickly immersed in freshly prepared cold lysis solution (2.5 *M* NaCl, 100 m*M* Na_2EDTA, 10 m*M* Tris, pH 10, with 1% Triton X-100 added just before use) for 1 h at 4°C. In the case of slides containing cells to be studied for repair, 5 Gy irradiation was administered and the slides were subsequently immersed in repair medium at 37°C for 15, 30 or 60 min, after which they were drained and placed in lysis solution for 1 h at 4°C.

After removal from the lysis solution, slides were placed side by side in a horizontal gel electrophoresis tank. The tank was filled with fresh, cold electrophoresis buffer (300 m*M* NaOH, 1 m*M* Na_2EDTA, pH >13). The slides were left in the alkaline buffer for 20 min to allow DNA unwinding to occur. Electrophoresis was then conducted at 25 V (0.66 V/cm) and 300 mA for 20 min. Slides were drained, placed on a tray, and flooded slowly with three changes of neutralization buffer (0.4 *M* Tris, pH 7.5) for 5 min each to remove alkali and detergents. The slides were then stained with ethidium bromide (2 μg/ml) or processed for FISH. To prepare the slides for FISH, they were placed in cold 100% ethanol for at least 30 min. They were then drained and placed in 0.5 *M* NaOH for 25 min at room temperature to fully denature the DNA. They were subsequently dehydrated in an ascending series of ethanol solutions (70%, 85%, 100%; 5 min each) and left to air-dry overnight on dry tissue paper in the dark. All experiments were carried out under yellow light.

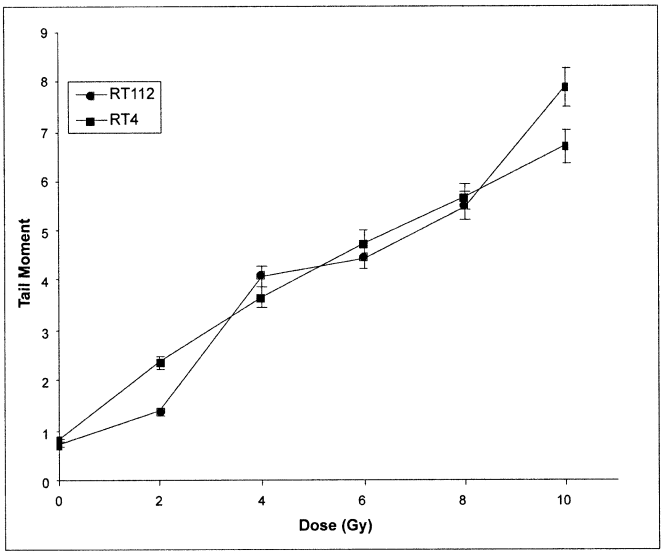
Fluorescence In Situ Hybridization (FISH) on Comet Slides

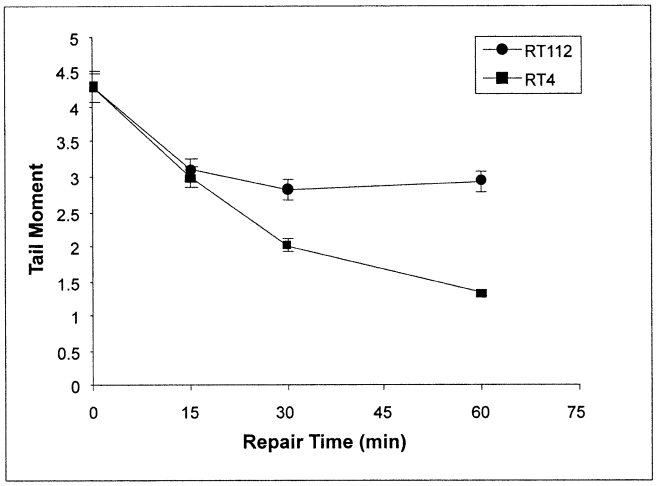
FISH was performed on prepared comet slides according to the manufacturer's instructions using a fragmented locus-specific identifier Spectrum Orange-labeled DNA probe spanning a region of 200 kb containing the *TP53* gene (Vysis, Surrey, UK). For each individual slide, a probe mixture, containing 1 μl probe solution, 7 μl locus-specific identifier hybridization buffer (Vysis), and 2 μl sterile water was heated at 73°C for 5 min and quickly added to the slide. A 22 × 22-mm cover slip was placed on top, and the edges were sealed with cow gum rubber cement. Hybridization took place at 37°C for 16 h in a dark, humidified chamber.

