Induction of Delayed Mutations and Chromosomal Instability in Fibroblasts after UVA-, UVB-, and X-Radiation

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ABSTRACT

Mutations in critical genes are believed to be a necessary part of cancer induction. The conventional view of radiation mutagenesis is that radiation induces most mutations in cells shortly after irradiation, because of false repair or lack of repair of DNA damage before or during DNA replication. In contrast, we here show that delayed mutations in the hypoxanthine phosphoribosyltransferase locus of Chinese hamster fibroblasts (V79) arise many cell generations after three types of carcinogenic irradiation: (a) UVA-; (b) UVB-; or (c) X-radiation. The frequency of mutations at the hypoxanthine phosphoribosyltransferase locus was measured in clones 14 days after irradiation with doses killing 80% of the cells. The proportion of unstable clones, as indicated by mutant fractions 10–7500-fold above background, was higher for the cells treated with UVA (13.2%) than for cells treated with UVB (9.2%) and X-radiation (9.6%). In contrast, UVA produces few immediate mutations compared with UVB and X-radiation. Thus, UVA-radiation, which is suspected to cause melanomas, produces few immediate mutations but more delayed mutations than UVB or X-radiation. Clones of cells that developed delayed mutations were examined for markers of chromosome instability, such as increased numbers of centrosomes, DNA content, and variability in the number of chromosomes. All radiation types increased the variability in the number of chromosomes in unstable clones. Although UVB and X-radiation, which damages DNA by direct interaction, resulted in an increased number of centrosomes in cell clones, the oxidative UVA-radiation did not. Thus, the mechanism of UVA-induced chromosomal instability is apparently different from that of UVB and X-radiation.

INTRODUCTION

UV radiation is a complete carcinogen responsible for initiation and promotion of the skin cancers basal and squamous cell carcinoma (1) and has also a role in induction of malignant melanoma (2). Skin cancer is the most common type of cancer in the United States. The melanoma rates and mortality have increased dramatically during the last 40 years. Thus, there is a great need to better understand the basic mechanisms of skin carcinogenesis.

Studies of mutagenesis by ionizing radiation have demonstrated unexpected mutations in the progeny of exposed cells (3, 4). There are many reports showing that the progeny of cells exposed to ionizing radiation exhibit delayed genetic changes, including specific gene mutations (5–8) and chromosome aberrations (9, 10). The delayed mutations and chromosome aberrations are regarded as consequences of a destabilization of the genome termed radiation-induced genomic instability. The delayed mutations are usually point mutations, similar to spontaneous mutations, and not deletions that are early effects of ionizing radiation (8), suggesting that instability represents an increase in spontaneous damage. The mechanisms underlying the phenomenon of radiation-induced genomic instability are not understood.

But a persistent increase in reactive oxygen species after irradiation has been implicated as a possible mechanism (11).

There is now evidence that most cancers, including skin cancer, may be genetically unstable (12). The instability exists at two distinct levels. In a few cancer types, the instability is observed at the nucleotide level and results in base substitutions, deletions, or insertions of a few nucleotides (13). However, in most types of cancer, the instability is observed at the chromosome level, leading to losses, rearrangements, and gains of whole chromosomes or large portions thereof (14, 15). In the UV-induced melanoma and squamous cell carcinoma, both types of instability have been shown to occur (12, 13, 16, 17).

The two types of UV radiation, UVA (320–400 nm) and UVB (290–320 nm), interact with skin in fundamentally different ways. UVB-radiation is strongly absorbed by DNA, which leads to direct DNA damage, whereas DNA hardly absorbs any UVA-radiation. The biological effect of UVA-radiation depends almost totally on the presence of oxygen, flavin-containing oxidases, and endogenous photosensitizers (18, 19). Reactive oxygen species damage DNA as well as many other biomolecules. UVA-radiation induces, in contrast to UVB-radiation, low levels of mutations immediately after irradiation (20). Thus, the mechanism of UVA-induced cancer is not well understood. In this study, we have compared the ability of UVA-, UVB-, and X-radiation to induce genomic instability and the types of instability induced by the radiation types. We have determined the relative abundance of delayed mutations in clones derived from single cells surviving radiation. The clones were also tested for chromosomal instability by three different methods.

MATERIALS AND METHODS

Cell Culture. Chinese hamster fibroblasts (V79 cells) were cultured in RPMI 1640 (PAA, Linz, Austria) supplemented with 10% FCS (PAA), penicillin (100 units/ml), streptomycin (100 μg/ml), and L-glutamine (2 mM).

UV Measurements. The irradiance from the lamps was routinely measured with a United Detector Technology detector (Universal Instruments) before each treatment. The lamp spectra and absolute irradiance from the lamps were measured by an irradiance-calibrated United States Biochemical 2000 spectrometer (Avantes, Eerbeck, Holland). The spectrometer was calibrated by a DH-2000-CAL calibration light source (Avantes); The DH-2000-CAL was calibrated by Avantes using National Institute of Standards and Technology traceable light sources. The error in irradiance from the DH-2000-CAL was ±9%. The UV doses used in this study, 9.1 kJ/m² for UVB and 265 kJ/m² for UVA, were physiologically relevant because they correspond to midday sun exposure times in midsummer of 4.6 and 2.5 h, respectively, in Finland (21) and 33 min for UVB in Italy (22).

UV-Irradiation Protocols. One million cells were seeded in 25 cm² flasks (Nunc, Inc., Naperville, IL) the day before irradiation. The cells were irradiated from above in PBS. The UVA lamp (Solland 3000, Gevelsberg, Germany) contained a 3 kW Hg-Xe light bulb and filters to remove all unwanted UVB. The irradiance from the UVA lamp was 436 W/m². The flasks absorbed 18 W/m²; the UVB lamp contained a bank of six fluorescing tubes (Philips TL-20W/01RS). The irradiance from the UVB lamp was 23.3 W/m². The flasks absorbed 4.7 W/m².

X-Irradiation Protocols. Half a million cells were seeded in a 10 cm² flask (Nunc) the day before irradiation. A Siemens stabiplan X-ray unit, operated at 200 kV and 20 mA and filtered with 0.5 mm copper, was used for irradiation at a dose rate of 3.3 Gy/min. The cells were irradiated in PBS.

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3 Internet address: http://www.cancer.gov.
Survival Curves. After irradiation with different doses, trypsin was added immediately, and 200-1000 detached cells were seeded in triplicate 60-mm dishes (Becton-Dickinson) per dose. To detach the cells irradiated with UVA, a cell scraper was used in addition to trypsin. The cells were incubated for 6 days, washed with 9 mg/ml NaCl, fixed with absolute alcohol, and stained with a saturated methylene blue solution.

Detection of Early HPRT Mutations. Cells were exposed to UVA-, UVB-, or X-radiation. The cells were subcultured every 2nd day after irradiation. On the 6th day after irradiation, one million cells were seeded in triplicate 100-mm dishes in medium with 5 μg/ml 6-thioguanine and 200 cells in triplicate 60-mm dishes in medium without 6-thioguanine. Six days after seeding, the cells were washed, fixed, and stained as described above. The mutant rate, m (number of mutants per million cells), was calculated by equation 1:

\[ m = \left( \frac{t^2}{M} \right) \] (number of mutants per million cells), was calculated by equation 1:

\[ m = \left( \frac{t^2}{M} \right) \times \frac{C}{C} \]

In equation 1, M is the total number of mutants, C is the total number of cells seeded (usually 3 million), and c.e. is the cloning efficiency.