After hybridization, the cow gum seals were carefully removed and the slides were placed in a solution of 50% formamide and 2× SSC for 10 min at 45°C. After the slides were placed in the solution, they were agitated gently to detach the cover slips. This wash was repeated twice, followed by a 10-min wash in 2× SSC at 45°C and a 5-min wash in 2× SSC containing 0.1% Igepal. Slides were left to air-dry for 10 min before being counterstained with 15 μl 125 ng/ml 4′6-diamidino-2-phenylindole (DAPI) in antifade mounting solution (Vysis). The slides were placed on damp tissue paper in a dark chamber and left at 4°C for no longer than 2 h prior to observation. All reagents were purchased from Sigma, Poole, UK unless otherwise indicated.

Comet-FISH Analysis

Analysis was performed using an epifluorescence microscope (Nikon Eclipse E400) fitted with a Nikon 60× fluor lens (Plan Apo 60:1 N.A. 1.4) and equipped with a Hamamatsu Orca digital CCD camera. A filter set (Chroma HiQ) tuned for ethidium bromide (excitation 510 nm, emission 595 nm) was used for standard comet analysis. A double bandpass filter set (Chroma HiQ) tuned for DAPI (excitation 370 nm, emission 450 nm) and Spectrum Orange (excitation 560 nm, emission 590 nm) was used for FISH, allowing simultaneous detection of DAPI and Spectrum Orange labels. Comet analysis was performed using the Komet 4.0 digital imaging system (Kinetic Imaging Ltd., Liverpool, UK), which measures a wide range of densitometric and geometric parameters for each comet. The primary measurements used in this study were percentage DNA in tail, defined as 100 − [(head optical intensity/total optical intensity) × 100], and tail moment, defined as the distance between the center of the comet head and the center of the comet tail, multiplied by the percentage





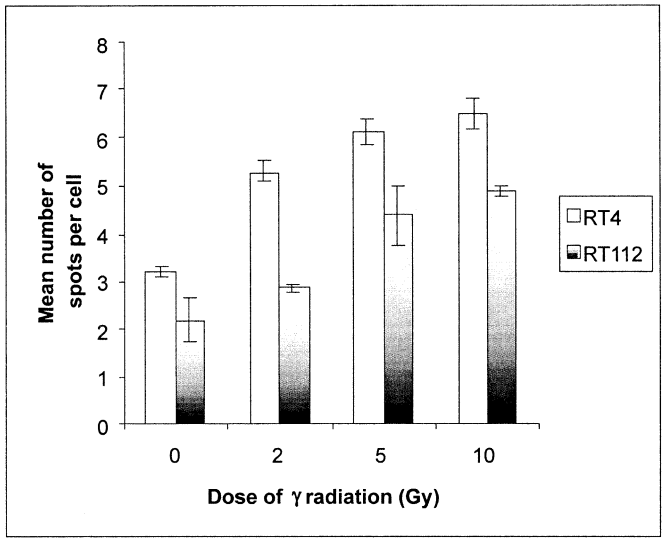


FIG. 1. Dose response to γ radiation. Top panel: Both RT4 and RT112 cells show a clear dose response to γ radiation. As the radiation dose is increased, the amount of DNA damage also increases, as shown by increased tail moment values at each point. Three independent experiments were performed to generate each data point. Error bars shown are 95% confidence limits. Middle panel: As repair time increases after irradiation with 5 Gy, both RT4 and RT112 cells show a steady decrease in the amount of DNA damage as shown by decreased tail moment values at each time. Error bars shown are 95% confidence limits. Bottom panel: Data from comet-FISH experiments show that both RT4 and RT112 cells demonstrate a clear dose response to γ radiation as the mean number of hybridization spots per cell increases with increased dose. Error bars shown are mean ± SEM.

TABLE 1
Chromosome Numbers of the RT112 and RT4 Cell Lines

Cell line	Sample no.	Median	Mode	Sample mean ± SD	Population mean ± SE
RT112	1	46	46	44.75 ± 4.95	
	2	46	47	43.94 ± 6.94	44.3 ± 0.4
RT4	1	83	73	81.25 ± 4.95	
	2	84	83	83.75 ± 5.75	82.5 ± 1.3

DNA in the tail, divided by 100 [(tail mean − head mean) × percentage DNA in tail/100]. Fifty cells were analyzed from each slide, and the number and location of hybridization spots within each comet were recorded for comet-FISH experiments. Three separate experiments were conducted for each data point.

RESULTS

With the alkaline comet assay, the RT112 and RT4 cell lines both exhibited a clear dose response to γ radiation for doses up to 10 Gy (Fig. 1a). A dose of 5 Gy was selected for the repair studies since the dynamic range of the comet parameter tail moment was not exceeded at this dose. Similar results were obtained using the parameter percentage DNA in the tail as a measurement of DNA damage (data not shown). Studies of repair demonstrated a reduction in DNA damage for both cell lines as the time increased after 5 Gy irradiation (Fig. 1b). Both cell lines had similar levels of initial damage, but RT112 cells had significantly higher levels of residual damage at 60 min. With the alkaline comet-FISH assay, a clear dose response was also observed in both cell lines when the mean number of TP53 hybridization spots per cell was calculated (Fig. 1c). The number of spots increased as the radiation dose increased.

The mean, median and modal chromosome numbers for each cell line are shown in Table 1. Calculation of the population mean demonstrated that RT112 cells were approximately diploid, while RT4 cells were polyploid. This was reflected in the lower mean number of spots per RT112 cell compared to RT4 cells at each dose of radiation (Fig. 1c). Frequency analysis of TP53 spot distribution in cells from the comet-FISH experiments confirmed this result (Fig. 2). The majority of the control RT112 cells displayed two TP53 hybridization spots, indicating that the cell line was diploid (Fig. 2a). A small percentage of the cells showed four spots, most likely reflecting a small subpopulation of mitotic cells. After irradiation, the RT112 cells showed an increase in spot number, with many showing four or more

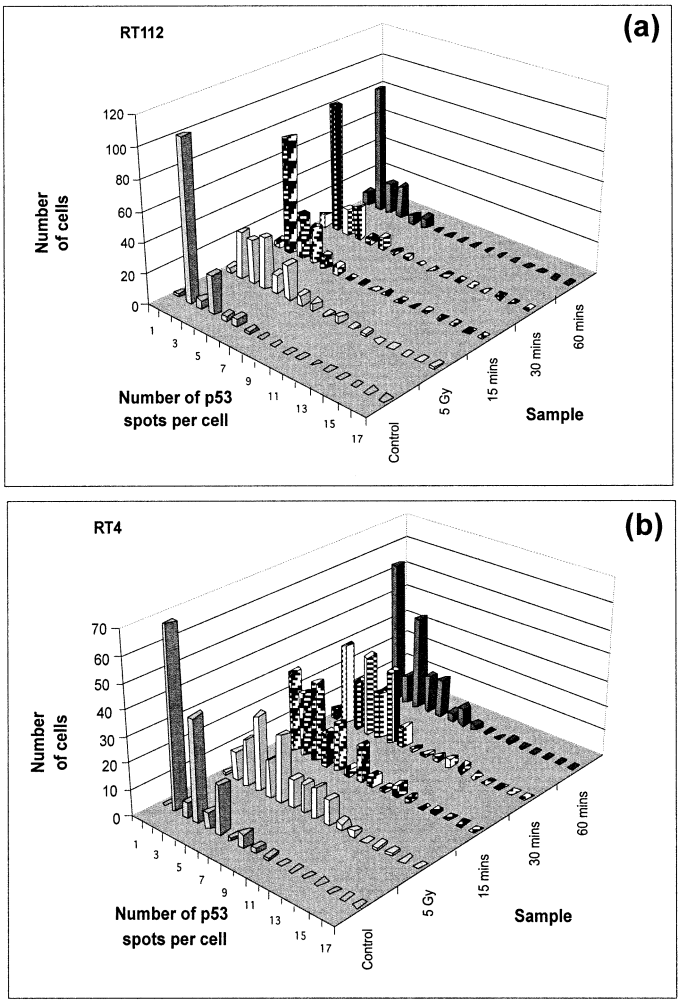


FIG. 2. Frequency distribution analysis of TP53 (p53) hybridization spot number. Panel a: 3D histogram showing the frequency of TP53 hybridization spot numbers in RT112 cells for each treatment. Panel b: 3D histogram showing the frequency of TP53 hybridization spot numbers in RT4 cells for each treatment.