Detection of Delayed Mutations. Delayed mutations were detected by a method developed by Little et al. (8). Cells were irradiated with D20 doses of UVA-, UVB-, or X-radiation. Immediately after irradiation, trypsin was added, and 400–500 cells were seeded in 10 100-mm cell culture dishes. The cells were incubated for 7 days. Then, two to three colonies were isolated from each dish using sterile tweezers and cloning disks (Sigma Chemical Co., St. Louis, MO) soaked in trypsin. One cloning disk was put in each well of a 24-well plate (Becton-Dickinson) per treatment. After 3 days of incubation, the wells were confluent, and the cells were transferred to six-well plates where they grew for 1 day and were then transferred to 100-mm dishes. After 3 days, the dishes were confluent. Then, for each of the 20–30 clones/treatment, 0.5–1 million cells (usually 1 million) were seeded in three 100-mm dishes in medium with 5 μg/ml 6-thioguanine to detect HPRT4 mutants. Two hundred cells from each clone were seeded in three 60-mm dishes with ordinary medium to determine the cloning efficiency. The cells were incubated for 6 days, and then the cells were washed, fixed, stained, and counted. The detection limit for the assay was 1.09 × 10^-6, which is the mutation frequency found with one mutant in one of three dishes seeded with 500,000 cells and the average plating efficiency for the control cells (0.61). Before fixation, colonies were isolated from some of the 100-mm dishes with a high number of mutants. These clones were expanded to 10^2 cells, and the frequency of mutations in the Nt°/kt° ATPase locus was determined by seeding 1 million cells in triplicate 100-mm dishes with medium containing 2 mM 6-thioguanine (Sigma). After 7 days of incubation, the cells were fixed, stained, and counted, and the mutation rate was calculated according to equation 1.

Labeling of Centrosomes. Cells were seeded on coated object glasses with wells (Nunc) and incubated for 18 h. Then, the cells were incubated for 2 h with 1 μg/ml nocodazole. The immunohistochemistry was performed according to a method developed by Purohit et al. (23). The cells were washed twice with PBS and fixed for 1 h with 3% paraformaldehyde. The cells were postfixed for 20 min in −20°C methanol. Fixed cells were washed twice with freshly made PBS with 0.1% Triton X-100 and 5% dried milk for 10 min. Then, pericentrin mouse IgG antibody (Becton-Dickinson) was added to each well to a final concentration of 5 μg/ml. The cells were incubated for 1 h on ice and washed 3 × 3 min with PBS with 0.1% Triton X-100 and 5% dried milk before staining with biotinylated horse antimouse IgG antibody (Molecular Probes, Eugene, OR) diluted 1/500 in PBS with 0.1% Triton X-100 and 5% dried milk for 0.5 h. Subsequently, the cells were washed for 3 × 3 min and stained with streptavidin-alexa (Molecular Probes) diluted 1/50 in PBS with 0.1% Triton X-100 and 5% dried milk for 0.5 h. Then, the cells were washed 2 × 3 min with PBS with 0.1% Triton X-100 and 5% dried milk and 3 × 3 min with PBS. The wells were removed, 7 μl of vectashield with 2 μg/ml Hoechst 33258 were added to each area, and the cells were covered by a cover glass. The cells were observed with a Zeiss Axiosoplan microscope using a 63 × oil immersion objective and a filter block with 450–490-nm excitation filter, 510-nm dichroic mirror, and 520-nm emission filter (pericentrin) or a filter block with 365-nm excitation filter, 395-nm dichroic mirror, and 420-nm emission filter (Hoechst 33258).

Measurement of DNA Content. V79 cells and human PBLs were stained according to a detergent-trypsin method developed by Vindelov et al. (24). The cells were treated with 30 μg/ml trypsin (Sigma) dissolved in a stock solution [3.4 mM trisodium citrat, 1.5 mM Spermidinetrhydrochloride (Sigma), 0.5 mM Tris (hydroxymethyl)-aminomethane (Sigma), and 0.1% volume for volume NP40 (BDH Chemicals, Poole, England) (pH 7.6)] for 10 min at room temperature to strip off the cytoplasm. Subsequently, a 500 μg/ml solution of trypsin inhibitor (Sigma) and 100 μg/ml solution of RNase A (Sigma) in stock solution were added, and the cells were incubated for 10 min at room temperature. During the incubation periods, the tubes were mixed by inversion five times. At last, the nuclei were stained by adding 416 μg/ml propidium iodide (Calbiochem, La Jolla, CA) for 10 min at room temperature before the cells were put on ice until DNA histograms were obtained by flow cytometry (FACS Calibur; Becton-Dickinson).