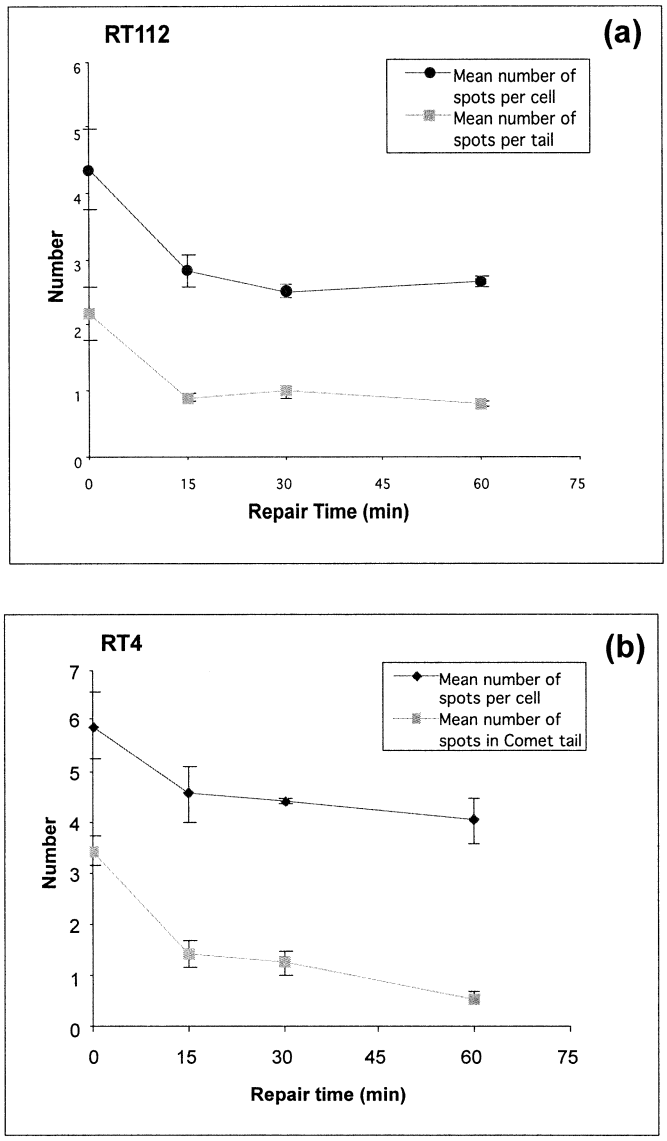


FIG. 3. Mean number of TP53 hybridization spots per cell after 5 Gy γ irradiation. Panel a: RT112 cells display a rapid rate of decrease in the mean number of spots per cell during the first 15 min after irradiation. This trend is also evident when the mean number of spots per comet tail is calculated. Panel b: RT4 cells also display a rapid rate of decrease in the total number of spots per cell during the first 15 min after irradiation. This trend is also evident when the total number of spots per comet tail is calculated. For all graphs, three independent experiments were performed to generate each data point, and error bars shown are means ± SEM.

spots per cell. As the repair time increased, however, the number of cells showing high numbers of spots was reduced, until at 60 min most RT112 cells again displayed either two or four spots. As expected for a polyploid cell line, the majority of the control RT4 cells displayed two, four or six spots, with some exhibiting as many as 10 spots (Fig. 2b). Immediately after irradiation, RT4 cells demonstrated a wide range in the number of hybridization spots, with some cells showing as many as 15 spots. However, as with the RT112 cells, with increasing repair time the number of cells showing high numbers of spots decreased, until at 60 min, the profile was similar to that for control RT4 cells.

Comet-FISH repair experiments demonstrated that both cell lines exhibited a decrease in the mean number of TP53 spots per cell immediately after 5 Gy irradiation (Fig. 3). When repair was allowed to occur for up to 60 min after irradiation, the RT112 cells displayed a decrease in the mean number of TP53 hybridization spots per cell up to 30 min (Fig. 3a). The number of spots per cell was significantly reduced within the first 15 min, but thereafter showed a much slower reduction. A similar decrease during the first 15 min was noted, when the mean number of spots per comet tail was calculated (Fig. 3a). The results obtained for the RT4 cell line also showed a similar pattern (Fig. 3b). The mean number of spots, both per cell and per comet tail, was markedly reduced after 15 min of repair and thereafter decreased at a slower rate.

Representative images of cells processed in the comet-

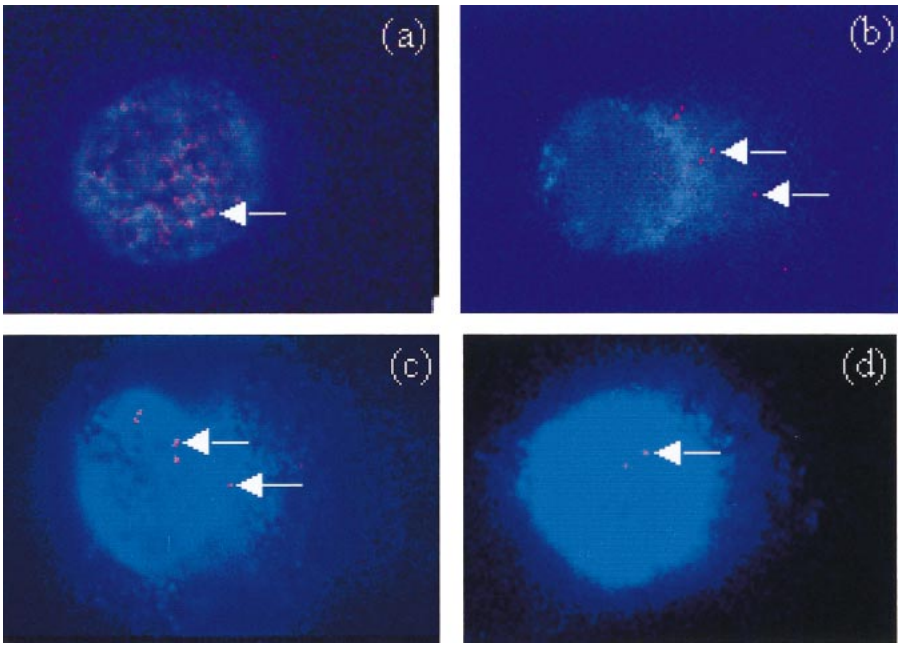


FIG. 4. Representative images of RT112 cells after comet-FISH protocol. Panel a: Control cell displays hardly any DNA damage as seen by the absence of a comet tail. Two TP53 hybridization spots (arrows) are clearly visible in the intact head. Panel b: Immediately after exposure to γ radiation, a large comet tail is visible, reflecting the extent of DNA damage. Several TP53 hybridization spots are located in the comet tail. Panel c: After 15 min repair time, the comet tail is reduced and most of the TP53 hybridization spots are now positioned in the comet head. Panel d: After 60 min repair time, the comet tail is further reduced and all TP53 hybridization spots are found in the head. Original magnification 600× for all panels.

FISH assay illustrate the decrease in spot number as repair time increased. Figure 4a shows control RT112 cells with two TP53 hybridization spots visible. Immediately after irradiation, several hybridization spots were detected, most of which were positioned in the comet tail, due to radiation-induced strand breaks in the region of the TP53 probe (Fig. 4b). After 15 min, the number of spots decreased as the breaks rejoined and most spots were positioned in the comet head (Fig. 4c). After 60 min, the number of spots was comparable to that of control cells (Fig. 4d). At each repair time, a comet tail was clearly visible, indicating that there was still a significant amount of overall genomic DNA damage, even after 60 min.

To demonstrate repair of the overall genomic DNA in each cell line after γ irradiation, the number of cells with induced DNA damage was calculated for each time (Fig. 5). A cell was considered to have γ-radiation-induced DNA damage if its tail moment measurement had a greater value than the mean tail moment value for control cells of that cell line. Control RT112 cells had a mean tail moment of 1.25 (±0.02) compared to 0.97 (±0.14) for control RT4 cells. Immediately after irradiation, 98% (49/50) of the RT112 cells exhibited radiation-induced DNA damage. As the repair time increased, the mean number of damaged cells decreased steadily up to 60 min, when 72% (36/50) of the cells displayed γ-radiation-induced damage. For comparison, the mean number of cells showing TP53 tail spots (and therefore damage within or near the probed region) was also calculated at each time (Fig. 5a). Seventy-seven percent (38.5/50) of the cells displayed tail spots immediately after irradiation. This was significantly reduced at 15 min, when 51% (25.5/50) contained tail spots. Thereafter, the number of cells with TP53 tail spots decreased at a rate similar to that of overall genomic DNA, until at 60 min 42% (21/50) of cells displayed tail spots. The results were similar for RT4 cells (Fig. 5b). Immediately after irradiation, all RT4 cells showed induced DNA damage, and 95% (47.5/50) of these exhibited tail spots, compared to 77% (38.5/50) for RT112 cells. As repair time increased, the number of cells showing overall DNA damage decreased at a steady rate to 61% (30.5/50) at 60 min. However, the number of cells showing TP53 tail spots decreased significantly from 95% (47.5/50) to 64% (32/50) at 15 min and subsequently to 32% (16/50) at 60 min, at a rate comparable to that of overall genomic DNA. The same trends were obtained for both cell lines when the number of cells showing tail spots was compared with the number of cells showing radiation-induced DNA damage, using the comet parameter percentage DNA in the tail (data not shown).