Measurement of the Number of Chromosomes in V79 Cells. The cells were treated with 0.1 μg/ml colcemid (Sigma) for 0.5 h at 37°C to accumulate cells in metaphase. The cells were stained by incubating with a hypotonic solution of freshly made 75 mM KCl (Sigma) at 37°C for 20 min. Subsequently, 1 ml of freshly made 3:1 methanol:acetic acid was added, the cells were centrifuged, and the pellet was carefully resuspended in 3:1 methanol:acetic acid while vortex mixing slowly. Two drops of cell solution were placed on object glasses at 4°C. After drying, the cells were stained with 1 μg/ml 4',6-diamidino-2-phenylindole (Sigma) in Vectashield (Vector Laboratories, Inc., Burlingame, CA). The number of chromosomes per metaphase cell was determined using the same microscope as described above.

Results and Discussion

We here report the first direct comparison of delayed mutations induced with the carcinogenic UVA-, UVB-, and X-radiations. By cloning cells immediately after irradiation, killing 80% of the cells, we have shown that UVA-, UVB-, and X-radiation induce delayed mutations at a frequency 10–7500 times above background.

Survival Curves. Fig. 1 shows the survival of V79 cells as a function of dose for UVA-, UVB-, and X-radiation. The doses killing 80% of the cells were 265 kJ/m² for UVA-irradiation, 9.1 kJ/m² for UVB-irradiation, and 4 Gy for X-radiation. The survival curves were in accordance with data published previously on V79 cells (20, 25).

Early Mutations. Induction of early mutations in V79 cells treated with D20 doses was measured as the fraction of cells resistant to 5 μg/ml 6-thioguanine after an expression period of 6 days after irradiation. UVB-radiation was most effective in induction of mutations, followed by X-radiation (Fig. 2). UVA-irradiation was only weakly mutagenic. X-radiation was used as a positive control in the studies of both early and delayed mutations, and our results were similar to those reported by others (8, 26). The results for early mutations for UV radiation were in accordance with data published previously on V79 cells (20, 25, 26).

Delayed Mutations. There are very few papers on UV-induced genomic instability in the literature. The most convincing studies were performed by Stamato et al. (27), who showed that delayed mutations in the glucose 6-phosphate dehydrogenase locus occurred from 4 to 11 cell generations after irradiation with nonsolar UVC-radiation. UVC-radiation induces direct DNA damage of the same type as UVB-radiation, suggesting that UVB-radiation may also induce genomic instability. Two other studies claim UVA-radiation to induce delayed cell death (28) and delayed HPRT mutation (29). However, the low doses of UVA-radiation needed for cell killing indicated UVB contamination. In the only previous study on UVA-induced genomic instability, Phillipson et al. (30) found an increase in the fraction of human keratinocytes with micronuclei ≤ 15 generations after UV exposure. However, cells with micronuclei are probably nonclonogenic (31). Using aminopterin, claimed to selectively kill early HPRT...
mutants, Phillipson et al. also found a small increase in the fraction of cells with HPRT mutations produced five to seven generations after UVA exposure (30). However, no information on the effect of aminopterin on survival of normal cells or of HPRT mutant cells was reported. Additionally, the UVA lamp used had low intensity, resulting in long irradiation times. To properly investigate late mutations, we decided to use the cloning method developed by Little et al. (8).

To measure delayed mutations, cell populations were cloned immediately after UVA-, UVB-, or X-radiation, killing 80% of the cells. The expression period after cloning was prolonged to 14 days. The probability of cloning an HPRT mutant can be calculated from Fig. 2 to be from 1 in 10^5 for control cells to 42 in 10^5 for UVB-treated cells, so it is very unlikely to clone a HPRT mutant when cloning 70–100 cells. At the time of analysis, the cells had undergone 23.3 ± 0.3 (control), 22.9 ± 0.9 (UVA-irradiated), 23.2 ± 1.2 (UVB-irradiated), or 23.2 ± 0.8 (X-irradiated) population doublings after irradiation [number of generations = ln(number of cells after 14 days)/ln(2)]. For each clone, 3 x 10^6 cells were examined for mutations. Table 1 and Fig. 3 show the frequency of mutations in clones derived from single cells surviving D_20 doses of UVA-, UVB-, and X-radiation. All types of radiation seem to result in an increased mutability in a significant fraction of the clones. The data are not in accordance with the idea that mutagenesis occurs as a consequence of false repair or lack of repair of DNA damage shortly after irradiation. Table 1 and Fig. 3 show that UVA-radiation induces the largest proportion of clones with delayed HPRT mutants. In fact, the distribution of mutation rates for cells treated with UVA-radiation was significantly different from the distributions for cells treated with UVB- and X-radiation (P < 0.02), indicating that UVA-radiation induces a significantly larger proportion of unstable cells at equitoxic doses. However, UVA-radiation was much less efficient in inducing early mutations.

The HPRT gene used for mutation studies probably has no role in genomic instability. However, it is likely that cells surviving radiation also have delayed mutations in many other genes. It is conceivable

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
<th>Total no. of clones examined</th>
<th>Number of clones (percentage of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1.09 x 10^-6</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
<td>102</td>
<td>79 (77.5%)</td>
</tr>
<tr>
<td>UVA 265 kJ/m²</td>
<td>20</td>
<td>68</td>
<td>43 (63.2%)</td>
</tr>
<tr>
<td>UVB 9.1 kJ/m²</td>
<td>20</td>
<td>87</td>
<td>58 (66.7%)</td>
</tr>
<tr>
<td>X 4 Gy</td>
<td>20</td>
<td>73</td>
<td>47 (64.4%)</td>
</tr>
</tbody>
</table>

*Clonogenic survival was measured in separate experiments described in Fig. 2 and normalized to 100% for control.

* Remaining clones had frequencies of 1.10-9.9 x 10^-6. The detection limit for the assay was 1.09 x 10^-6.
that most, if not all, of the cells surviving radiation are genetically unstable. The way to investigate this question would be to use other mutation tests, such as the Ouabain test for Na⁺/K⁺-ATPase mutation, and measure the mutation rate in cells cloned after irradiation. With the fraction of clones unstable with respect to HPRT mutations in mind, it is unlikely that clones that are unstable with respect to Na⁺/K⁺-ATPase gene also should have mutations in the HPRT gene. We found that the unstable clones with delayed HPRT mutations had mutation frequencies in the Na⁺/K⁺-ATPase gene similar to background, that is, below and just above the detection limit for the assay (Fig. 4). Our findings are consistent with those of Limoli et al. (32), which showed no correlation between four end points of genomic instability. In contrast, Little et al. found a correlation between delayed mutations in the HPRT gene and increased mutation frequency in the Na⁺/K⁺-ATPase gene when the unstable clones in addition were selected for slow growth (8), suggesting that a fraction of the cells was particularly unstable.

On the basis of the assumption that mutant cells and normal cells have similar doubling times and that only one mutational event occurs in each clone, it is possible to calculate, from the data in Fig. 3, approximately at how many generations after irradiation the mutational event occurred. Using these assumptions, the mutational events after UVB- or X-radiation occurred between 17 and 20 generations after irradiation, whereas for UVA-radiation, mutational events occurred between 10 and 20 generations after irradiation. Because this is highly unlikely, the assumptions of normal proliferation of mutants must be incorrect. Nevertheless, the larger time window for unstable UVA clones indicates that the delayed mutants in this case have a doubling time more similar to normal cells and/or are able to survive for more generations than the delayed mutants induced by UVB- and X-radiation. Thus, the oxidative damage induced by UVA-radiation may result in more viable delayed HPRT mutants than the direct DNA damage induced by UV and X-radiation.

Chromosomal Instability. To study the relationship between delayed mutations in the HPRT gene and chromosomal instability, the number of centrosomes of cells with delayed mutations was determined. Centrosomes have crucial functions in the correct separation of chromosomes during mitosis. Normal cells have one or two visible centrosomes, whereas cells that have more than two centrosomes and/or abnormal centrosomes have been shown to be chromosomally unstable (33). V79 control cells and unstable cell clones were stained with a monoclonal antibody to the centrosome protein pericentrin (Fig. 5). Table 2 and Fig. 5 show that V79 control cells and the UVA-treated clones with delayed mutations had a normal number of centrosomes, whereas the clones irradiated with X-rays or UVB had an increase in the number of centrosomes.