DISCUSSION

Since its conception by Östling and Johanson in 1984 (*22*), the comet assay has become a widely accepted and versatile method for measuring a variety of DNA lesions in individual cells (*1–3*). When broken DNA is subjected

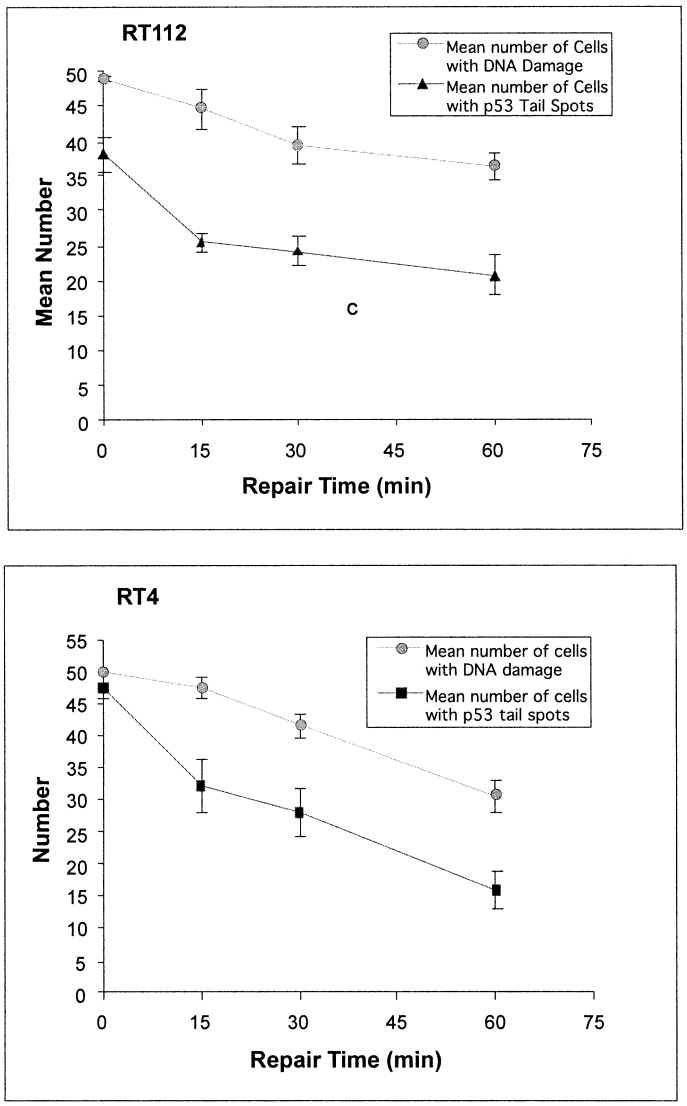


FIG. 5. Repair of overall genomic DNA and of *TP53* (p53) gene region. Upper panel: Mean number of RT112 cells showing γ-radiation-induced DNA damage (tail moment > 1.25) and mean number of RT112 cells having TP53 hybridization spots in the comet tail at various repair times. Lower panel: Mean number of RT4 cells showing γ-radiation-induced DNA damage (tail moment > 0.97) and mean number of RT4 cells having TP53 hybridization spots in the comet tail at various repair times.

to an electric current, DNA containing strand breaks migrates through the agarose while undamaged DNA remains immobile. After it is stained with a fluorescent DNA-specific dye, the resultant image resembles a comet, with undamaged DNA forming a ''head'' and damaged DNA forming a ''tail''. Combining the comet methodology with FISH affords the opportunity to determine whether or not a specific domain of interest contains strand breaks. The position of the hybridization spot in the head or tail of the comet indicates whether it lies in, or close to, a region of damaged DNA. Further information about whether or not DNA breakage occurs within the probed region is obtained by observing increases in spot number in the comet tail.