Given the important role of centrosomes in mitotic spindle organization, it is possible that centrosome aberration can result in chromosome instability (34). In fact, cells overexpressing a centrosome protein (pericentrin) have been shown to have abnormal centrosomes, disorganized spindle formation, and variability in chromosome numbers (35). We found clear differences in centrosome aberrations among the radiation types; 0% of the cells from UVA clones, 5% of the cells from UVB clones, and 15% of the cells from X clones had centromere aberrations (Table 2). UVA-radiation resulted in increased variation in chromosome number but not centrosome aberrations, indicating that UVA-radiation induces increased variation in chromosome number by disruption of another mechanism, such as the spindle, mitotic, or DNA damage checkpoint (36).

An aberrant number of centrosomes might lead to changes in DNA content and variations in the number of chromosomes of cells. However, DNA content was found to be quite similar among the various clones and V79 control cells (Table 3), but Fig. 6 shows that the number of chromosomes had a broader distribution for the unstable clones than for the control cells. In accordance with Table 3, the mean number of chromosomes was not significantly different (one-way ANOVA, P = 0.059).

Our results show a correlation between delayed HPRT mutations and chromosomal instability measured both as a variation in chromo-

![Fig. 4. Mutation in the Na⁺/K⁺-ATPase gene in V79 cells that formed unstable clones with mutation in the HPRT gene after treatment with UVA- (A15 and A16), UVB- (B3 and B7), or X-radiation (X12 and X15). Control, unirradiated V79 cells. /VIR, positive control (V79 cells irradiated with 9.1 kJ/m² UVB-radiation). Error bars = SE calculated from three independent experiments.](image)

![Fig. 5. The distribution of the number of centrosomes in V79 control cells and unstable clones. For each clone, ~100 cells were counted in two to four independent experiments. The centrosomes were labeled with an antibody against pericentrin.](image)

<table>
<thead>
<tr>
<th>Clone</th>
<th>No. of centrosomes</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>V79 control</td>
<td>84.2</td>
</tr>
<tr>
<td>A15</td>
<td>83.0</td>
</tr>
<tr>
<td>A16</td>
<td>75.4</td>
</tr>
<tr>
<td>B3</td>
<td>82.9</td>
</tr>
<tr>
<td>B7</td>
<td>74.8</td>
</tr>
<tr>
<td>X12</td>
<td>58.5</td>
</tr>
<tr>
<td>X15</td>
<td>65.1</td>
</tr>
</tbody>
</table>

* A15 and A16, UVB-irradiated 21 days before cloning. B3 and B7, UVB-irradiated. X12 and X15, X-irradiated. The clones were picked from dishes with frequency of HPRT mutants significantly above background levels.

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* Number of generations after cloning = 23 – [Ln(number of mutants per million)/Ln(2)].
some number and increased number of centrosomes (not for UVA-irradiated cells). Little et al. (8) also found a correlation between delayed mutations in the HPRT gene and chromosomal aberrations when the unstable clones in addition were selected for slow growth. However, Limoli et al. (32) found no increase in HPRT mutation frequency in clones with delayed chromosomal aberrations. We and Little et al. measured chromosomal instability after cloning cells with delayed mutation, whereas Limoli et al. did it the other way around. It seems that the order of which different end points are measured may decide if they are correlated or not. It is conceivable that separate molecular processes drive the different end points associated with genomic instability.

Mutations induced by UV radiation have been thought to be a direct consequence of DNA damage not being repaired correctly before subsequent cell divisions (37). A mutagenic event in a critical gene has been thought to represent the first step in carcinogenesis. Based primarily on studies of the age dependence of cancer incidence in humans, it is commonly assumed that in order for a cell to develop into cancer, the cell has to obtain approximately six mutations in genes critical for cancer development (38). Given the low rate of mutation per cell generation in normal cells, it is highly unlikely that

<table>
<thead>
<tr>
<th>Clone</th>
<th>G1_{clone}/G1_{PBL}</th>
<th>Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>V79 control</td>
<td>0.93 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>A15</td>
<td>0.91 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>A16</td>
<td>0.90 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>0.89 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>B7</td>
<td>0.88 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>X12</td>
<td>0.91 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>X15</td>
<td>0.90 ± 0.06</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 DNA content in unstable clones as compared with V79 cells and human PBLs. Mean fluorescence in G1 for V79 cells divided by mean fluorescence in G1 for PBLs. Error = ([SD(clone)] + [SD(PBL)]/[mean(clone)] + [mean(PBL)]).


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