In the current study, we successfully observed hybridization of a *TP53* locus-specific identifier probe to the DNA of individual cells with variable amounts of γ-radiation-induced DNA damage, which were processed in the alkaline comet assay. We recorded the number of hybridization spots per comet and used this measure as an indicator of the extent of damage, since breaks within the probe target DNA will result in two or more fluorescent signals rather than one. We also recorded the location of the spots in the head or tail of the comet to further determine whether the gene of interest is in the vicinity of a broken region of DNA.

To assess the intrinsic sensitivity of the cell lines to radiation, the cells were exposed to doses of radiation up to 10 Gy. A clear dose response to γ radiation was observed for both cell lines up to a dose of 10 Gy (Fig. 1a). Above this level, cellular damage was too great for the Komet 4.0 software to analyze consistently. From these results, we selected 5 Gy as a suitable dose for studying repair. As the repair time increased after 5 Gy irradiation, the amount of DNA damage was reduced (Fig. 1b). Although both cell lines had comparable levels of initial damage immediately after irradiation, the RT112 cell line had significantly higher levels of residual damage at 60 min. This is likely to be because it has a mutation in its *TP53* gene, which results in a dysfunctional TP53 product (*21*). Since the correct function of the *TP53* gene product is a major factor in mediating global genomic DNA repair in response to damage (*10*), the levels of residual damage observed in the RT112 cell line after 60 min of repair is not an unexpected finding. Using the comet-FISH assay, a clear dose response was also observed in the RT4 and RT112 cell lines when the mean number of TP53 hybridization spots per cell was counted (Fig. 1c). The increased spot number that occurs as the dose is increased indicates that strand breaks are occurring within the probed region; otherwise only movement of spots between the comet head and tail would be observed, with no overall increase in the number of spots. It is probable that radiation-damaged DNA in the *TP53* gene region is separated during the comet protocol, and consequently the probe fragments will bind to each separated region, resulting in an increased number of spots in irradiated cells.

Using metaphase spreads, we were able to determine the ploidy of each cell line (Table 1). RT112 cells were found to be approximately diploid, while RT4 cells were shown to be polyploid. These observations are in accordance with previous studies (*20, 21*) and with the supplier's datasheets for these cell lines, which list RT112 as diploid and RT4 as hyperdiploid and hypotetraploid to hypertetraploid. The ploidy status for the two cell lines was also confirmed by frequency distribution analysis of the TP53 hybridization spot numbers from cells processed in comet-FISH experiments. As expected, most control RT112 cells showed two hybridization spots (Fig. 2a), although a small but notable proportion displayed four spots. It is likely that these cells were in the process of mitosis when they were processed for the comet-FISH assay. The majority of the RT4 control

cells show two, four or six spots, with some showing as many as 10 (Fig. 2b). However, irrespective of their ploidy status, treatment with 5 Gy radiation results in both cell lines showing an increased frequency of cells with high numbers of hybridization spots (Fig. 2a and b). Thereafter, as repair time increases, the number of cells showing high numbers of spots was reduced, until at 60 min both cell lines exhibited profiles similar to those of their respective controls.

Results from repair experiments present evidence that the *TP53* gene region is rapidly repaired within the first 15 min in both RT4 and RT112 cells in response to γ radiation. The number of hybridization spots per comet and the number of spots located in the comet tail are markedly reduced within the first 15 min of repair (Fig. 3a and b). Thereafter the rate of repair is significantly slower. Images of representative cells from selected times illustrate this observation. Figure 4a shows a control RT112 cell with two spots corresponding to the two *TP53* gene loci. Immediately after irradiation, however, several TP53 spots are visible and are located mostly in the comet tail (Fig. 4b). The increased number of spots and the location of the spots in the comet tail indicate that they lie within or in the vicinity of a damaged region of DNA. However, after 15 min of repair, it is noticeable that most hybridization spots are located within the comet head, indicating that they now lie within or near an intact (i.e. repaired) region of DNA (Fig. 4c). At 60 min repair, only two spots are visible, and this reduction in spot number from that after 15 min of repair indicates that strand breaks within the probed region have been repaired (Fig. 4d). Taken together, these results imply that the cancer cell lines studied here rejoin strand breaks in the *TP53* gene region quickly, i.e. within 15 min, presumably in an attempt to restore its function. Significantly, early rapid repair of the *TP53* gene has been observed previously in response to UV radiation (*11, 12*), and it has been suggested that the diverse mutational spectrum exhibited by the *TP53* gene in several tumor types results from more rapid repair of its transcribed strand (*12*). Given these observations, it seems reasonable to propose that the rapid repair observed in this study also reflects the crucial role of the *TP53* gene in the cellular response to γ radiation.

It may also be significant that in both cell lines, the movement of TP53 spots into the comet head is markedly faster than that of overall genomic DNA during the first 15 min after damage is induced. The cell images shown in Fig. 4 demonstrate that a comet tail is visible at each repair time, indicating that there is still a significant amount of overall DNA damage at each repair time, while the TP53 hybridization spots are mostly positioned within the intact head after 15 min. Figure 5 also demonstrates that the decrease in the number of cells showing TP53 tail spots is significantly more evident than the decrease in the number of cells showing overall DNA damage during the first 15 min. From 15 to 60 min, however, the rates of decrease are similar for the two cell lines. From these observations, it is tempting to speculate that the *TP53* gene is preferentially repaired in comparison to the overall genome. Indeed, previous studies have shown that *TP53* is repaired at a faster rate than the overall genome in human cells exposed to UV radiation (*11, 12*). Furthermore, preferential repair of other transcriptionally active genes has also been reported in mammalian cells after UV-radiation damage (*16–19*). On the basis of these studies, it has been suggested that cells can prioritize the process of DNA repair after UV-radiation damage to restore the function of important genes first (*23, 24*), and it is feasible that a similar order of gene repair occurs in response to γ radiation. The results presented here suggest that differences in repair rate between the *TP53* gene region and overall DNA do indeed appear to occur at an early stage after damage in γ-irradiated cells. However, further work using probes for actively transcribed and nontranscribed genes is required to prove conclusively that a gene repair hierarchy exists after γ irradiation in these cell lines. Indeed, ongoing work in our laboratory is focusing on the development of a multicolor comet-FISH assay, whereby three different fluorescence-labeled probes of equal size, specific for the *TP53* gene, the housekeeping gene *DHFR*, and the inactive gene locus 754, respectively, will be hybridized to cells on a single slide. In this manner, any differences in repair rates between these sequences can be observed within an individual cell. It is also our intent to label each end of the *TP53* gene with two different probes. The separation between the two different fluorescent labels after the comet assay will allow us to ascertain definitively and more easily whether a strand break has occurred within the gene itself.

It is also worth noting that immediately after 5 Gy irradiation, 77% (38.5/50) of RT112 cells exhibit TP53 tail spots, compared to 95% (47.5/50) of RT4 cells (Fig. 5a and b). This suggests that the *TP53* gene region in RT112 cells is protected in some manner. It is possible that differences in the chromatin configuration of the cell lines may play a role in this protective effect, since the presentation and location of genes within the nuclear architecture is known to be a determining factor in their susceptibility to damaging agents such as γ radiation (*23*). An ongoing study in our laboratory has employed a digital cell imaging technique called nuclear texture analysis to measure characteristics of chromatin distribution in bladder cancer cell lines (*25*). Future work will assess whether this technique can be used to determine if the chromatin distribution in the region of the *TP53* gene is different in the two cell lines studied here.

It is worth remembering that the nature of the comet assay precludes us from stating categorically that damage and repair are occurring within a given gene. Rather, it enables us to conclude whether the damage and repair are occurring in the vicinity of the gene of interest. Nevertheless, the findings of this study present evidence that the comet-FISH assay can be used to measure repair of the *TP53* gene region after γ-radiation-induced DNA damage in two bladder cancer cell lines. Significantly, the repair is

similar in both cell lines irrespective of their *TP53* mutational status. Moreover, it is suggested that this repair may be preferential in comparison to overall genomic repair, which gives a measure of the average DNA repair response of the whole genome, including preferentially repaired domains. Therefore, we suggest that the comet-FISH assay is a rapid and clinically feasible technique that offers considerable potential for further study of gene-specific repair.

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